

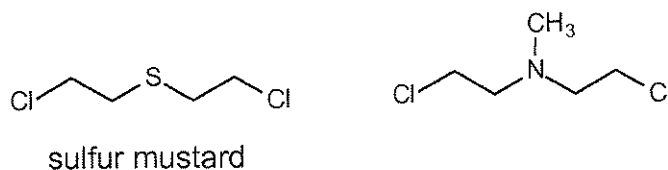
SYNTHESIS AND FORMULATIONS OF SALTS OF ISOPHOSPHORAMIDE MUSTARD AND ANALOGS THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

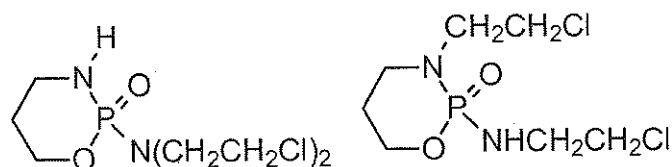
This application claims the benefit of U.S. Provisional Application Serial No. 61/137,538 filed July 31, 2008, which is incorporated by reference herein in its entirety.

BACKGROUND

Autopsies of soldiers killed by mustard gas in World War I indicated that sulfur mustard has a disproportionate effect on rapidly dividing cells and suggested that sulfur mustard compounds might have antitumor effects. Indeed, early researchers attempted to treat cancer by direct injection of sulfur mustard into tumors. This research was limited by the extreme toxicity of sulfur mustard compounds and nitrogen mustard analogs, such as mechlorethamine, were investigated as less toxic alternatives.



Because of the lack of selectivity of most mechlorethamine analogs, prodrugs, such as phosphoramidate compounds, which can be activated by the high concentration of phosphoramidases present in neoplastic cells, have been investigated. Two phosphoramidate alkylating agents, cyclophosphamide (CPA) and the isomeric compound ifosfamide (IFOS), have demonstrated effectiveness in the treatment of a broad range of solid tumors and hematological cancers (Zhang *et al.*, Current Drug Therapy 1: 55-84 (2006)). CPA and IFOS are used both as single agents as well as in combination with other anticancer agents to obtain synergistic antitumor effects. In addition to its application in cancer, CPA can also be used as an immunosuppressant to treat autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) (Petri *et al.*, Lupus 13:366-371 (2006); Leandro *et al.* Ann., Rheum. Dis. 61: 883-888 (2002); Verberg *et al.*, Arthritis Rheum. 52: 421-424 (2005)).



Cyclophosphamide (CPA) Ifosfamide (Ifos)

The metabolism of CPA and IFOS has been described in detail by Zhang *et al.* (Zhang *et al.*, Current Drug Therapy 1: 55-84 (2006)). CPA and IFOS are prodrugs that are activated intracellularly by 4-hydroxylation by the cytochrome (CYP) P450 oxidases, primarily CYP3A4, CYP2C9 and CYP2B6 in the liver, to produce cytotoxic nitrogen mustards that can react with DNA. Acrolein is a byproduct of this reaction. The *in vivo* metabolism of CPA and IFOS also involves inactivation by N-dechloroethylation by CYP3A4/5 and CYP2B6 prior to their conversion to the nitrogen mustards, resulting in production of dechloroethylated metabolites and the byproduct chloroacetaldehyde (CAA). Acrolein and CAA are implicated in toxicities of CPA and IFOS that are unrelated to the cytotoxic mechanism of action of the nitrogen mustard molecules (Zhang *et al.*, Current Drug Therapy 1: 55-84 (2006)). Acrolein causes the urotoxicity, hemorrhagic cystitis, and liver damage, and CAA causes neurotoxicity and has also been implicated in renal toxicity. Co-administration of the sulfhydryl compounds, mesna and amifostine, which react specifically with acrolein in the urinary tract, can reduce the urotoxicity of acrolein but does not eliminate other toxicities (Zaki *et al.*, Toxicol. In Vitro 17: 397-402 (2003)).

The nitrogen mustards of CPA and IFOS, phosphoramidate mustard and isophosphoramidate mustard, are bifunctional alkylating agents that bind covalently to nucleophilic groups of nucleic acids. At pH ≥ 7 , the mustards are dechlorinated to produce carbonium ions that react covalently with N⁷ of guanine residues. The reaction is referred to as DNA alkylation. Both inter- or intra-strand crosslinks result from the ability of each mustard molecule to react with two guanine residues (Zhang *et al.*, Current Drug Therapy 1: 55-84 (2006)). Because the inter-strand crosslinks prevent strand separation required for DNA replication, DNA-alkylation is considered to be the major mechanism responsible for the inhibition of cell division by CPA and IFOS. In addition to the antiproliferative (cytostatic) effect, the DNA damage also induces apoptosis, i.e., programmed cell death (O'Conner *et al.*, Cancer Res. 1: 6550-6557; (1991); Bahtia *et al.*, Clin. Cancer Res. 1: 873-880 (1995)). The

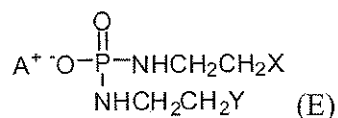
cytotoxic/cytostatic effects of the nitrogen mustards are mainly responsible for the antitumor activity of CPA and IFOS and, by preventing the proliferative expansion of autoreactive lymphocytes, also for the immunosuppressant activity of CPA in autoimmune disease. However, cross-linking of DNA in normal tissues by the nitrogen mustards also causes cytotoxic, mechanism-based collateral damage, particularly myelosuppression resulting in leucocytopenia, which is the principal dose-limiting hematological toxicity (Zhang *et al.*, Current Drug Therapy 1: 55-84 (2006)).

Although phosphoramidate mustard and isophosphoramidate are chemically similar, isophosphoramidate mustard interacts with DNA with a higher affinity than phosphoramidate mustard (Boal *et al.*, J. Med. Chem. 32: 1768-1773; 1989). Structural differences involving the intramolecular distance between the chloroethyl groups and their orientation appear to be responsible for the different affinities of the two mustards (Springer *et al.*, J. Org. Chem. 63: 7218-7222 (1998)).

By administering cytotoxic nitrogen mustards directly to cancer patients, the "off-target" toxicities and the drug resistance associated with the prodrugs may be reduced. IPM has been synthesized and preliminary biological evaluations of the compound have been conducted; but, unfortunately, IPM itself is unstable and difficult to use directly for human treatment. Stabilized formulations of IPM might further reduce toxicity and allow metronomic administration of doses that are sufficient for both direct cytotoxicity against the tumor and antiangiogenic activity. Improved methods for formulation and manufacture of IPM and analogues and salts thereof are needed.

SUMMARY

The invention discloses pharmaceutical formulations of isophosphoramidate mustard (IPM) salts and analogues thereof as well as methods for synthesizing IPM and salts and analogues thereof. IPM salts and analogues of the invention include compounds of formula (E):

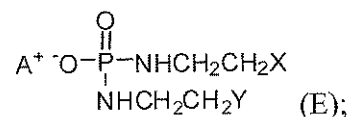


wherein X and Y independently represent leaving groups; and A⁺ is an ammonium cation.

In certain embodiments, the invention relates to pharmaceutical formulations comprising a compound of formula (E) and one or more pharmaceutically acceptable carriers. Methods of preparing such compounds and formulations are also described.

DETAILED DESCRIPTION

The disclosure concerns pharmaceutical formulations of isophosphoramidate mustard (IPM) salts and analogues thereof as well as methods for synthesizing IPM and salts and analogues thereof. IPM salts and analogues of the invention include compounds of formula (E):

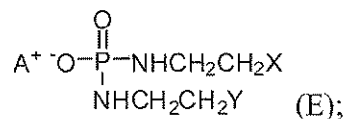


wherein X and Y independently represent leaving groups such as Cl, Br, I, or a sulfonate, e.g., toluenesulfonate, methanesulfonate, 2,4,6-triisopropylbenzenesulfonate, or 2,4,6-trimethylbenzenesulfonate; and A⁺ is an ammonium cation. Compounds disclosed in U.S. Application No. 11/257,766, filed October 25, 2005 are useful in the compositions and methods of the invention and are herein incorporated by reference. Formulations of the invention include IPM salts and analogues together with a lubricant, a diluent and a disintegrant. Formulations of the invention may further comprise additional excipients such as a binder and a compression filler. The disclosure further concerns the synthetic preparation of IPM salts and analogues thereof.

I. Salts of IPM and IPM analogs

The formulations disclosed herein include IPM and IPM analogs that are formulated with one or more equivalents of base. In certain embodiments, the disclosed compounds are salts of isophosphoramidate mustard or isophosphoramidate mustard analogs including one or more cations. In one embodiment, the cations can be a conjugate acid of an amine base or can be a quaternary ammonium cation. Suitable counterions for isophosphoramidate and its analogs include the conjugate acids (as used herein, terms that refer to amines should be understood to include their conjugate acids unless the context indicates that the free amine is intended) of bases including basic amino acids, aliphatic amines, heterocyclic amines, aromatic amines, pyridines, guanidines, and amidines.

In certain embodiments, group A of formula (E):



is selected from a primary, secondary or tertiary amine. For example, in certain embodiments, A of formula (E) represents at least one primary amine such as lysine, arginine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine, 2-aminoethanol, or tris(hydroxymethyl)aminomethane. In certain embodiments, A of formula (E) represents at least one secondary amine such as diethylamine, 2-(methylamino)ethanol, aziridine, azetidine, pyrrolidine and piperidine. In certain embodiments, A of formula (E) represents at least one tertiary amine, such as triethylamine, trimethylamine, *N,N*-diisopropylethylamine, and 2-(dimethylamino)ethanol.

Primary, secondary, and tertiary amine as used herein refers to the number of direct single bonds between a nitrogen and carbon atom(s), the remainder of the three valencies of the neutral amine being filled by hydrogen atoms. A primary amine has only one direct bond between nitrogen and carbon, while a secondary amine is singly bound to exactly two carbons, a tertiary amine is singly bound to exactly three carbons. Quarternary ammonium cations have four single bonds to carbon atoms, the fourth bond involving the lone pair of the nitrogen atom as well as the three valencies ordinarily present in primary, secondary, and tertiary amines. Accordingly, as the name implies, such species carry a positive charge. In order for a molecule to be classified within a particular amine type, e.g., primary, secondary or tertiary amine, the molecule must comprise at least one nitrogen with the indicated bonding pattern to carbon(s). For example, a primary amine comprises at least one nitrogen that is singly bound to exactly one carbon atom. A molecule may however comprise more than one type of amine, e.g., 2-aminopiperidine comprises both primary and secondary amine functionality.

In certain embodiments, the amine is an aliphatic amine, such as an acyclic aliphatic amine. In certain embodiments, the amine is an acyclic aliphatic amine, e.g., an amine having 2-3 branched or straight chain alkyl substituents. In certain embodiments, each branched or straight chain alkyl substituent is a C₃-C₁₀ alkyl amine, such as a C₃-C₅ alkyl amine. In certain such embodiments, one or more of the

branched or straight chain alkyl substituents are optionally substituted, such as with one or more hydroxyl substituents, e.g., 1, 2, 3 or 4 hydroxyl substituents.

