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(54) Title: ANTIPROLIFERATIVE SUBSTITUTED 5-THIAPYRIMIDINONE AND 5-SELENOPYRIMIDINONE COMPOUNDS		
(57) Abstract Novel derivatives of 5-thia- and 5-selenopyrimidinone are found to inhibit the enzyme glycinamide ribonucleotide formyl transferase (GARFT) and amino imidazole carboxamide ribonucleotide formyl transferase (AICARFT). Novel intermediates of these compounds are also disclosed. A novel method of preparing such compounds is also disclosed, as well as methods and compositions for employing the compounds as antiproliferative agents.		

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ANTIPROLIFERATIVE SUBSTITUTED
5-THIAPYRIMIDINONE and 5-SELENOPYRIMIDINONE COMPOUNDS

This application is a continuation-in-part of parent application U.S. Serial No. 07/991,259. This CIP is not being filed in response to the U.S. Patent and Trademark Examiner's rejections in the parent application. The filing of the CIP application should in no way be construed as an acquiescence that the Examiner's § 112 rejections (second and fourth paragraphs) in the parent application are correct.

The present invention relates to certain substituted 5-thia- and 5-selenopyrimidinonyl compounds which inhibit the enzyme glycineamide ribonucleotide formyl transferase (GARFT), certain substituted 5-thia- and 5-selenopyrimidinonyl compounds which inhibit the enzyme amino imidazole carboxamide ribonucleotide formyl transferase (AICARFT), to intermediates thereof, to pharmaceutical compositions containing these compounds, and to the use thereof to inhibit the growth and proliferation of the cells of higher organisms and microorganisms such as bacteria, yeast and fungi. Such effects include antitumor, antiinflammatory, antipsoriatic and immunosuppressive activity. A process for the preparation of these compounds is also disclosed.

The large class of antiproliferative agents includes antimetabolite compounds. A particular subclass of antimetabolites known as antifolates or antifoies are antagonists of the vitamin folic acid. Typically, antifolates closely resemble the structure of folic acid and incorporate the characteristic P-benzoyl glutamate moiety of folic acid. The glutamate moiety of folic acid takes on a double negative charge at physiological pH. Therefore, this compound and its analogs have an active energy driven transport system to cross the cell membrane and exert a metabolic effect.

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GARFT and AICARFT are folate dependent enzymes in the *de novo* purine biosynthesis pathway. This pathway is critical to cell division and proliferation. Shutting down this pathway is known to have an antiproliferative effect, in particular, an antitumor effect. Thus, a number of folate analogs have been synthesized and studied for their ability to inhibit GARFT. A prototypic specific tight binding inhibitor of GARFT, 5,10-dihydro-5,10-methylenetetrahydrofolate, has been reported to show antitumor activity. See F.M. Muggia, "Folate antimetabolites inhibitory to *de novo* purine synthesis" in New Drugs, Concepts and Results in Cancer Chemotherapy, pp.65-87, Kluwer Academic Publishers, Boston (1992).

The present invention introduces a novel class of 5-thia- or 5-selenopyrimidinonyl compounds containing a glutamic acid moiety. These compounds can be effective in inhibiting GARFT and/or AICARFT and the growth and proliferation of cells of higher organisms and of microorganisms such as bacteria, yeast and fungi. The invention further relates to pharmaceutical compositions containing these compounds or suitable salts thereof and the use of these compounds as inhibitors of the enzymes GARFT and/or AICARFT.

As stated above, compounds of the invention possess anti-proliferative activity, a property which may express itself in the form of anti-tumor activity. A compound of the invention may be active *per se*, or it may be a precursor which is converted *in vivo* to an active compound. Compounds of the present invention possess at least one chiral center. Thus compounds of the invention include mixtures of diastereomers or enantiomers, as well as diastereomers and enantiomers substantially free of other diastereomers or enantiomers.

Preferred compounds of the invention are active in inhibiting the growth of the L1210 cell line, a mouse leukemia cell line which can be grown in tissue culture. Compounds of the invention can also be active in inhibiting

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the growth of bacteria such as Escherichia coli gram negative bacteria which can be grown in culture.

The compounds according to the invention, as well as the pharmaceutically acceptable salts thereof, may be incorporated into convenient dosage forms such as capsules, tablets, or injectable preparations. Solid or liquid pharmaceutically acceptable carriers may also be employed. Solid carriers include starch, lactose, calcium sulphate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline solution and water.

The carrier or diluent may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, sterile injectable liquid (e.g. solution) or a non-aqueous or aqueous liquid suspension.