Exemplary amines (and their corresponding ammonium ions) for use in the formulations or methods of the invention include pyridine, *N, N*-dimethylaminopyridine, diazabicyclononane, diazabicycloundecene, *N*-methyl-*N*-ethylamine, diethylamine, triethylamine, *N, N*-diisopropylethylamine, mono-, bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, tris(hydroxymethyl)methylamine, *N, N*-dimethyl-*N*-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine and *N*-methyl-D-glucamine.

In a further aspect, the salts described above in formula (E) can include a second amine or ammonium group. In certain embodiments, the compounds disclosed herein include more than one equivalent of an amine for each equivalent of isophosphoramidate mustard or isophosphoramidate mustard analog. Such embodiments include those having non-integer ratios of amine to isophosphoramidate mustard or isophosphoramidate mustard analogs. In certain embodiments, the compounds have a two to one or three to one ratio of amine to isophosphoramidate mustard or an isophosphoramidate mustard analog. In working embodiments, salts were produced containing two equivalents of amine base per equivalent of isophosphoramidate mustard. In some embodiments, an amine base used to form isophosphoramidate mustard and isophosphoramidate mustard analog salts includes more than one amino group; such bases can be termed "multibasic." More specifically, certain examples of multibasic bases that can be used have two amino groups; such compounds can be referred to as "dibasic." For example, one suitable dibasic molecule is *N, N*-dimethylaminopyridine, which includes two basic amino groups. In a particular embodiment of a compound disclosed herein, a compound includes isophosphoramidate mustard or an isophosphoramidate mustard analog and one equivalent of a dibasic amine.

In one embodiment, the disclosed compounds include one or more zwitterionic bases. Examples of such bases include basic amino acids, which are zwitterionic at physiological pH.

As used herein, "aliphatic amine" refers to a compound of the formula $\text{NR}^1\text{R}^2\text{R}^3$, wherein at least one of R^1 , R^2 or R^3 is an aliphatic group.

The term "acyclic aliphatic amine" refers to an aliphatic amine as above, wherein at least one, and preferably all, of the aliphatic groups is acyclic.

The term "heterocyclic amine" refers to a compound of the formula $\text{NR}^1\text{R}^2\text{R}^3$, wherein at least one of R^1 , R^2 or R^3 is a heterocyclic group or R^1 , R^2 and/or R^3 taken together with their common nitrogen atom form a ring.

The term "leaving group" refers to a group that can be displaced by a nucleophile. With reference to the presently disclosed compounds, leaving group refers to a group that can be displaced to form an aziridinium intermediate, or can be directly displaced by a biomolecular nucleophile, such as a nucleic acid nucleophile, to form, for example, a 7-alkylated guanidinium species. Examples of suitable leaving groups include the halogens and the sulfonates ($-\text{SO}_2\text{R}$). In certain embodiments, for the isophosphoramidate analog salts disclosed herein, the compound is a "mixed" leaving group compound, including two different types of leaving groups, for example a halogen and a sulfonate or two different halogens, such as a bromide and a chloride. U.S. Pat. No. 6,197,760 to Struck teaches methods for making such mixed leaving group compounds.

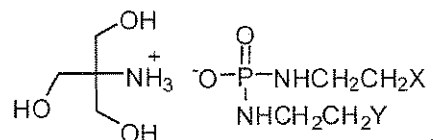
II. *Formulation of IPM salts and analogues thereof*

An aspect of the disclosure includes pharmaceutical formulations, such as an oral dosage form, prepared for administration to a subject and which include a therapeutically effective amount of one or more of the IPM salts and analogues thereof disclosed herein or elsewhere. The formulation may be in the form of a pill, a tablet or a capsule to be administered orally. In certain embodiments, the formulation is in the form of a capsule for oral administration.

In certain embodiments, the formulation comprises a lubricant, a diluent and a disintegrant in addition to, e.g., admixed with, the IPM salt or analogue thereof. The formulation may comprise, for example, 0.25-5% of a lubricant, up to 98% of a diluent such as from 80 to 98%, such as 85 to 95% such as about 90% of a diluent, and up to 90% of a disintegrant such as from 0.5 to 10%, such as from 0.5 to 5%, such as about 1% of a disintegrant by weight of the formulation. The formulation may further comprise one or more additional diluents, disintegrants or lubricants and additional carriers.

In certain embodiments, an oral dosage form comprises from 1 to 250 mg of the compound of formula (E) such as from about 1 to about 100 mg or from about 10

mg to about 50 mg. In certain embodiments, the formulation comprises from 5-25 mg of the compound of formula (E) such as about 5 mg, about 7.5 mg, about 10 mg, about 12 mg, about 15 mg, about 20 mg and about 25 mg. In certain embodiments, the compound of formula (E) is the tris(hydroxymethyl)aminomethane (Tris) salt:



wherein X and Y are independently selected from leaving groups, such as Cl, Br, or I, or a sulfonate, e.g., toluenesulfonate, methanesulfonate, 2,4,6-triisopropylbenzenesulfonate, or 2,4,6-trimethylbenzenesulfonate.

In certain embodiments, the lubricant of the formulation may be selected from any one or more of talc; fumed silicon dioxide such as Aerosil, Cab-O-Sil, or Syloid; starch; calcium silicate; magnesium carbonate (heavy); magnesium oxide (heavy); magnesium lauryl sulfate, sodium lauryl sulfate, calcium stearate, sodium stearyl fumarate, polyethylene glycol 4000 and 6000, sodium benzoate, light mineral oil, hydrogenated vegetable oils, stearic acid, or glyceryl behenate. In certain embodiments, the lubricant is magnesium stearate. In certain embodiments, the lubricant of the formulation comprises the salt of a fatty acid, such as the salt of a long chain, e.g., C₁₀-C₂₄, saturated or unsaturated fatty acid. In certain embodiments, the salt of a fatty acid is a metallic salt, such as the magnesium salt of a fatty acid, e.g., magnesium stearate. In certain embodiments, the lubricant of the formulation comprises a long chain fatty acid ester such as sodium stearyl fumarate. In other embodiments, the lubricant comprises a mixture of glycerides of fatty acids such as glyceryl behenate.

The formulation may comprise at least one of the following lubricants in the indicated amount (by weight of the formulation):

Talc	1-5%,
Fumed silicon dioxide	0.1-0.5%,
Starch	1-10%,
Calcium silicate	0.5-2.0%,
Magnesium carbonate (heavy)	1-3%,
Magnesium oxide (heavy)	1-3%,
Magnesium lauryl sulphate	0.2-2%,

Sodium lauryl sulphate	0.2-2%,
Calcium stearate	0.5-4%,
Sodium stearyl fumarate	0.5-2%,
Polyethylene glycol 4000 and 6000	2-10%,
Sodium benzoate	2-5%,
Light mineral oil	1-3%,
Hydrogenated vegetable oils	1-5%,
Stearic acid	0.25-2%, and
Glyceryl behenate	0.5-4%.

In certain embodiments, the formulation comprises magnesium stearate in an amount (by weight of formulation) selected from 0.25% and 2% or from 0.25% to 1% or about 0.5%.

The capsule formulation may comprise a diluent selected from any one or more of lactose, microcrystalline cellulose, mannitol, calcium hydroxy-dioxido-oxo-phosphorane, dextrose, glucose, sucrose, starch and derivatives, calcium carbonate, dicalcium phosphate and magnesium carbonate. In certain embodiments, the diluent is selected from microcrystalline cellulose, mannitol, lactose and calcium hydroxydiodioxido-oxo-phosphorane. In certain embodiments, the diluent is microcrystalline cellulose.

In certain embodiments, the diluent comprises a carbohydrate, such as sugar or sugar alcohols (e.g., lactose, α -lactose monohydrate, sucrose, mannitol, or sorbitol), or a cellulose polymer such as microcrystalline cellulose, silicified microcrystalline cellulose, or powdered cellulose.

The formulation may comprise at least one diluent in an amount up to 98% by weight of the formulation such as from about 50-85% or about 50-75%. In certain exemplary embodiments, the formulation comprises one or more of the following diluents in the indicated amount by weight of the formulation:

microcrystalline cellulose	5-98%,
mannitol	10-90%,
dextrose	up to 98%,
glucose	up to 98%,
starch and derivatives	up to 98%,
calcium carbonate	up to 98%,

dicalcium phosphate	up to 98%,
magnesium carbonate	up to 98%,
lactose	up to 98% and
calcium hydroxydiodioxido-oxo-phosphorane	10-80%.

In certain embodiments, the formulation comprises from 85-95% microcrystalline cellulose. The formulation may comprise from 88-92% microcrystalline cellulose such as about 91% microcrystalline cellulose. In exemplary embodiments, the formulation comprises 0.25-1% magnesium stearate and about 91% microcrystalline cellulose. In certain particular embodiments, the formulation comprises about 91% microcrystalline cellulose and about 0.5% magnesium stearate.

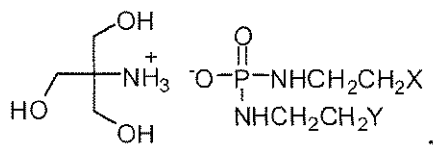
The formulation may comprise at least one disintegrant, e.g., a water-soluble polymer, preferably an anionic water-soluble polymer, such as cellulose or a derivative thereof or a salt thereof. In various embodiments, the disintegrant may be selected from any one or more of starch, microcrystalline cellulose, insoluble ion exchange resins, sodium starch glycolate, sodium carboxymethylcellulose, gums such as agar, guar and xanthan, alginic acid, sodium alginate and povidone. In certain embodiments, the disintegrant comprises a salt of cellulose or a derivative thereof. Derivatives of cellulose include molecules in which one or more of the hydroxyl functionalities of cellulose are bound to atoms or groups of atoms other than hydrogen. For example, the disintegrant may comprise carboxymethylcellulose (CMC) (e.g., a cellulose derivative with carboxymethyl groups ($-\text{CH}_2\text{-COOH}$) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone) or an anionic form thereof. In certain embodiments, the disintegrant is or comprises sodium carboxymethylcellulose, which may optionally be crosslinked. Preferably, the disintegrant is selected such that the formulation disintegrates in the stomach in less than 30 minutes, such as less than 15 minutes, or even less than 10 minutes.

The formulation may comprise at least one disintegrant in an amount up to 90% by weight of the formulation. In certain exemplary embodiments, the formulation comprises one of the following disintegrants in the indicated amount by weight of the formulation:

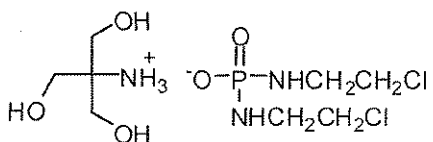
microcrystalline cellulose	5-90%,
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starch	3-25%,
sodium starch glycolate	2-8%,
sodium carboxymethylcellulose	up to 15%,
gum	less than 5%
alginic acid or sodium alginate	4-6%, and
crospovidone	1-5%.