The pharmaceutical preparations (not necessarily the compounds or salts thereof per se) are prepared following conventional techniques of the pharmaceutical chemist involving steps such as mixing, granulating, and compressing when necessary for tablet forms, or mixing, filling and dissolving the ingredients as appropriate to give the desired products for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural and rectal administration.

The compositions of the invention may further comprise one or more other compounds which are anti-tumor agents such as: a mitotic inhibitor (for example: vinblastine); alkylating agents; dihydrofolate reductase inhibitors or TS inhibitors; antimetabolites (for example, 5-fluorouracil and cytosinearabioside); intercalating antibiotics (for example, adriamycin and bleomycin); enzymes (for example, asparaginase); topoisomerase inhibitors (for example,

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etoposide); or biological response modifiers (for example, interferon).

The compositions of the invention may also comprise one or more other compounds including antibacterial, antifungal, antiparasitic, antiviral, antipsoriatic and anticoccidial agents. Exemplary antibacterial agents include, for example, sulfonamides such as sulfamethoxazole, sulfadiazine, sulfameter or sulfadoxine; dihydrofolic reductase inhibitors such as trimethoprim, bromodiazepim, or trimetrexate; penicillins; cephalosporins; aminoglycosides; bacteriostatic inhibitors of protein synthesis; the quinolone carboxylic acids and their fused isothiazolo- analogs.

Another aspect of the invention relates to a therapeutic process of inhibiting the growth and proliferation of cells of higher organisms and microorganisms which comprises administering to a host an effective amount of a compound according to the present invention. The compounds of the invention are particularly useful in the treatment of mammalian hosts such as human hosts and in the treatment of avian hosts. Therapeutic processes can comprise administering to a host an effective amount of a compound according to the present invention to inhibit GARFT and/or AICARFT.

Many of the antiproliferative drugs described herein or pharmaceutically acceptable salts thereof can be employed in the therapeutic process of the invention. The compounds may be administered in the form of a pharmaceutically acceptable composition comprising a diluent or carrier such as those described above.

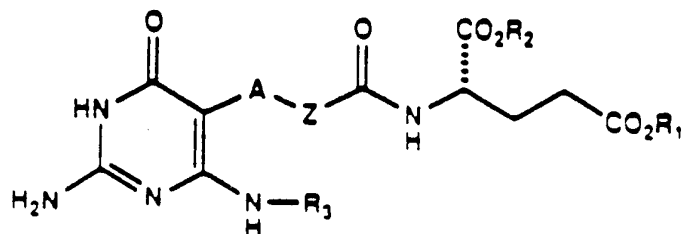
Doses of the compounds preferably include pharmaceutical dosage units comprising an effective quantity of the active compound. An "effective quantity" means a quantity sufficient to inhibit the folate metabolic pathways and derive the beneficial effects therefrom through administration of one or more of the pharmaceutical dosage units.

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An exemplary daily dosage unit for a vertebrate host comprises an amount up to one gram of active compound per kilogram of the host, preferably one half gram, more preferably 100 milligrams, and most preferably, about 50 milligrams or less per kilogram of the host weight. The selected dose may be administered to a warm-blooded animal or mammal, for example, a human patient in need of treatment mediated by folate metabolic pathways inhibition, by any known method of administering the dose including topically as, for example, an ointment or cream; orally; rectally, for example, as a suppository; parenterally by injection; or continuously by intravaginal, intranasal, intravenous, intrabronchial, intraaural or intraocular infusion. For oral administration, one can use the compounds of the invention in ester or free acid form.

The compounds according to the present invention may be characterized as producing any one or more of an antiproliferative effect, antibacterial effect, an antiparasitic effect, an antiviral effect, an antipsoriatic effect, an antiprotozoal effect, an anticoccidial effect, an antiinflammatory effect, an immunosuppressive effect or an antifungal effect. The compounds can be especially useful in producing an antitumor effect in a vertebrate host harboring a tumor.

The present invention relates to antiproliferative compounds having the formula I



I.

wherein:

A represents sulfur or selenium;

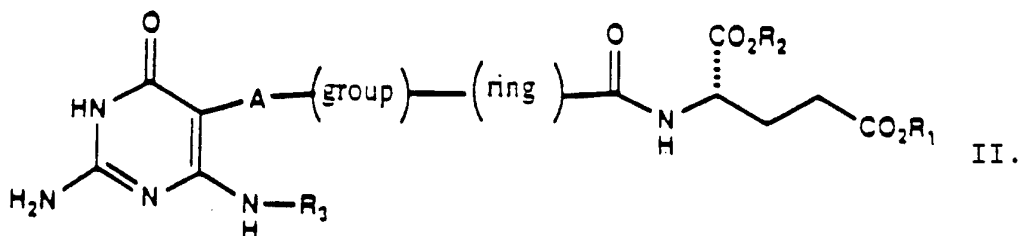
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Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen, and phosphorous; 2) a substituted or unsubstituted mono or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein when said non-cyclic spacer is bonded to A, said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms and further wherein when said non-cyclic spacer is bonded to $-C(O)-$, said non-cyclic spacer separates $-C(O)-$ from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R_1 and R_2 represent, independently, H or C_1 to C_6 alkyl or other readily lyzable, preferably hydrolyzable, groups; and