In certain embodiments, the disintegrant is sodium carboxymethylcellulose. The formulation may comprise from 0.5-2% sodium carboxymethylcellulose. In certain embodiments, the formulation comprises about 1% sodium carboxymethylcellulose. In certain embodiments, the formulation comprises 0.5-2.0% sodium carboxymethylcellulose, 0.25-1% magnesium stearate, at least about 90% microcrystalline cellulose, and from 5-9% of the compound of formula (E). In exemplary embodiments, the formulation comprises about 1% sodium carboxymethylcellulose, about 91% microcrystalline cellulose, about 0.5% magnesium stearate and about 15 mg of the compound of formula (E):



wherein X and Y are leaving groups, such as Br, Cl, or I. In an exemplary embodiment, the compound of formula (E) is:



The formulation may further comprise one or more additional carriers such as a binder from 3-90% and a compression filler up to 98%. The formulation may further comprise a carrier selected from a second diluent, a second disintegrant, and a second lubricant. Other pharmaceutically acceptable carriers useful for these formulations are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition (1995), describes formulations suitable for pharmaceutical delivery of the compounds herein disclosed.

In certain embodiments, the formulation comprises a component that performs the function of two or more of a lubricant, a diluent, and a disintegrant, e.g., acts as

both a lubricant and a disintegrant. For example, the formulation may comprise microcrystalline cellulose as both the diluent and the disintegrant. In certain such embodiments, there may or may not be one or more additional diluents and/or disintegrants in a formulation, and/or the multi-acting component is present in an amount equal to the amounts of all of the components whose functions it performs. In certain embodiments, a single component of the formulation may act as all three of a diluent, a lubricant and a disintegrant. In certain embodiments, each of a lubricant, diluent and disintegrant are compounds that are distinct from one another.

Pharmaceutical formulations can also include one or more additional active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like.

In one aspect, certain embodiments of pharmaceutical formulations are formulated into unit dosage forms. For example such unit dosage forms can contain from about 1 mg to about 250 mg, such as from about 5 mg to about 100 mg, such as about 5 mg to about 50 mg, such as about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg or about 50 mg of a disclosed isophosphoramidate mustard salt or analog thereof per dosage unit.

In certain embodiments, the formulations of IPM salts or analogs thereof disclosed herein, are stable at room temperature for at least two weeks, at least one month, at least two months at 5 °C, at least three months at 5 °C, at least six months at 5 °C, at least 9 months at 5 °C, at least 12 months at 5 °C, at least 18 months at 5 °C, or even at least 24 months at 5 °C. In certain embodiments, the active ingredient of such stable formulations undergoes less than 10% decomposition, preferably less than 5%, 2%, or even less than 1% decomposition, e.g., as measured by assaying for the presence of decomposition by-products such as phosphoric acid and its salts and substituted ethylamines, such as by HPLC or GC, for at least two weeks, at least one month, at least two months, at least three months, or even at least six months. In other embodiments, the stable formulations maintain >90%, >95%, or even >98% potency at room temperature for at least two weeks, at least one month, at least two months at 5 °C, at least three months at 5 °C, at least six months at 5 °C, at least 9 months at 5 °C, at least 12 months at 5 °C, at least 18 months at 5 °C, or even at least 24 months at 5 °C, e.g., as determined by HPLC analysis.

As used herein, the term “stable” means that the purity of the IPM salt or analog thereof after a period of time (e.g., one month, two months, three months, six months, one year, etc.) is at least 90%, at least 95%, at least 97%, or even at least 99% of the initial purity, which may be determined e.g., by HPLC using evaporative light scattering detection (ELSD). Such an assay may be performed, for example, using a C18 column and an isocratic system with a mobile phase comprising 0.005 M heptafluorobutyric acid and 0.1% trifluoroacetic acid in water.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin (“gelcaps”), as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with carriers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

Methods are disclosed herein for treating conditions characterized by abnormal or pathological proliferative activity or neoplasia by administering one or more of the disclosed compounds or formulations to a subject. “Neoplasia” refers to the process of abnormal and uncontrolled cell growth. Neoplasia is one example of a proliferative disorder. The product of neoplasia is a neoplasm (a tumor), which is an abnormal growth of tissue that results from excessive cell division. A tumor that does not metastasize is referred to as “benign.” A tumor that invades the surrounding tissue and/or can metastasize is referred to as “malignant.”

Conditions that can be treated according to the disclosed method include those characterized by abnormal cell growth and/or differentiation, such as cancers and other neoplastic conditions. Typical examples of proliferative disorders that can be treated using the disclosed compounds and formulations are listed below.

Examples of hematological tumors that can be treated using the compounds and formulations disclosed herein include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's

macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

Additional examples of conditions that can be treated using the disclosed compounds and formulations include solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, bladder carcinoma, and CNS tumors (such as a glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma).

In certain embodiments, the methods disclosed herein include treating a subject having a CPA-resistant neoplastic condition with an isophosphoramidate mustard salt or analog thereof as disclosed herein.

In one embodiment of the method a subject is administered from about 0.2 mg/kg/day to about 20 mg/kg/day of a disclosed isophosphoramidate mustard salt or analog thereof. For example, from about 0.5 to about 10 mg/kg/day, such as from about 1 to about 7.5 mg/kg/day of a disclosed compound can be administered to a subject.

In another embodiment of the method, a subject is administered from about 10 to about 700 mg/m²/day, such as from about 20 to about 400 mg/m²/day or from about 100 to about 500 mg/m²/day. For example, from about 30 to about 100 mg/m²/day, such as from about 40 to about 90 mg/m²/day of a compound disclosed herein.

In one embodiment of the method for treating hyperproliferative disorders disclosed herein, a disclosed compound is administered to a subject on a multiple daily dosing schedule. In such embodiments the compound is administered on at least two days and on as many as five different days. In one aspect of multiple daily dosing

schedules, the compound is administered to the subject on consecutive days, such as from two to five consecutive days.

In one embodiment of the method one or more additional therapeutic agents is administered to a subject in addition to the presently disclosed compounds and formulations. For example, additional therapeutic agents that can be used include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and/or RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and/or angiogenesis inhibitors.

"Microtubule binding agent" refers to an agent that interacts with tubulin to stabilize or destabilize microtubule formation thereby inhibiting cell division. Examples of microtubule binding agents that can be used in conjunction with the presently disclosed isophosphoramidate mustard salts and analogs thereof include, without limitation, paclitaxel, docetaxel, vinblastine, vindesine, vinorelbine (navelbine), the epothilones, colchicine, dolastatin 15, nocodazole, podophyllotoxin and rhizoxin. Analogs and derivatives of such compounds also can be used and will be known to those of ordinary skill in the art. For example, suitable epothilones and epothilone analogs for incorporation into the present compounds are described in International Publication No. WO 2004/018478, which is incorporated herein by reference. Taxoids, such as paclitaxel and docetaxel are currently believed to be particularly useful as therapeutic agents in the presently disclosed compounds. Examples of additional useful taxoids, including analogs of paclitaxel are taught by U.S. Patent Nos. 6,610,860 to Holton, 5,530,020 to Gurram et al. and 5,912,264 to Wittman et al. Each of these patents is incorporated herein by reference.

Suitable DNA and/or RNA transcription regulators, including, without limitation, actinomycin D, daunorubicin, doxorubicin and derivatives and analogs thereof also are suitable for use in combination with the presently disclosed compounds.

DNA intercalators and cross-linking agents that can be incorporated into the disclosed compounds include, without limitation, cisplatin, carboplatin, oxaliplatin, mitomycins, such as mitomycin C, bleomycin, chlorambucil, cyclophosphamide and derivatives and analogs thereof.

DNA synthesis inhibitors suitable for use as therapeutic agents include, without limitation, methotrexate, 5-fluoro-5'-deoxyuridine, 5-fluorouracil and analogs thereof.

Examples of suitable enzyme inhibitors for use in combination with the presently disclosed compounds include, without limitation, camptothecin, etoposide, formestane, trichostatin and derivatives and analogs thereof.

Suitable therapeutics for use with the presently disclosed compounds that affect gene regulation include agents that result in increased or decreased expression of one or more genes, such as, without limitation, raloxifene, 5-azacytidine, 5-aza-2'-deoxycytidine, tamoxifen, 4-hydroxytamoxifen, mifepristone and derivatives and analogs thereof.

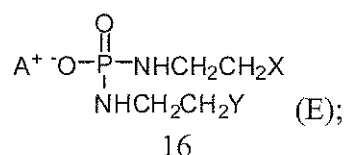
The term "angiogenesis inhibitor" is used herein, to mean a molecule including, but not limited to, biomolecules, such as peptides, proteins, enzymes, polysaccharides, oligonucleotides, DNA, RNA, recombinant vectors, and small molecules that function to inhibit blood vessel growth. Angiogenesis is implicated in certain pathological processes, such as those involved in disorders such as diabetic retinopathy, chronic inflammatory diseases, rheumatoid arthritis, dermatitis, psoriasis, stomach ulcers, and most types of human solid tumors.

Angiogenesis inhibitors are known in the art and examples of suitable angiogenesis inhibitors include, without limitation, angiostatin K1-3, staurosporine, genistein, fumagillin, medroxyprogesterone, suramin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thrombospondin, endostatin, thalidomide, and derivatives and analogs thereof.

Other therapeutic agents, particularly anti-tumor agents, that may or may not fall under one or more of the classifications above, also are suitable for administration in combination with the presently disclosed compounds. By way of example, such agents include adriamycin, apigenin, rapamycin, zebularine, cimetidine, and derivatives and analogs thereof.

III. *Preparation of IPM salts and analogues thereof*

In one aspect, the methods are used to prepare compounds of Formula (E):



or any salt, prodrug, tautomer, or isomer thereof,

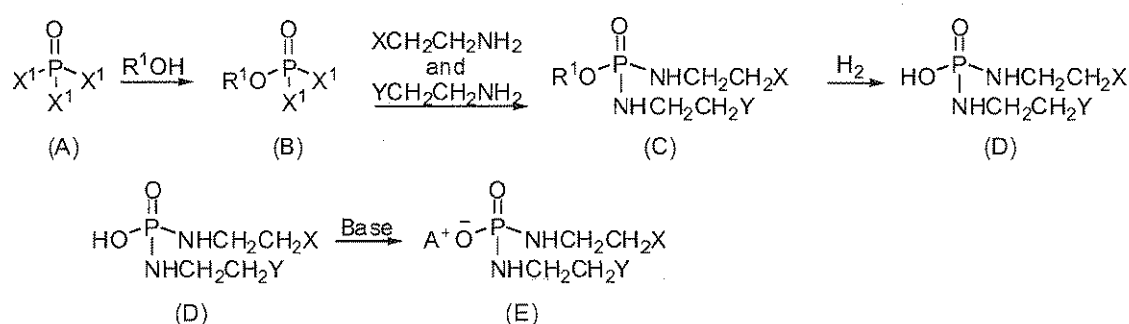
wherein:

X and Y independently represent leaving groups; and

A⁺ is an ammonium cation.

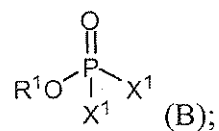
In certain embodiments, the present disclosure provides methods for preparing compounds of Formula (E) via the pathway in Scheme 1.

Scheme 1. Preparation of IPM and salts and analogues thereof

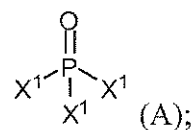


According to Scheme 1, phosphine oxide (A) is treated with alcohol R¹OH affording monoester (B). Monoester (B) is treated with one or more amines, e.g., XCH₂CH₂NH₂ and YCH₂CH₂NH₂ or salts thereof in concert or in series, under condensation conditions affording phosphordiamidate (C). Phosphordiamidate (C) is converted under hydrogenolysis conditions to phosphordiamidic acid (D). Phosphordiamidic acid (D) is then converted to a salt by treatment with a base.

In certain embodiments, the present invention provides a method for the preparation of a compound of formula (B):



comprising treating a compound of formula (A):



with an alcohol R^1 -OH under condensation conditions, wherein, as valence and stability permit,

X^1 independently for each occurrence, is selected from Cl, I, and Br,

R^1 is benzyl, optionally substituted with one or more substituents, e.g., selected from halogen, $-R^2$, $-OR^2$ and $-NR^2_2$, wherein R^2 is independently selected for each occurrence from H and lower alkyl.

In certain embodiments, R^1 is benzyl optionally substituted with one or more substituents. In certain embodiments, R^1 is unsubstituted benzyl. In certain embodiments, X^1 at each occurrence is independently selected from any of $-Cl$, $-Br$ and $-I$. In certain embodiments, the compound of formula (A) is $P(O)Cl_3$ and/or R^1OH is $PhCH_2OH$.

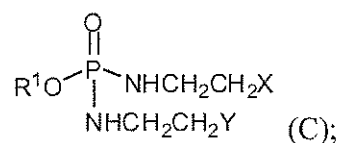
In certain aspects, the condensation conditions comprise an amine base. In certain such embodiments, the amine base is selected from any of N-methyl morpholine, triethylamine, pyridine or diisopropylethylamine, e.g., triethylamine.

In certain embodiments, the condensation conditions may comprise an aprotic organic solvent, e.g., acetone, 2-butanone, butyl acetate, ethyl acetate, acetonitrile, toluene, THF, dioxane, N,N-DMF, DMSO, 1,2-dichloroethane, or methylene chloride. In certain embodiments, the aprotic organic solvent comprises acetonitrile, wherein the acetonitrile may represent, for example, more than about 10, 20, 30, 50, 70, or 90% of the solvent system, or substantially all of the solvent system, for example about 95% or greater of the solvent system.

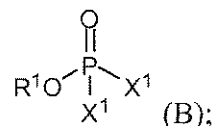
In some embodiments, phosphine oxide A and R^1OH are combined under condensation conditions, e.g., in a ratio in the range of 2:1 to 1:1.2 of compound of formula (A) to R^1OH , such as in a ratio in the range of 1.2:1 to 1:1.2, preferably approximately equimolar amounts of the compound of formula (A) and alcohol R^1OH .

In certain embodiments, the condensation conditions comprise maintaining a reduced temperature, e.g., in the range of about -50 to about -10 °C, such as from about -35 to about -25 °C, e.g., about -30 °C.

In certain embodiments, the present invention provides a method for the preparation of a compound of formula (C):



comprising treating a compound of formula (B):



with amines $\text{XCH}_2\text{CH}_2\text{NH}_2$ and $\text{YCH}_2\text{CH}_2\text{NH}_2$ or salts thereof under condensation conditions, wherein, as valence and stability permit,

X and Y independently represent leaving groups which may be the same or different; and

R^1 is benzyl optionally substituted with one or more substituents, e.g., selected from halogen, $-\text{R}^2$, $-\text{OR}^2$ and $-\text{NR}^2_2$, wherein R^2 is independently selected for each occurrence from H and lower alkyl.

In certain embodiments, R^1 is benzyl optionally substituted with one or more substituents. In certain embodiments, R^1 is unsubstituted benzyl. In certain embodiments, X^1 at each occurrence is independently selected from any of Cl, Br and I. In certain embodiments, R^1 is unsubstituted benzyl and/or X^1 is selected at each occurrence from Cl or Br.

X and Y of amines $\text{XCH}_2\text{CH}_2\text{NH}_2$ and $\text{YCH}_2\text{CH}_2\text{NH}_2$ may be independently selected from halogen, e.g., Cl, Br, or I, or a sulfonate, e.g., toluenesulfonate, methanesulfonate, 2,4,6-triisopropylbenzenesulfonate, or 2,4,6-trimethylbenzenesulfonate. In certain embodiments, X and Y are independently selected from Cl, Br or I. In certain embodiments, X and Y are identical, e.g., X and Y are both Cl. In any of the embodiments of the present disclosure, $\text{XCH}_2\text{CH}_2\text{NH}_2$ and $\text{YCH}_2\text{CH}_2\text{NH}_2$ may be employed in the form of salts such as the HCl salt.

The amine or amines may be introduced to the compound of formula (B) in a single dose, in portions over time, or in multiple smaller doses. In certain

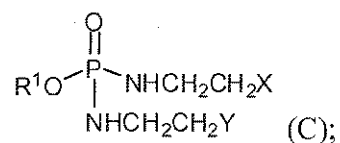
embodiments, the amine is steadily added to the compound of formula (B) over a period of time, e.g., with a continuous feed, a syringe pump or an addition funnel.

In embodiments where X and Y of amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are different from one another, e.g., X is Cl and Y is Br, or X is Br and Y is I, the amines, e.g., $ClCH_2CH_2NH_2$ and $BrCH_2CH_2NH_2$, can be introduced to a compound of formula (B) in concert or, preferably, in series. In an example of a serial addition, the amine $ClCH_2CH_2NH_2$ is introduced to the compound of formula (B) in one dose and, e.g., after reacting to completion, amine $BrCH_2CH_2NH_2$ is added.

In certain embodiments, the sum of molar equivalents of amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ relative to the compound of formula (B) is a ratio in the range of 2.5:1 to 1.8:1. In certain embodiments, wherein X and Y are leaving groups with the same molecular formula, such that $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are compounds of the same formula, the ratio of amine components to the compound of formula (B) is approximately 2:1. In certain embodiments, wherein X and Y are not identical substituents, amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are approximately equimolar to each other, and together are added in a ratio of approximately 2:1 relative to the compound of formula (B).

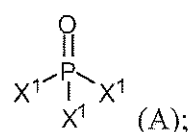
The condensation conditions may comprise an amine base such as N-methylmorpholine, triethylamine, pyridine or diisopropylethylamine. In certain embodiments, the amine base is in a ratio of 5:1 to 3:1 relative to the compound of formula (B). In certain embodiments, the amine base is in a ratio of approximately 4:1 relative to the compound of formula (B). The amine base may be triethylamine in a ratio of 4:1 relative to the compound of formula (B). It will be recognized by those of skill in the art that more amine base will be advantageous when amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are added in their salt form than if they are added in their free-base form. Specifically, the condensation reaction will be facilitated by the addition of two or more equivalents of amine base when $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are used in their free-base form, whereas four or more equivalents of amine base are preferred when $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are added as amine salts.

In certain embodiments, the present invention provides a method for the preparation of a compound of formula (C):

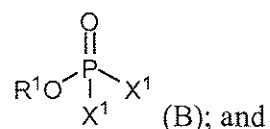


comprising

a. treating a compound of formula (A):



with an alcohol $\text{R}^1\text{-OH}$ in a reaction mixture under condensation conditions to generate a compound of formula (B):



b. adding to the reaction mixture amines $\text{XCH}_2\text{CH}_2\text{NH}_2$ and $\text{YCH}_2\text{CH}_2\text{NH}_2$ or a salt or salts thereof under condensation conditions, wherein, independently for each occurrence and as valence and stability permit,

X and Y independently represent leaving groups;

X^1 , independently for each occurrence, is selected from Cl, I, and Br,

R^1 is benzyl optionally substituted with one or more substituents, e.g., selected from halogen, $-\text{R}^2$, $-\text{OR}^2$ and $-\text{NR}^2_2$, wherein R^2 is independently selected for each occurrence from H and lower alkyl.

In certain embodiments, R^1 is benzyl optionally substituted with one or more substituents. In certain embodiments, R^1 is unsubstituted benzyl. In certain embodiments, X^1 at each occurrence is independently selected from any of Cl, Br and I. In certain embodiments, R^1 is unsubstituted benzyl and/or X^1 is selected at each occurrence from Cl or Br, e.g., the compound of formula (A) may be $\text{P}(\text{O})\text{Cl}_3$.

In certain aspects, the condensation conditions comprise an amine base. In certain such embodiments, the amine base is selected from any of N-methyl morpholine, triethylamine, pyridine or diisopropylethylamine, e.g., triethylamine.

The condensation conditions may comprise an aprotic organic solvent, e.g., one or more of acetone, 2-butanone, butyl acetate, ethyl acetate, acetonitrile, toluene, THF, dioxane, N,N-DMF, DMSO, 1,2-dichloroethane, or methylene chloride. In certain embodiments, the aprotic solvent comprises acetonitrile, wherein the acetonitrile may represent, for example, more than about 10, 20, 30, 50, 70, or 90% of the solvent system, or substantially all of the solvent system, for example about 95% or greater of the solvent system.

In some embodiments, phosphine oxide (A) and R^1OH are combined under condensation conditions, e.g., in a ratio in the range of 2:1 to 1:1.2 of compound of formula (A) to R^1OH , such as in a ratio in the range of 1.2:1 to 1:1.2, e.g., in approximately equimolar amounts of the compound of formula (A) and substitution reagent R^1OH .

In certain embodiments, the condensation conditions comprise maintaining an reduced temperature, e.g., in the range of about -50 to about -10 °C, or from about -35 to about -25 °C, or about -30 °C.

X and Y of amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ may be independently selected from halogen, e.g., Cl, Br, or I or a sulfonate, e.g., toluenesulfonate, methanesulfonate, 2,4,6-triisopropylbenzenesulfonate, or 2,4,6-trimethylbenzenesulfonate. In certain embodiments, X and Y are independently selected from Cl, Br or I. In certain embodiments, X and Y are identical, e.g., both Cl. In any of the embodiments of the present disclosure, $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ may be employed in the form of salts, such as the HCl salt.

The amine may be introduced to the reaction mixture in a one-time dose, in portions over time, or in multiple smaller doses. In certain embodiments, the amine is steadily added to the compound of formula (B) over a period of time, e.g., with a syringe pump, an addition funnel, or continuous feed.

In embodiments wherein X and Y of amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are distinct from one another, e.g., X is Cl and Y is Br, or X is Br and Y is I, the amines, e.g., $ClCH_2CH_2NH_2$ and $BrCH_2CH_2NH_2$, may be introduced to the reaction mixture in concert or, preferably, in series. In an example of a serial addition, the amine $ClCH_2CH_2NH_2$ is introduced to the reaction mixture in one dose and, e.g., after reacting to completion, amine $BrCH_2CH_2NH_2$ is added.

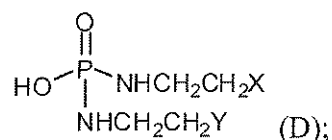
In certain embodiments, the sum of molar equivalents of amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ relative to the compound of formula (A) are in a ratio in the range of 2.5:1 to 1.8:1. In certain embodiments, wherein X and Y are leaving groups with the same molecular formula, such that $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are compounds of the same formula, the ratio of amine components to the compound of formula (A) is approximately 2:1. In certain embodiments, wherein X and Y are not identical substituents, amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are approximately equimolar to each other, and together are added in a ratio of approximately 2:1 relative to compound of formula (A).