R_3 represents H or a straight, branched or cyclic C_1 to C_6 alkyl group (cyclic obviously applies only to C_3 to C_6 alkyl groups) optionally carrying one or more halogen, hydroxyl or amine groups; or a pharmaceutically acceptable salt thereof.

A preferred subgenus of the compounds of the invention has the formula II



wherein:

A is sulfur or selenium;

(group) represents a non-cyclic spacer which separates A from (ring) by 1 to 5 atoms, said atoms being independently

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selected from carbon, oxygen, sulfur, nitrogen and phosphorus and optionally carrying one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings;

(ring) represents one or more of a substituted or unsubstituted monocyclic, carbo- or heterocyclic ring or a fused or non-fused polycarbocyclic or heterocyclic ring optionally substituted with one or more substituents selected from those recited for (group);

R_1 and R_2 represent, independently, hydrogen, C_1 to C_6 alkyl or other readily lyzable, preferably hydrolyzable, groups; and

R_3 represents hydrogen or a straight, branched or cyclic C_1 to C_6 alkyl group (obviously cyclic only involves C_3 to C_6 alkyl groups) optionally carrying halogen, hydroxyl, or amine substitution; or a pharmaceutically acceptable salt thereof.

In a preferred embodiment of the compound of formula I, the moiety Z is represented by Q-X-Ar wherein:

Q represents a C_1 - C_5 alkylene, or a C_2 - C_5 alkenylene or alkynylene radical optionally carrying one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings;

X represents a methylene, monocyclic carbo- or heterocyclic ring, sulfur, oxygen or amino radical, optionally carrying one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to

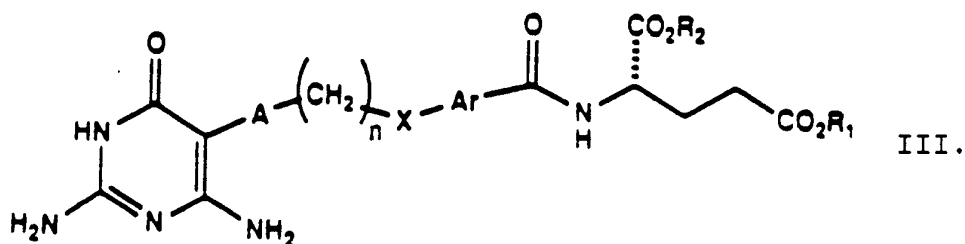
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C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused polycarbocyclic or poly-heterocyclic rings; and

Ar represents a monocyclic carbo- or heterocyclic aromatic ring or a bicyclic carbo- or heterocyclic ring, all or a portion of which may be aromatic, and wherein the Ar may be fused to the monocyclic carbo- or heterocyclic ring of X, and wherein the Ar optionally carries one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused polycarbocyclic or poly-heterocyclic rings; or a pharmaceutically acceptable salt thereof.

In a preferred embodiment of the compound of formula II, the moiety (group) represents a C_1 to C_4 alkylene group and the moiety (ring) represents a substituted or unsubstituted, fused or non-fused carbocyclic or heterocyclic bicyclic ring system, or a substituted or unsubstituted, carbocyclic or heterocyclic monocyclic ring system, or at least two monocyclic ring systems linked by a single bond, said monocyclic ring systems being independently substituted or unsubstituted.

Another preferred embodiment of the invention has the formula III



wherein n is an integer from 0 to 5, A represents sulfur or selenium, X is methylene, monocyclic carbo- or heterocyclic

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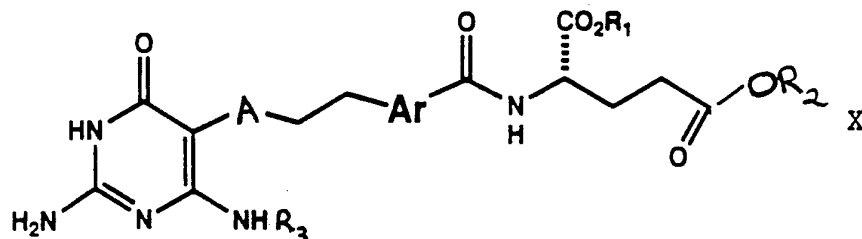
ring, O, S, or -NH-, Ar is an aromatic radical, wherein Ar can form a fused bicyclic ring system with said ring of X, and R₁ and R₂, which can be the same or different, are hydrogen or alkyl radicals having 1 to 6 carbon atoms or a pharmaceutically acceptable salt thereof.

Ar in formula III can be any substituted or unsubstituted 5 or 6 membered aromatic ring such as, for example, 3-methyl-2,5-thienyl, 4-methyl-2,5-thienyl, 3-ethyl-2,5-thienyl, 1,4-phenylene, 1,3-phenylene, 2,5-thienyl, 2,4-thienyl, 2,5-pyrrole, 2,4-pyrrole, 2,5-furyl, 2,4-furyl, 2,5-pyridyl, 2,4-pyridyl, 2-methyl-1,4-phenylene, and the like.

Although the compounds are depicted throughout this description in the formulae in the 4-oxo form and are referred to as such throughout this description, the oxo group exists in tautomeric equilibrium with the corresponding 4-hydroxy group and it will be understood that in each case the tautomeric hydroxyl form is also indicated.

The compounds of formula I in which each of R₁ and R₂ is hydrogen are active anti-tumor and antiproliferative compounds. The compounds of formula I wherein R₁ and R₂ are lower alkyl groups or other readily lyzable groups are novel intermediates for forming the free glutamic acid forms of the compounds and can also be metabolized *in vivo* to polyglutamates and thus act as prodrugs.

The invention also relates to compounds useful as AICARFT inhibitors of the formula X



wherein:

A represents sulfur or selenium;

Ar represents an unsubstituted phenylene or thienylene radical;

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R_1 and R_2 represent, individually, hydrogen or C_1 to C_6 alkyl or other readily lyzable, preferably hydrolyzable, groups;

R_3 represents hydrogen or a straight, branched or cyclic C_1 - C_6 alkyl group (obviously, when the alkyl is cyclic, C_3 - C_6 is intended), optionally carrying one or more halogen, hydroxyl or amine groups; or

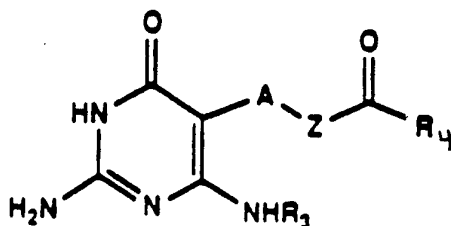
a pharmaceutically acceptable salt thereof.

Preferably, R_3 in formula X is hydrogen. A in formula X is preferably -S-. Preferably, R_1 and R_2 independently represent hydrogen, methyl and ethyl in formula X.

The compounds of formula X in which each of R_1 and R_2 is hydrogen are active anti-tumor and antiproliferative compounds. The compounds of formula X wherein R_1 and R_2 are lower alkyl groups or other readily lyzable groups are novel intermediates for forming the free glutamic acid forms of the compounds and can also be metabolized *in vivo* to polyglutamates and thus act as prodrugs.

As discussed above, the invention also includes pharmaceutically acceptable salts, including, for example, alkaline metal, alkaline earth metal, other non-toxic metals, ammonium and substituted ammonium salts of the glutamic acid embodiments of the invention such as, but not limited to, the sodium, potassium, lithium, calcium, magnesium, aluminum, zinc, ammonium, trimethyl ammonium, triethyl ammonium, tetrabutyl ammonium, pyridinium and substituted pyridinium salts.

Novel compounds of the formula V



V.

wherein:

A represents sulfur or selenium;

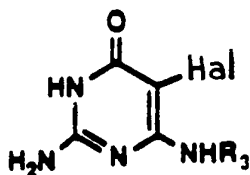
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Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms; and

R₃ represents H or a straight, branched or cyclic (C₁ to C₆) alkyl group (obviously, when the alkyl is cyclic, C₃-C₆ is intended), optionally carrying one or more hydroxyl or amine groups;

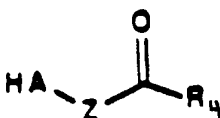
R₄ represents hydroxy, (C₁ to C₆) alkyloxy group optionally carrying one or more hydroxyl or amine groups, or a protected or unprotected amino acid linked to the acyl group of formula V by the amine portion of the amino acid; or a pharmaceutically acceptable salt thereof;

can be prepared by reacting a compound having the formula VI



VI.

wherein hal is bromine, chlorine, fluorine, or iodine, preferably bromine, and R₃ is as defined above with a compound having the formula IV



IV.

wherein A, Z, and R₄ are as defined above, in the presence of another base, preferably a non-nucleophilic auxiliary base, in a solvent in which at least one of the reactants is at

least partially soluble under conditions sufficient to obtain the compound of formula V.