The condensation conditions may comprise an amine base such as N-methyl morpholine, triethylamine, pyridine or diisopropylethylamine. In certain embodiments, the amine base is in a ratio of 5:1 to 3:1 relative to the compound of formula (A). In certain embodiments, the amine base is in a ratio of approximately 4:1 relative to the compound of formula (A). The amine base may be triethylamine in a ratio of 4:1 relative to the compound of formula (A). It will be recognized by those of skill in the art that more amine base will be advantageous when amines $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are added in their salt form than if they are added in their free-base form. Specifically, the condensation reaction will be facilitated by the addition of two or more equivalents of amine base when $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are used in their free-base form, whereas four or more equivalents of amine base are preferred when $XCH_2CH_2NH_2$ and $YCH_2CH_2NH_2$ are added as amine salts (in addition to any amine base used in the condensation with alcohol R^1OH , e.g., typically at least one further equivalent).

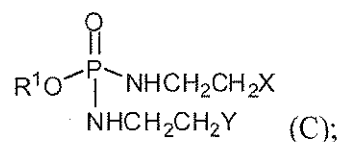
In certain embodiments the reaction mixture comprising the product of the reaction of the compound of formula (B) to form the compound of formula (C) is used in the hydrogenolysis reaction, e.g., without first purifying or partially or completely

removing solvents from the reaction mixture. For example, the reaction mixture may be filtered to remove solids, e.g., salt by-products of the condensation conditions, prior to subjecting the solution to the hydrogenolysis conditions affording the compound of formula (D). The reaction mixture may be filtered by any method known in the art to remove solids from the solvent of the reaction mixture. In some embodiments, the reaction mixture is filtered and substantially directly subjected to hydrogenation conditions, e.g., the compound of formula (C) is not further purified or isolated from the reaction mixture (e.g., no extractions, chromatography, or quenches are performed).

In certain embodiments, the present invention provides a method for the preparation of a compound of formula (D):



comprising treating a compound of formula (C):



with a reducing agent under hydrogenolysis conditions, wherein, as valence and stability permit,

X and Y independently represent leaving groups; and

R¹ is benzyl optionally substituted with one or more substituents, e.g., selected from halogen, -R², -OR² and -NR²₂, wherein R² is independently selected for each occurrence from H and lower alkyl.

In certain embodiments, R¹ is benzyl optionally substituted with one or more substituents. In certain embodiments, R¹ is unsubstituted benzyl. In certain embodiments, X and Y at each occurrence are independently selected from halogens

such as Cl, Br and I. In certain embodiments, R¹ is unsubstituted benzyl and/or X and Y are both Cl.

In certain embodiments the reaction mixture comprising the product of the reaction of the compound of formula (B) to form the compound of formula (C) is used in the hydrogenolysis reaction, e.g., without first purifying or partially or completely removing solvents from the reaction mixture. For example, the reaction mixture may be filtered to remove solids, e.g., salt by-products of the condensation conditions, prior to subjecting the solution to the hydrogenolysis conditions affording the compound of formula (D). The reaction mixture may be filtered by any method known in the art to remove solids from the solvent of the reaction mixture. In some embodiments, the reaction mixture is filtered and substantially directly subjected to hydrogenation conditions, e.g., the compound of formula (C) is not further purified or isolated from the reaction mixture (e.g., no extractions, chromatography or quenches are performed).

The hydrogenolysis conditions may comprise treating the compound of formula (C) with hydrogen gas in the presence of a catalyst. The catalyst may be selected from any suitable hydrogenolysis catalyst, such as Pd/carbon, Pd black, Pd EnCat (polymer encapsulated active metal catalyst) Raney nickel, Rh/carbon, Ru/carbon, Re/carbon, palladium oxide, palladium chloride, PtO₂ or RuO₂. In certain embodiments, the hydrogenolysis catalyst comprises palladium, e.g., Pd/carbon.

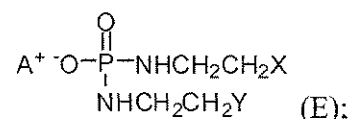
In certain embodiments, the hydrogenolysis conditions comprise a partial pressure of hydrogen greater than 1 atmosphere. In certain embodiments, the partial pressure of hydrogen is less than or equal to 100, 75 or 50 psi. In certain embodiments, the partial pressure of hydrogen is selected from between 10 psi and 50 psi. The partial pressure of hydrogen may be from 20-50 psi, such as from 40-50 psi. Alternatively, the partial pressure of hydrogen during hydrogenolysis may be about 50 psi, or even between 50 and 75 psi.

In certain embodiments, the hydrogenolysis conditions may comprise an aprotic organic solvent, e.g., toluene, xylene, benzene, furan, acetonitrile, dioxane, tetrahydrofuran, chloroform or mixtures thereof. In certain embodiments, the aprotic solvent comprises acetonitrile, wherein the acetonitrile may represent, for example, more than about 10, 20, 30, 50, 70, or 90% of the solvent system, or substantially all of the solvent system, for example about 95% or greater of the solvent system.

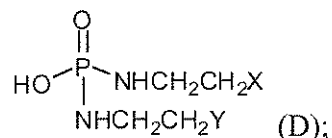
In certain embodiments, the separation of the product from residual catalyst is achieved by the formation of a basic salt under aqueous conditions, e.g., using sodium hydroxide or another base in water, which may be performed in situ. The resulting salt may be dissolved in a suitable solvent and the residual catalyst may be filtered to remove solids from the solvent of the reaction mixture.

In certain embodiments, the separation of the product from residual catalyst is achieved by the formation of a basic salt under anhydrous conditions, e.g., using triethylamine or another base, e.g., an amine base, preferably a sterically hindered base such as diisopropylethylamine or proton sponge. The subsequent salt may be dissolved into the reaction solvent and the residual catalyst may be filtered to remove solids from the solvent of the reaction mixture.

In certain embodiments, the present invention provides a method for the preparation of a compound of formula (E):



comprising treating a compound of formula (D):



with a base under salt-forming conditions, wherein as valence and stability permit,

X and Y independently represent leaving groups; and

A⁺ represents an ammonium cation.

In certain embodiments, X and Y at each occurrence are independently selected from halogens such as -Cl, -Br and -I. In certain embodiments, X and Y are both Cl.

In certain embodiments, A⁺ represents BH⁺ and B is an amine selected from the basic amino acids, pyridine, *N,N*-dimethylaminopyridine, diazabicyclononane, diazabicycloundecene, *N*-methyl-*N*-ethylamine, diethylamine, triethylamine,

diisopropylethylamine, mono-, bis- or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, tris(hydroxymethyl)methylamine, *N,N*-dimethyl-*N*-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine and *N*-methyl-D-glucamine. In certain embodiments, B is tris(hydroxymethyl)methylamine.

In certain embodiments, the salt-forming conditions comprise combining approximately equimolar amounts of an amine base and the compound of formula (D). In certain embodiments the compound of formula (D) and the amine base are combined in a molar ratio in the range of 1:1 to 1:10. In certain embodiments, the salt-forming conditions comprise combining approximately equimolar amounts of tris(hydroxymethyl)aminomethane and the compound of formula (D).

In certain embodiments, the salt-forming conditions comprise a polar aprotic solvent, such as *N,N*-DMF, acetone, DMSO, THF, 2-butanone, butyl acetate, ethyl acetate, acetonitrile, and combinations thereof. In certain embodiments, the aprotic solvent comprises *N,N*-DMF, wherein the *N,N*-DMF may represent, for example, more than about 10, 20, 30, 50, 70, or 90% of the solvent system, or substantially all of the solvent system, for example about 95% or greater of the solvent system.

In certain embodiments, the invention relates to a compound comprising a crystalline salt of IPM or analog thereof wherein the IPM or analog thereof, and the counterion, preferably tris (hydroxymethyl) amino methane (tris), are present in a ratio from 2:1 to 1:2, preferably 1:1. In certain embodiments, the crystalline formulation comprises more than one polymorphic form of crystals, such as two, three, four, or even five polymorphic forms of crystals. In certain alternative such embodiments, the crystalline formulation comprises a single polymorphic form of crystals. In certain embodiments, such salts are more stable than IPM and IPM analogs as free acids.

In certain such embodiments, the compound is a crystalline salt of a 1:1 ratio of IPM and Tris. In certain such embodiments, the melting point of the crystalline solid is between about 100 and about 110 °C, about 102 to about 108 °C, about 103 to about 106 °C, or even 105 to 106 °C.

In certain embodiments, the compound, e.g., a crystalline salt of a 1:1 ratio of IPM and Tris, is at least about 80% pure, at least about 85% pure, at least 90% pure, at least 95% pure, at least 97% pure, at least 98% pure, or even at least 99% pure. In certain such embodiments, no single impurity exceeds 1% by weight. In certain

embodiments, purity is measured relative to all other components of the formulation, while in other embodiments (e.g., where the compound is part of a pharmaceutical formulation or lyophilisate mixture), purity may be measured relative to degradation products of the compound (e.g., phosphorous-containing degradation products of the compound) or by-products of the manufacture of the compound (e.g., phosphorous-containing degradation products of the compound), thereby excluding other components purposefully added to the formulation.

EXEMPLIFICATION

The foregoing disclosure is further explained by the following non-limiting examples.

Exemplary Formulations

I. Formulation Development

IPM tris drug formulation comprises a blend of IPM tris salt, Microcrystalline Cellulose (Avicel PH112), Sodium Croscarmellose (Ac-Di-Sol) and Magnesium Stearate (vegetable source).

A. Batch formulation of 10 mg strength Tris IPM capsules

Table 1. Quantitative Composition of IPM Tris Capsule Drug Product – 10 mg strength

Component	Reference to Quality Standard	Function	Amount per Batch (10 mg strength)	Amount per Capsule (10 mg strength)
IPM-tris salt	In-house standard	API	154.0 g ¹	15.4 mg
Microcrystalline Cellulose, NF (Avicel PH112)	NF	Diluent	1912.0 g	191.2 mg
Croscarmellose Sodium, NF (Ac-Di-Sol)	NF	Water-absorbing agent; capsule disintegrant	21.0 g	2.1 mg
Magnesium Stearate, NF,	NF	Lubricant	10.5 g	1.05 mg

Ph.Eur. (Vegetable Source – Grade 905-G)				
Total			2097.5 g/batch	209.75 mg/capsule
Size 1, hard- gelatin white opaque capsule	Capsugel certificate of conformance	Product delivery	10,000 capsules	1 capsule

¹154 g of IPM tris salt (molecular weight = 342.16) will deliver 100.0 g of IPM active pharmaceutical ingredient (molecular weight = 221.0).

API = active pharmaceutical ingredient; NA = not available; NF = National Formulary.