In a preferred embodiment of the compound of formula V, Z represents $-(CH_2)_n-X-Ar-$ wherein n is an integer from 0 to 5, A represents sulfur, X is methylene, monocyclic carbo- or heterocyclic ring, O, S, or $-NH-$, and Ar is an aromatic radical, wherein Ar can form a fused bicyclic ring system with said ring of X.

The reaction is preferably carried out in a suitable solvent in which at least one or both reactants are soluble at the reaction temperature. The solvent and the reaction environment are preferably purged of oxygen prior to introduction of the reactants by bubbling an inert gas, such as argon or nitrogen, through the solvent. Bubbling of the inert gas is preferably continued until the reaction has gone to completion and been quenched, such as by pouring into water. Suitable preferred solvents are dipolar aprotic solvents such as, e.g., dimethylsulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide, or N-methyl-2-pyrrolidinone.

The basic medium is preferably provided via a non-nucleophilic auxiliary base which is defined as a base capable of neutralizing hydrogen halide, preferably hydrogen bromide, gas generated by the substitution reaction. The base is preferably an alkali or earth metal carbonate or a trialkylamine such as, e.g., trimethylamine, triethylamine or diisopropylethylamine.

A more specific method for conducting the reaction of compounds of the formulae IV and VI is to suspend the compound of formula VI, preferably, 5-bromo-2,6-diamino-4(3H)-oxo-pyrimidine, in the solvent; compound of the formula IV and the auxiliary base are then added sequentially. The reaction vessel is then immersed in an oil bath which has been heated to the appropriate temperature (20-200°, preferably 70-120°C). The reaction mixture is stirred at this temperature for the requisite length of time (usually 30-330 minutes), then cooled to room temperature and poured

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into water. The product is then isolated by filtration or extraction with an organic solvent and purified either by recrystallization or by chromatography.

The compound of the formula V can be hydrolyzed, in basic medium, to its free carboxylic acid form, peptide coupled, by means well-known to those skilled in the art, with a glutamic acid diester hydrochloride and, finally, hydrolyzed to the free glutamic acid form depicted in formula I (R_1 and $R_2 = H$). Detailed syntheses for various compounds of formula I will be presented in the examples that follow.

Specific examples of novel compounds of formula I include:

N-[4-(3-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)propyl]benzoyl-(S)-glutamic acid (Example 2);

N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)benzoyl]-S-glutamic acid (Example 5);

N-[(4-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]thio)benzoyl]-S-glutamic acid (Example 4);

N-[2-(5-[3-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)propyl]-thienoyl)]-(S)-glutamic acid (Example 3);

N-[5-(3-[2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio)-propyl]-3-methyl-thieno-2-yl]-L-glutamic acid (Example 6);

N-[5-(3-[2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio)-propyl]-4-methyl-thieno-2-yl]-L-glutamic acid (Example 7);

N-(6-([2,6-diamino-4(3H)-oxopyrimidino-5-yl]thio)-methyl)-4,5,6,7-tetrahydrobenzothieno-2-yl]-S-glutamic acid (Example 8);

N-(5-[2-([2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]thieno-2-yl)-L-glutamic acid (Example 9); and

N-(4-[4-[2-([2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]benzoyl]-L-glutamic acid (Example 10).

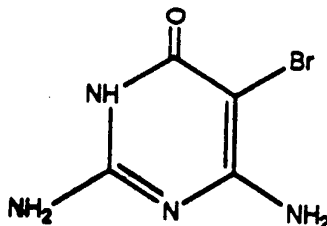
It is to be understood that application of the teachings of the present invention to a specific problem or environment will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their preparation and recovery

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appear in the following examples.

EXAMPLE 1

Synthesis of 5-bromo-2,6-diamino-4(3H)-oxo-pyrimidine



This compound was prepared according to a procedure described by C.O. Okafor, J. Heterocyclic Chem., 17, 1587 (1980).

Eighty mmol (10.09 g) of commercially available 2,6-diamino-4(3H)-pyrimidinone was deposited in a one liter flask suspended in a mixture of 60 ml of methanol and 60 ml of water. This suspension was vigorously stirred and 12 grams of sodium bicarbonate was added in a single portion. Eight ml (155 mmole) of liquid bromine was added dropwise to the reaction flask over a 40-minute interval. An additional 30 ml of 50% aqueous methanol was then added to the reaction mixture to facilitate stirring. After stirring for a further 30 minutes, an additional 8 grams of sodium bicarbonate was added in a single portion. The resultant reaction mixture was stirred for 75 minutes and left standing at room temperature overnight. The product was collected by filtration and recrystallized from 300 ml of water, yielding 6.4 grams (39% yield) of yellow needles having a M.P. of 242°C (decomposed).

EXAMPLE 2

Synthesis of N-[4-(3-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio]propyl)benzoyl-(S)-glutamic acid

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This compound of formula III wherein A is sulfur, $n = 2$, $x = -CH_2-$, Ar = phenylene, and R_1 & R_2 = hydrogen was synthesized via the following 10-step process.

a. Methyl-4-iodobenzoate

Fifty (50) mmol (12.4 g) of commercially available 4-iodobenzoic acid was dissolved in 200 ml of tetrahydrofuran and then added to an ether solution containing freshly prepared diazomethane. The excess diazomethane was subsequently consumed by addition of glacial acetic acid and the resultant solution was concentrated *in vacuo*. The residue thus obtained was partitioned between ethyl acetate and saturated $NaHCO_3$. The layers were separated and the aqueous phase was extracted with 100 ml of ethyl acetate. The organic extracts were combined, dried over Na_2SO_4 , and concentrated *in vacuo*, yielding 13.01 grams (99.3% yield) of a yellow solid which melted at $111^\circ C$.

The following analyses indicate that methyl 4-iodobenzoate were obtained.

1H NMR (300 MHz, GE QE-300, $CDCl_3$)

δ = 7.80 (2H, d, J = 8.6Hz); 7.74 (2H, d, J = 8.6Hz); 3.91 (3H, s).

Elemental Analysis:

Calculated as $C_8H_7O_2I$: C = 36.37; H = 2.69 and
I = 48.43

Found: C = 36.91; H = 2.74 and
I = 48.23

b. Methyl 4-(3-hydroxypropynyl)benzoate

Methyl 4-iodobenzoate (9.0g, 34.4 mmol) was dissolved in 90 ml of diethylamine. The solution was stirred vigorously and, sequentially, 121 milligrams of bis(triphenylphosphine) palladium chloride and 65 milligrams of cuprous iodide were added, each in a single portion, followed by 1.93 grams of propargyl alcohol. The resultant mixture was stirred for about 20 hours at room temperature in an argon atmosphere. At the end of this period, diethylamine was removed by

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concentration *in vacuo*. The residue was diluted with 200 ml of water and extracted three times with 100 ml of benzene and 75 ml of ethyl acetate. The organic extracts were combined, dried over Na_2SO_4 , and concentrated *in vacuo*, yielding a brown solid. This crude residue was purified by flash chromatography. Elution with a 2 to 1 (V:V) mixture of hexane and ethyl acetate yielded 5.44 grams (83% yield) of a pale yellow solid melting at 81 to 82°C.

The following analyses were obtained for this material, indicating that it was methyl 4-(3-hydroxy propynyl) benzoate:

NMR (CDCl_3) δ = 7.98 (2H, d, J = 8.3Hz), 7.49 (2H, d, J = 8.3Hz), 4.52 (2H, s), 3.92 (3H, s)
Anal. ($\text{C}_{11}\text{H}_{10}\text{O}_3$) C, H

c. Methyl 4-(3-hydroxypropyl)benzoate

Three grams (15.8 mmol) of methyl 4-(3-hydroxy propynyl) benzoate was dissolved in 200 ml of ethanol in a Parr flask and 0.3 g (10% wt. eq.) of 5% Pd/C was added. This mixture was shaken under 45 psi of hydrogen for 3.5 hours. The crude reaction mixture was filtered through a pad of Celite® and the filtrate was then concentrated, *in vacuo*, yielding a green oil which was purified by vacuum flash chromatography. Elution with a 2 to 1 (V:V) mixture of hexane and ethyl acetate yielded 2.9 grams (95% yield) of a yellow oil.

The following analyses were obtained for this material, indicating that it was methyl 4-(3-hydroxypropyl) benzoate:

NMR (CDCl_3) δ = 7.96 (2H, d, J = 8.3Hz), 7.27 (2H, d, J = 8.3Hz), 3.90 (3H, s), 3.68 (2H, J = 6.4Hz), 2.77 (2H, t, J = 7.5Hz), 1.91 (2H, tt, J = 6.4, 7.5Hz)
Anal. ($\text{C}_{11}\text{H}_{14}\text{O}_3 \cdot 0.3\text{H}_2\text{O}$) C, H

d. Methyl 4-3(bromopropyl)benzoate

Methyl 4-(3-hydroxypropyl) benzoate, (3.49g, 18mmol) and 4.21 grams of carbon tetrabromide were dissolved in 25 milliliters of methylene chloride. This solution was stirred vigorously and cooled to about 0°C centigrade. Triphenyl

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phosphine (5.67 grams dissolved in 25 ml of methylene chloride) was added dropwise to this solution over a 10 minute period, with the reaction temperature holding at about 0°. The resultant yellow solution was stirred at 0° for about 30 minutes, then overnight at room temperature. The crude reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography. Elution with 9 to 1 (v:v) hexane ethyl acetate yielded 4.38 grams (95% yield) of product as a yellow oil.