B. Batch Analysis of 10 mg Tris IPM capsules

Product: Tris IPM capsules of Table 1

Lot Disposition: Stability, CTM

Stability Storage Condition: -20 °C, 5 °C or 25 °C/60%RH

Table 2. Batch Analyses of IPM Tris Drug Product

Test	cGMP Batch
Appearance	Size 1 hard shell white opaque capsule, containing white to off-white powder
Brittleness	No cracking or breaking observed
Identification (HPLC Retention Time)	0.999
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)
Purity	100.0%
Related Substances (LC/MS)	No impurities, 0.0%
Capsule Weight (mg)	Capsule 1: 285.46 Capsule 2: 283.69 Capsule 3: 289.99 Capsule 4: 286.17 Capsule 5: 287.22 Capsule 6: 289.43 Capsule 7: 285.23 Capsule 8: 286.80 Capsule 9: 277.25 Capsule 10: 288.25 Mean: 285.95
Content Uniformity	Capsule 1: 99.0% Capsule 2: 96.6%

Test	cGMP Batch
	Capsule 3: 93.1% Capsule 4: 93.2% Capsule 5: 104.1% Capsule 6: 102.9% Capsule 7: 96.7% Capsule 8: 101.4% Capsule 9: 98.0% Capsule 10: 97.9% Mean: 98.3%
Disintegration	Vessel #/ Rupture Time 1.....1 min 43 sec 2.....1 min 37 sec 3.....1 min 41 sec 4.....1 min 35 sec 5.....1 min 39 sec 6.....1 min 48 sec
Water Content	1.2%
Microbial Limits: Total Aerobic Count Total combined yeast and molds P. aeruginosa E. coli S. aureus Salmonella sp.	Less than 10 CFU/g of specimen 20 CFU/g of specimen Absent Absent Absent Absent

NA: not available; HPLC = high-performance liquid chromatography; CFU = colony forming unit; RRT= relative retention time

Table 3. Summary of -20°C Stability Data for 10 mg IPM Tris capsules

Test	Results			
	Initial	0.5 mo	1.0mo	3.0 mo
Identification (HPLC)	1.00	1.00	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	105.3% (mean IPM active per capsule: 10.5 mg)	102.1% (mean IPM active per capsule: 10.2 mg)	103.1% (mean IPM active per capsule: 10.3 mg)
Purity (% area)	100.0%	NA	NA	99.9%
Related Substances (% area)	ND	NA	NA	RRT 0.56 = 0.09%

Test	Results			
	Initial	0.5 mo	1.0 mo	3.0 mo
Total Related Substances	0.0%	NA	NA	0.1%
Water Content	1.2%	1.1%	2.0%	3.5%

CTM = clinical trials material; HPLC = high-performance liquid chromatography; ND = not detected; RRT = relative retention time; NA: not available

Table 4. Summary of 5°C Stability Data for 10 mg IPM Tris capsules

Test	Results			
	Initial	0.5 mo	1.0 mo	3.0 mo
Identification (HPLC)	1.00	1.00	1.01	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	98.2% (mean IPM active per capsule: 9.8 mg)	94.8% (mean IPM active per capsule: 9.5 mg)	101.8% (mean IPM active per capsule: 10.2 mg)
Purity (% area)	NA	99.9%	NA	99.9%
Related Substances (% area)	NA	RRT 0.558: 0.06%	NA	RRT 0.56 = 0.08%
Total Related Substances	NA	0.1%	NA	0.1%
Water Content	1.2%	1.3%	1.8%	4.2%

HPLC = high-performance liquid chromatography; ND = not detected; RRT = relative retention time; NA: not available

Table 5. Summary of 25°C Stability Data for 10 mg IPM Tris Capsules

Test	Results			
	Initial	0.5 mo	1.0 mo	3.0 mo
Identification (HPLC)	1.00	1.00	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	95.4% (mean IPM active per capsule: 9.5 mg)	100.9% (mean IPM active per capsule: 10.1 mg)	79.4% (mean IPM active per capsule: 7.9 mg)
Purity (% area)	NA	NA	NA	99.0%
Related Substances (% area)	NA	NA	NA	RRT 4.26 = 0.22% RRT 0.57 = 0.12% RRT 4.56 = 0.14% RRT 5.10 = 0.2%
Total Related Substances	NA	NA	NA	1.0%

Test	Results			
	Initial	0.5 mo	1.0 mo	3.0 mo
Water Content	1.2%	2.0%	2.6%	3.8%

CTM = clinical trials material; HPLC = high-performance liquid chromatography; ND = not detected; RRT = relative retention time; NA: not available

C. Batch Analysis of 50 mg Tris IPM capsules

Table 6 – Batch analysis of 50 mg Tris IPM capsules

Test	cGMP Batch
Appearance	Size 1 hard shell opaque capsule, containing white to off-white powder
Brittleness	No cracking or breaking observed
Identification (HPLC Retention Time)	1.002
Potency by HPLC (Assay)	102.8%, 51.4mg / capsule.
Purity	100.0%
Related Substances (LC/MS)	Total impurities – None Detected
Capsule Weight (mg)	Capsule 1: 286.87 Capsule 2: 276.81 Capsule 3: 271.31 Capsule 4: 283.70 Capsule 5: 291.33 Capsule 6: 277.61 Capsule 7: 281.76 Capsule 8: 279.91 Capsule 9: 285.73 Capsule 10: 272.00 Mean: 280.70
Content Uniformity	Capsule 1: 102.0% Capsule 2: 97.6% Capsule 3: 97.8% Capsule 4: 107.8% Capsule 5: 111.4% Capsule 6: 101.9% Capsule 7: 101.1% Capsule 8: 104.2% Capsule 9: 105.9% Capsule 10: 97.9% Mean: 102.8%
Water Content	2.4%
Microbial Limits:	
Total Aerobic Count	<10 cfu / g
Total combined yeast and molds	20 cfu / g
P. aeruginosa	

Test	cGMP Batch
E. coli	Absent
S. aureus	Absent
Salmonella sp.	Absent
	Absent

Table 7. Summary of -20°C Stability Data for 50 mg IPM Tris Capsules

Test	Results		
	Initial	0.5 mo	1.0 mo
Identification (HPLC)	RRT = 0.98 to 1.02	NA	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	NA	101% (mean IPM active per capsule: 50.6 mg)
Purity (% area)	NA	NA	100.0%
Related Substances (% area)	NA	NA	ND
Individual			
Total Related Substances	NA	NA	ND
Water Content	2.4%	2.5%	2.0%

NA = not available; HPLC = high-performance liquid chromatography

Table 8. Summary of 5°C Stability Data for 50 mg IPM Tris Capsules

Test	Results		
	Initial	0.5 mo	1.0 mo
Identification (HPLC)	RRT = 0.98 to 1.02	NA	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	NA	96% (mean IPM active per capsule: 48.1 mg)
Purity (% area)	NA	NA	99.9%
Related Substances (% area)	NA	NA	0.1%
Total Related Substances	NA	NA	0.1%
Water Content	2.4%	2.4%	2.4%

NA = not available; HPLC = high-performance liquid chromatography

Table 9. Summary of 25 °C/60%RH Stability Data for 50 mg IPM Tris Capsules

Test	Results		
	Initial	0.5 mo	1.0 mo
Identification (HPLC)	RRT = 0.98 to 1.02	NA	1.00
Potency by HPLC (Assay)	98.3% (mean IPM active per capsule: 9.8 mg)	NA	97% (mean IPM active per capsule: 48.4 mg)
Purity (% area)	NA	NA	99.9%
Related Substances (% area)	NA	NA	0.1%
Individual			
Total Related Substances	NA	NA	0.1%
Water Content	2.4%	2.5%	2.8%

NA = not available; HPLC = high-performance liquid chromatography

D. Batch Analysis of 10 mg Tris IPM capsules

Table 10. Summary of -20°C Stability Data for 10 mg Palifosfamide Tris

(Tris(hydroxymethyl)aminomethane (Tris)) Capsules ≤3 month

Test	Results				
	Initial	0.5 mo	1 mo	2 mo	3 mo
Appearance	Conforms	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	Conforms	Conforms
Identification (HPLC)	1.00	1.00	1.00	Not tested	1.01
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8 mg)	105.3% (mean IPM/cap: 10.5 mg)	102.1% (mean IPM/cap: 10.2 mg)	Not tested	103.1% (mean IPM/cap: 10.3 mg)
Purity(% area)	100.0%	99.9%	99.9%	Not tested	99.9%
Related Substances (% area) Individual	Not detected	RRT 0.555: 0.06%	RRT 2.198: 0.05%	Not tested	RRT 0.560: 0.09%
Total Related	0.0%	0.1%	0.1%	Not tested	0.1%

Substances						
	Vessel	Rupture Time	Vessel	Rupture Time	Vessel	Rupture Time
Dissolution (Capsule Rupture Method)	1	1 min	1	53 sec	1	1 min
		43 sec	2	1 min		38 sec
	2	1 min		19 sec	2	1 min
		41 sec	3	1 min		26 sec
	3	1 min		25 sec	3	1 min
		35 sec	4	1 min		38 sec
	4	1 min		10 sec	4	1 min
		35 sec	5	1 min		0 sec
	5	1 min		1 sec	5	1 min
		39 sec	6	40 sec		26 sec
	6	1 min			6	1 min
		48 sec				17 sec
Dissolution (HPLC)	<u>20 min</u> Mean % LC: 67.1% Mean IPM/cap: 6.7 mg		Not tested		<u>20 min</u> Mean % LC: 78.1% Mean IPM/cap: 7.8 mg	
	<u>40 min</u> Mean % LC: 59.3% Mean IPM/cap: 5.9 mg		Not tested		<u>40 min</u> Mean % LC: 66.4% Mean IPM/cap: 6.6 mg	
	<u>60 min</u> Mean % LC: 53.4% Mean IPM/cap: 5.3 mg				<u>60 min</u> Mean % LC: 58.8% Mean IPM/cap: 5.9 mg	
Water Content	1.2%		1.1%		2.0%	
					2.5%	
					3.5%	

HPLC=high-performance liquid chromatography; RRT=relative retention time

**Table 11. Summary of -20°C Stability Data for 10mg Palifosfamide Tris
(Tris(hydroxymethyl)aminomethane (Tris)) Capsules, 6–12 month**

Test	Results			
	Initial	6mo	9mo	12 mo
Appearance	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	Conforms
Identification (HPLC)	1.00	1.00	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8mg)	96.3.3% (mean IPM/cap: 9.6mg)	94.8% (mean IPM/cap: 9.5mg)	101.9% (mean IPM/cap: 10.2mg)
Purity (% area)	100.0%	99.8%	99.8%	99.8%
Related Substances (% area) Individual	Not detected	RRT 0.578: 0.17%	RRT 2.798: 0.08% RRT 0.610: 0.05% RRT 6.189: 0.05%	RRT 2.546: 0.05% RRT 0.382: 0.07% RRT 6.083: 0.05%
Total Related Substances	0.0%	0.2%	0.2%	0.2%
Dissolution (Capsule Rupture Method)	Vessel Rupture Time 7 1 min 43 sec 8 1 min 37 sec 9 1 min 41 sec 10 1 min 35 sec 11 1 min 39 sec 12 1 min 48 sec	Not tested	Not tested	Not tested