The following analyses were obtained for this material, indicating that it was methyl (4-(3-bromopropyl) benzoate:

NMR (CDCl₃) δ = 7.97 (2H, d, J = 8.2Hz),
7.27 (2H, d, J = 8.2Hz), 3.91 (3H, s), 3.39 (2H, t, J =
6.5Hz),

2.84 (2H, t, J = 7.4Hz), 2.18 (2H, tt, J = 6.5, 7.4Hz)

Anal. (C₁₁H₁₃O₂Br) C, H, Br

e. Methyl 4-[3-(acetylthio)propyl]benzoate

Five (5) mmol (1.29 grams) of methyl 4-(3 bromopropyl) benzoate was dissolved in 40 ml of acetone and vigorously agitated while 10 mmol (1.14 grams) of potassium thioacetate was added in a single portion. The mixture was heated at reflux for 35 minutes, then returned to room temperature and filtered. The filter cake was washed twice with acetone. The filtrate and washings were combined and concentrated under vacuum. The residue was partitioned between ether and water (25 ml each). The layers were separated and the aqueous phase extracted with 25 ml of ether. The organic extracts were combined, dried over Na₂SO₄, and concentrated *in vacuo*, yielding a red oil. This oil was purified by flash chromatography. Elution with hexane/ethyl acetate (9 to 1 V:V) yielded 1.22g (97% yield) of an amber colored oil.

The following analyses were obtained for this material indicating that it was methyl 4-[3-(acetylthio)propyl] benzoate:

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NMR (CDCl_3) δ = 7.95 (2H, d, J = 8.3Hz),
7.24 (2H, d, J = 8.3Hz), 3.90 (3H, s), 2.88 (2H, t, J =
7.2Hz), 2.74 (2H, t, J = 7.7Hz), 2.34 (3H, s), 1.91 (2H, tt,
 J = 7.2, 7.7Hz)

Anal. ($\text{C}_{13}\text{H}_{16}\text{O}_3\text{S}$) C,H,S

f. Methyl 4-(3-thiopropyl)benzoate

One ml (1.1 gram) of acetyl chloride was added slowly to 10 ml of methanol in a 100 ml flask. This solution was rapidly stirred and to it was added a solution of methyl 4-[3-(acetylthio)propyl] benzoate in 5 ml of methanol. The reaction mixture was heated at reflux for two hours, then cooled to room temperature. The crude reaction mixture was diluted with 10 ml of water. Methanol was removed by concentration under vacuum, and the aqueous residue was extracted twice with 25 ml of ether. The organic extracts were combined, dried over Na_2SO_4 , and concentrated under vacuum, yielding 815 milligrams (97.3% yield) of an amber colored oil.

The following analyses were obtained for this material, indicating that it is the desired methyl 4-(3-thiopropyl) benzoate:

NMR (CDCl_3) δ = 7.96 (2H, d, J = 8.2Hz),
7.25 (2H, d, J = 8.2Hz), 3.90 (3H, s), 2.79 (2H, t, J =
7.6Hz),
2.57-2.50 (2H, m), 2.00 (2H, m), 1.37 (1H, t, J = 7.9Hz)

Anal. ($\text{C}_{11}\text{H}_{14}\text{O}_2\text{S}$) C,H,S

g. Methyl 4-(3-[2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio]propyl)benzoate

Argon was bubbled through a slurry of 1.01 grams (4.9 mmol) of 5-bromo-2,6-diamino-4(3H)pyrimidinone in 15 ml of DMF. To this slurry was added a solution of 1.11 gram (5.3 mmol) of methyl 4-(3-thiopropyl)benzoate in 10 ml of DMF and 1.8 ml (1.34g) of diisopropylethylamine. This mixture was heated at 100°C for 30 minutes, then poured over ice. The

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resultant precipitate was collected by filtration, washed twice with 30 ml of H₂O, then twice with 30 ml of ether, yielding 711 mg of a white powder (73% yield) melting at 248 to 251°C (decomposed).