Dissolution (HPLC)	<u>20 min</u> Mean % LC: 67.1% Mean IPM/cap: 6.7mg	<u>20 min</u> Mean % LC: 79.4% Mean IPM/cap: 7.9mg	<u>20 min</u> Mean % LC: 67.5% Mean IPM/cap: 6.8 mg	<u>20 min</u> Mean % LC: 69.6% Mean IPM/cap: 7.0 mg
	<u>40 min</u> Mean % LC: 59.3% Mean IPM/cap: 5.9mg	<u>40 min</u> Mean % LC: 66.3% Mean IPM/cap: 6.6 mg	<u>40 min</u> Mean % LC: 56.0% Mean IPM/cap: 5.6 mg	<u>40 min</u> Mean % LC: 58.6% Mean IPM/cap: 5.9 mg
	<u>60 min</u> Mean % LC: 53.4% Mean IPM/cap:5.3mg	<u>60 min</u> Mean % LC: 57.0% Mean IPM/cap:5.7mg	<u>60 min</u> Mean % LC: 48.0% Mean IPM/cap: 4.8 mg	<u>60 min</u> Mean % LC: 51.6% Mean IPM/cap: 5.2 mg
Water Content	1.2%	2.9%	3.7%	3.5%

HPLC=high-performance liquid chromatography; RRT=relative retention time

Table 12. Summary of 5°C Stability Data for 10mg Palifosfamide Tris

(Tris(hydroxymethyl)aminomethane (Tris)) Capsules, ≤3 month

Test	Results				
	Initial	0.5 mo	1 mo	2 mo	3 mo
Appearance	Conforms	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	Conforms	Conforms
Identification (HPLC)	1.00	1.00	1.00	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8 mg)	98.2% (mean IPM/cap: 9.8 mg)	94.8% (mean IPM/cap: 9.5 mg)	Not tested	101.8% (mean IPM/cap: 10.2 mg)
Purity (% area)	100%	99.9%	99.9%	Not tested	99.9%
Related Substances (% area)	Not detected	RRT 0.558: 0.06%	NA	Not tested	RRT 0.561: 0.08%

Test	Results				
	Initial	0.5 mo	1 mo	2 mo	3 mo
Individual					
Total Related Substances	0.0%	0.1%	0.1%	Not tested	0.1%
Dissolution (Capsule Rupture Method)	Vessel Rupture Time 1 1 min 43 sec 2 1 min 37 sec 3 1 min 41 sec 4 1 min 35 sec 5 1 min 39 sec 6 1 min 48 sec	Vessel Rupture Time 1 1 min 10 sec 2 51 sec 3 1 min 5 sec 4 1 min 9 sec 5 1 min 29 sec 6 1 min 19 sec	Vessel Rupture Time 1 1 min 11 sec 2 1 min 24 sec 3 1 min 18 sec 4 1 min 1 sec 5 1 min 24 sec 6 47 sec	Not tested	Not tested
Dissolution (HPLC)	<u>20 min</u> Mean % LC: 67.1% Mean IPM/cap: 6.7 mg <u>40 min</u> Mean % LC: 59.3% Mean IPM/cap: 5.9 mg <u>60 min</u> Mean % LC: 53.4% Mean IPM/cap: 5.3 mg	Not tested	Not tested	<u>20 min</u> Mean % LC: 65.5% Mean IPM/cap: 6.6 mg <u>40 min</u> Mean % LC: 59.0% Mean IPM/cap: 5.9 mg <u>60 min</u> Mean % LC: 53.6% Mean IPM/cap: 5.4 mg	<u>20 min</u> Mean % LC: 72.9% Mean IPM/cap: 7.3 mg <u>40 min</u> Mean % LC: 65.0% Mean IPM/cap: 6.5 mg <u>60 min</u> Mean % LC: 58.1% Mean IPM/cap: 5.8 mg
Water Content	1.2%	1.3%	1.8%	2.3%	4.2%

HPLC=high-performance liquid chromatography; NA=not applicable (None $\geq 0.1\%$); RRT=relative retention time

**Table 13. Summary of 5°C Stability Data for 10mg Palifosfamide Tris
(Tris(hydroxymethyl)aminomethane (Tris)) Capsules, 6–12 month**

Test	Results			
	Initial	6mo	9mo	12 mo
Appearance	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	1 of 6 capsules cracked
Identification (HPLC)	1.00	1.00	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8 mg)	94.4% (mean IPM/cap: 9.4 mg)	92.4% (mean IPM/cap: 9.3 mg)	97.9% (mean IPM/cap: 9.8 mg)
Purity (% area)	100%	99.8%	99.8%	99.9%
Related Substances (% area)	Not detected	RRT 0.576: 0.13%	RRT 2.822: 0.06% RRT 0.579: 0.06% RRT 8.294: 0.03%	RRT 2.651: 0.05% RRT 0.391: 0.07%
Individual				
Total Related Substances	0.0%	0.2%	0.2%	0.1%
Dissolution (HPLC)	<u>20 min</u> Mean % LC: 67.1% Mean IPM/cap: 6.7 mg <u>40 min</u> Mean % LC: 59.3% Mean IPM/cap: 5.9 mg <u>60 min</u> Mean % LC: 53.4% Mean IPM/cap: 5.3 mg	<u>20 min</u> Mean % LC: 69.7% Mean IPM/cap: 7.0 mg <u>40 min</u> Mean % LC: 60.6% Mean IPM/cap: 6.1 mg <u>60 min</u> Mean % LC:	<u>20 min</u> Mean % LC: 70.8% Mean IPM/cap: 7.1 mg <u>40 min</u> Mean % LC: 57.6% Mean IPM/cap: 5.8 mg <u>60 min</u> Mean % LC:	<u>20 min</u> Mean % LC: 68.4% Mean IPM/cap: 6.8 mg <u>40 min</u> Mean % LC: 58.9% Mean IPM/cap: 5.9 mg <u>60 min</u> Mean % LC: 50.8% Mean IPM/cap: 5.1 mg

Test	Results			
	Initial	6mo	9mo	12 mo
		53.6% Mean IPM/cap: 5.4 mg	48.1% Mean IPM/cap: 4.8 mg	
Water Content	1.2%	2.6%	3.9%	3.5%

HPLC=high-performance liquid chromatography; RRT=relative retention time

**Table 14. Summary of 25°C Stability Data for 10mg Palifosfamide Tris
(Tris(hydroxymethyl)aminomethane (Tris)) Capsules ≤3 month**

Test	Results				
	Initial	0.5 mo	1 mo	2 mo	3 mo
Appearance	Conforms	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	Conforms	Conforms
Identifica- tion (HPLC)	1.00	1.00	1.01	1.00	1.00
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8 mg)	95.4% (mean IPM/cap: 9.5 mg)	100.9% (mean IPM/cap: 10.1 mg)	Not tested	79.4% (mean IPM/cap: 7.9 mg)
Purity (% area)	100.0%	99.9%	99.9%	Not tested	99.0%
Related Substances (% area) Individual	NA	RRT 0.558: 0.06%	NA	Not tested	RRT 4.264: 0.22% RRT 0.568: 0.12% RRT 4.562: 0.14% RRT 5.104: 0.20%
Total Related Substances	0.0%	0.1%	0.1%	Not tested	1.0%

Test	Results									
	Initial		0.5 mo		1 mo		2 mo	3 mo		
Dissolution (Capsule Rupture Time)	Vessel	Rupture Time	Vessel	Rupture Time	Vessel	Rupture Time	Not tested	Not tested		
	1	1 min 43 sec	1	1 min 10 sec	1	57 sec 1 min				
	2	1 min 37 sec	2	1 min 3 sec	3	1 min 14 sec				
	3	1 min 41 sec	3	1 min 20 sec	4	39 sec				
	4	1 min 35 sec	4	1 min 25 sec	5	1 min 20 sec				
	5	1 min 39 sec	5	1 min 9 sec	6	49 sec				
	6	1 min 48 sec	6	52 sec						
Dissolution (HPLC)	<u>20 min</u> Mean % LC: 67.1% Mean IPM/cap: 6.7 mg		Not tested		Not tested		<u>20 min</u> Mean % LC: 70.2% Mean IPM/cap: 7.0mg	<u>20 min</u> Mean % LC: 59.5% Mean IPM/cap: 6.0mg		
	<u>40 min</u> Mean % LC: 59.3% Mean IPM/cap: 5.9 mg						<u>40 min</u> Mean % LC: 61.3% Mean IPM/cap: 6.1mg	<u>40 min</u> Mean % LC: 54.1% Mean IPM/cap: 5.4mg		
	<u>60 min</u> Mean % LC: 53.4% Mean IPM/cap: 5.3 mg						<u>60 min</u> Mean % LC: 56.7% Mean IPM/cap: 5.7mg	<u>60 min</u> Mean % LC: 49.1% Mean IPM/cap: 4.9mg		
Water Content	1.2%		2.0%		2.6%		2.3%	3.8%		

HPLC=high-performance liquid chromatography; NA=not applicable (None $\geq 0.1\%$); RRT=relative retention time

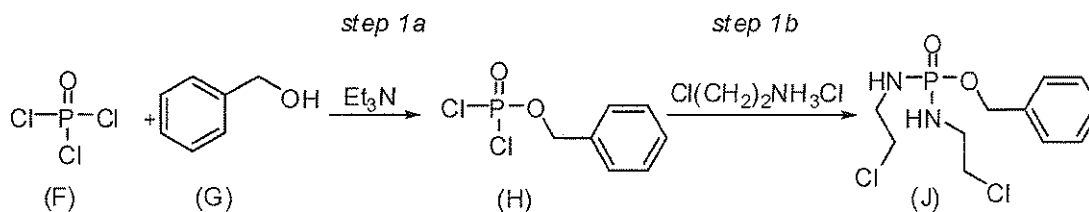
**Table 15. Summary of 25°C Stability Data for 10mg Palifosfamide Tris
(Tris(hydroxymethyl)aminomethane (Tris))Capsules 6–12 month**

Test	Results			
	Initial	6mo	9mo	12 mo
Appearance	Conforms	Conforms	Conforms	Conforms
Brittleness	Conforms	Conforms	Conforms	Conforms
Identification (HPLC)	1.00	0.98	Not tested	Not tested
Potency by HPLC (Assay)	98.3% (mean IPM/cap: 9.8 mg)	32.8% (mean IPM/cap: 3.3 mg)	Not tested	Not tested
Purity (% area)	100.0%	1.6%	Not tested	Not tested
Related Substances (% area)	NA	**	Not tested	Not tested
Individual				
Total Related Substances	0.0%	98.4%	Not tested	Not tested

Test	Results			
	Initial	6mo	9mo	12 mo
Dissolution (HPLC)	<u>20 min</u>	<u>20 min</u>	Not tested	Not tested
	Mean % LC: 67.1%	Mean % LC: 0.0%		
	Mean IPM/cap: 6.7 mg	Mean IPM/cap: ND *		
	<u>40 min</u>	<u>40 min</u>		
	Mean % LC: 59.3%	Mean % LC: 0.0%		
	Mean IPM/cap: 5.9 mg	Mean IPM/cap: ND *		
	<u>60 min</u>	<u>60 min</u>		
	Mean % LC: 53.4%	Mean % LC: 0.0%		
	Mean IPM/cap: 5.3 mg	Mean IPM/cap: ND *		
Water Content	1.2%	2.6%	3.9%	3.5%

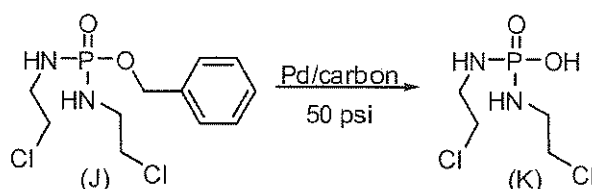
HPLC=high-performance liquid chromatography; RRT=relative retention time; ND *=No IPM peak was detected

II. Synthetic preparation of IPM salts and analogues thereof

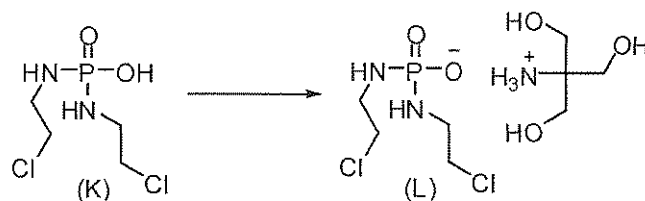


Procedure for the preparation of benzyl isophosphoramidate (J): Into a 2 L 3-neck flask, 100 g of $\text{P}(\text{O})\text{Cl}_3$ was charged, followed by addition of 400 mL of acetonitrile. The content was then cooled to $-30 \pm 5^\circ\text{C}$. Into another flask, 70.5 g of benzyl alcohol and 90.7 mL of triethylamine (TEA) were added, followed by addition of 200 mL of acetonitrile and stir until the mixture becomes homogeneous. To the cold solution of $\text{P}(\text{O})\text{Cl}_3$ was added the solution of benzyl alcohol and TEA *via* syringe pump while maintaining the reaction temperature at to $-30 \pm 5^\circ\text{C}$. This addition lasts 140 min. After the addition, the reaction mixture was allowed to stir for 1 h at $-30 \pm 5^\circ\text{C}$. To

the reaction mixture, 151.3 g of $\text{ClCH}_2\text{CH}_2\text{NH}_3\text{Cl}$ was weighed. Subsequently, 362.6 mL of TEA was charged over 140 min *via* syringe pump while maintaining the temperature at $-30 \pm 5^\circ\text{C}$. After the addition, the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was filtered to remove triethylamine hydrochloride salt and the reactor rinsed with 3 x 200 mL acetonitrile. The filtrate was taken on to the next step without further purification.



Procedure for the preparation of benzyl isophosphoramidate (K): The filtrate resulting from step 1b above was transferred into a hydrogenation reactor and 3.4 g of Pd/C was charged. Hydrogenolysis was conducted under 50 psi of hydrogen. After 15h, the reaction was complete and no Bz-IPM was observed (Note: The pH for the reaction mixture was 3-4). For work up and isolation of the product, the reaction mixture was transferred into a flask and the content was cooled to 0°C to 10°C. To the cooled reaction mixture was added 100 mL (719.4 mmol, 1.1 equiv) of TEA to increase the pH to 9.5-10 (Note: This addition lasted less than 5 min). The resulting mixture was filtered to remove catalyst and rinsed with 2 × 100 mL of acetonitrile. The filtrate was cooled to 0°C to 10°C, and to it 76 mL (924.5 mmol, 1.4 equiv) of 37% HCl was added to lower the pH to 1-2 (Note: This addition took 15 min). The resulting slurry was filtered and the wet cake was made into a slurry with two portions of 400 mL of 5% water in acetonitrile, and stirred for 5 min each. The wet cake was then rinsed with 3 × 200 mL of acetonitrile to give 105.8871 g (73.5%) product.



A. Procedure for the preparation of IPM salt (L): Into a 250 mL 3-neck flask 26.5791 g of tris(hydroxymethyl)-aminomethane was added, followed by addition of 78.0 mL of *N,N*-DMF. The mixture was then heated to 100°C to dissolve the base.

After a clear solution formed, the batch was cooled to 20-25°C (a slurry then formed). To the above slurry, 49.1322 g (98.7% purity based on NMR, 0.2194 moles) of IPM analogue (K) (1766-026-11) was weighed, and rinsed with 48 mL of *N,N*-DMF. The mixture was stirred for 1 h until a clear solution formed. The resulting solution proceeds polish filtration with Whatman #1 filter paper. The filtrate was transferred into a 2 L flask, and to it, 144 mL of acetonitrile was added over 5 min. To the batch 852 mL of methyl tert-butyl ether (MTBE) was introduced to form cloudy solution (Solid seeds were generated by scratching against the glass wall with a spatula). The batch was stirred for 1 h. The product was collected by filtration, followed by washing with 242 mL of MTBE in a yield of 72.3868 g (96.4%) as white solid. Residual palladium content was <10ppm by ICP analysis.

B. Procedure for the preparation of IPM salt (L): Into a 500 mL 3-neck flask, 54.1043 g of tris(hydroxymethyl)-aminomethane was added, followed by addition of 158 mL of *N,N*-DMF. The mixture was then heated to 100°C to dissolve the base. After a clear solution formed, the batch was cooled to 20-25°C (a slurry then formed). To the above slurry, 100.0135 g (98.7% purity based on NMR, 0.4466 moles) of IPM (1766-028-11) was weighed, and rinsed with 99 mL of *N,N*-DMF. The mixture was stirred for 140 min until a clear solution formed. The resulting solution proceeds polish filtration with Whatman #1 filter paper. The filtrate was transferred into a 5 L flask, and to it, 293 mL of acetonitrile was added over 5 min. To the batch, a slurry mixture of 0.3 g of salt L in 4.8 mL of DMF and 5.7 mL of acetonitrile was added. To the batch, 1734 mL of MTBE was introduced. The batch was stirred for 1 h. The product was collected by filtration, followed by washing with 494 mL of MTBE in a yield of 146.7 g (96.0%) as white solid.

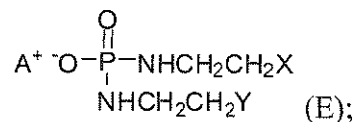
EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

All of the above-cited references and publications are hereby incorporated by reference.

We claim:

1. An oral formulation of the compound of formula (E):



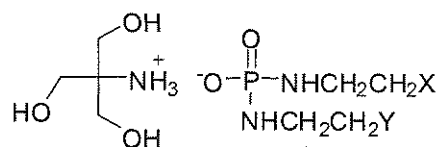
wherein the formulation comprises 0.25-5% lubricant, up to 98% diluent, and up to 90% disintegrant by weight of formulation, and the compound wherein

X and Y independently represent leaving groups; and

A⁺ is an ammonium cation, and

wherein two or more of the lubricant, diluent, and disintegrant may be a single component.

2. The oral formulation of claim 1, wherein the compound of formula (E) is represented by the formula:



3. The oral formulation of claim 1, wherein the lubricant comprises the salt of a fatty acid, such as magnesium stearate.

4. The oral formulation of any of claims 1-3, wherein the formulation comprises at least one of the following diluents in the indicated amount (by weight of the formulation):

microcrystalline cellulose 5-98%,

mannitol 10-90%,
lactose up to 98% and
calcium hydroxydiodioxido-oxo-phosphorane 10-80%.

5. The oral formulation of any of claims 1-3, wherein the diluent comprises a carbohydrate, such as microcrystalline cellulose.

6. The oral formulation of claim 5, wherein the formulation comprises from 80-98% microcrystalline cellulose.

7. The oral formulation of claim 6, wherein the formulation comprises about 91% microcrystalline cellulose.

8. The oral formulation of claim 1, wherein the formulation comprises 0.25-1.0% magnesium stearate and about 91% microcrystalline cellulose.

9. The oral formulation of any of claims 1-8, wherein the formulation comprises at least one of the following disintegrants in the indicated amount (by weight of the formulation):

microcrystalline cellulose from 5-90%,
starch 3-25%,
sodium starch glycolate 2-8% and
sodium carboxymethylcellulose up to 15%.

10. The oral formulation of any one of claims 1-8, wherein the disintegrant comprises a water soluble polymer, such as sodium carboxymethylcellulose.

11. The oral formulation of claim 10, wherein the formulation comprises up to 15% sodium carboxymethylcellulose.

12. The oral formulation of claim 11, wherein the formulation comprises from 0.5-2.0% sodium carboxymethylcellulose.

13. The oral of any of claims 2-12, wherein the disintegrant is a desiccant.

14. The oral formulation of claim 1, wherein the formulation comprises:

magnesium stearate 0.25-1.0%,

microcrystalline cellulose about 91%, and

sodium carboxymethylcellulose 0.5-2.0%;

in the indicated amount (by weight of the formulation).

15. The oral formulation of any of claims 1-14, wherein the formulation further comprises an additional carrier selected from:

a binder from 3-90% and

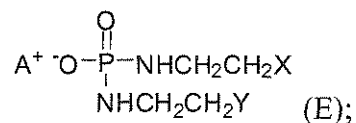
a compression filler up to 98%;

in the indicated amount (by weight of the formulation).

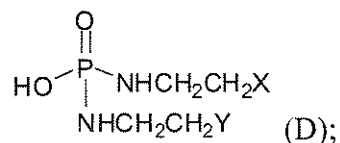
16. The oral formulation of any of claims 1-15, further comprising a second diluent, a second disintegrant and a second lubricant.

17. The oral formulation of claim 10, wherein both the diluent and the disintegrant are microcrystalline cellulose.

18. A method for the preparation of a compound of formula (E):



comprising treating a compound of formula (D):



with a base under salt-forming conditions, wherein as valence and stability permit,

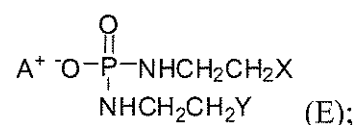
X and Y independently represent leaving groups; and

A⁺ represents an ammonium cation;

wherein the salt-forming conditions comprise combining approximately equimolar amounts of tris(hydroxymethyl)aminomethane and the compound of formula (D).

19. The method of claim 18, wherein A⁺ represents BH⁺ and B is an amine selected from the basic amino acids, pyridine, *N,N*-dimethylaminopyridine, diazabicyclononane, diazabicycloundecene, *N*-methyl-*N*-ethylamine, diethylamine, triethylamine, diisopropylethylamine, mono-, bis- or tris-(2-hydroxyethyl)amine, 2-hydroxy-*tert*-butylamine, tris(hydroxymethyl)methylamine, *N,N*-dimethyl-*N*-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine and *N*-methyl-*D*-glucamine.

20. The method of claim 19, wherein B is tris(hydroxymethyl)methylamine.
21. The method of claim 18, wherein the salt-forming conditions comprise *N,N*-dimethylformamide.
22. The use an oral formulation of any of claims 1-17, in the manufacture of a medicament for treating a condition characterized by abnormal cell growth and/or differentiation.
23. Use of a compound of formula (E):



wherein

X and Y independently represent leaving groups; and

A⁺ is an ammonium cation, in the manufacture of a medicament for oral administration for treating a condition characterized by abnormal cell growth and/or differentiation, the medicament comprising: 0.25-5% lubricant, up to 98% diluent, and up to 90% disintegrant by weight of formulation, and the compound, wherein two or more of the lubricant, diluent, and disintegrant may be a single component.