The following analyses indicated that this material was the desired 4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]propyl) benzoate:

NMR (DMSO-d₆) δ = 9.95 (1H, s), 7.84 (2H, d, J = 8.2hz),
7.33 (2H, d, J = 8.2hz), 6.28 (4H, broad), 3.82 (3H, s),
2.76 (2H, t, J = 7.2Hz), 2.42 (2H, t, J = 7.0Hz),
1.70 (2H, tt, J = 7.0, 7.2hz)

Anal. (C₁₅H₁₈N₄O₃S·0.3H₂O)C,H,N,S

h. 4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]propyl) benzoic acid

A suspension of 669 mg (2 mmol) of 4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5yl)thio]propyl)benzoate in 30 ml of 1N NaOH was stirred overnight at room temperature, then filtered. The filtrate was acidified to pH 5.0 with acetic acid. The precipitate that formed was collected by filtration and washed 3 times with 5 ml of H₂O yielding 589 mg (91.9% yield) of an off-white powder product.

The following analyses indicated that this was the desired 4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5yl)thio]propyl)benzoic acid:

NMR (DMSO-d₆) δ = 12.72 (1H, broad), 9.97 (1H, broad),
7.82 (2H, d, J = 8.1Hz), 7.29 (2H, d, J = 8.1Hz), 6.29 (4H, broad)
2.74 (2H, t, J = 7.2Hz), 2.43 (2H, t, J = 7.0Hz), 1.70 (2H, tt, d = 7.0, 7.2Hz)

Anal. (C₁₄H₁₆N₄O₃S) C,H,N,S

i. Diethyl N-[4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]propyl)benzoyl]-S-glutamate

4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]propyl)benzoic acid (577 mg) was dissolved in 40 ml of 1-methyl-2-pyrrolidinone. This mixture was vigorously stirred and 920

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milligrams of 4-methyl morpholine were added followed by 723 milligrams of phenyl N-phenylphosphoroamidochloridate in a single portion. The mixture was stirred under an argon atmosphere at room temperature for 60 minutes followed by the addition of 863 milligrams of S-glutamic acid diethyl ester hydrochloride. This mixture was left stirring overnight under argon 20 hours, then concentrated *in vacuo*. The residue obtained was partitioned between 30 ml of water and 30 ml of chloroform. The layers were separated and the aqueous phase was extracted with 30 ml of chloroform. The combined organic extracts were washed with 30 ml of H₂O and dried over MgSO₄ then concentrated *in vacuo* to yield a yellow gum, which was purified by flash chromatography. Elution with 5% methanol in ethyl acetate yielded 212 mg of a white solid melting at 78-81°C.

The following analyses indicated that this material was the desired diethyl N-[4-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5yl)thio]propyl)benzoyl]-S-glutamate.

NMR(CDCl₃) δ = 7.67(2H, d, J = 8.1Hz), 7.28(1H, d, J = 8.0Hz), 7.16(2H, d, J = 8.1 Hz), 6.64(2H, br s), 5.84(2H, br s), 4.78(1H, ddd, J = 5.0, 8.0, 12.8Hz), 4.21(2H, q, J = 7.1H), 4.09(2H, q, J = 7.1Hz), 2.64(2H, t, J = 7.3Hz), 2.57-2.25(4H,m), 2.21-2.09(2H, m), 1.83(2H, tt, J = 7.0, 7.3Hz), 1.29(3H, t, J = 7.1Hz), 1.21(3H, t, J = 7.1Hz)

Anal. (C₂₃H₃₁N₅O₆S) C,H,N,S

j. N-[4-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5yl)thio]propyl)benzoyl]-S-glutamic acid

A solution of 192 mg (0.4 mmol) of diethyl N-[4-(3-[(2,6-thiamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)benzyl]-S-glutamate in 15 ml of 1N NaOH was stirred at room temperature for about 70 hours, then neutralized with 6N HCl. The precipitate that formed was collected by filtration and washed 3 times with 10 ml of H₂O yielding 147 mg of a white solid which melted at 205-206°C.

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The following analyses indicate that the product was the desired N-[4-(3-[(2,6-diamino-4(3H)-oxypyrimidin-5yl)thio]-propyl) benzoyl]-S-glutamic acid:

NMR(DMSO-d₆) δ = 12.34(2H, broad), 9.96(1H, broad), 8.51(1H, d, J = 7.7Hz), 7.76(2H, d, J = 8.2Hz), 7.27(2H, d, J = 8.2Hz), 6.29(4H, br s), 4.37(1H, ddd, J = 4.8 7.7, 9.7Hz), 2.73(2H, t, J = 7.2Hz), 2.43(2H, t, J = 6.9Hz), 2.34(2H, t, J = 7.9Hz), 2.11-2.01(1H, m), 1.99-1.89 (1H, m), 1.69 (2H, tt, J = 6.9, 7.2 Hz)

Anal. (C₁₉H₂₃N₅O₆S) C,H,N,S

EXAMPLE 3

Synthesis of N-[2-(5-[3-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)propyl]-thienoyl)]-(S)-glutamic acid

This compound of formula III, wherein A is sulfur, n = 2, x = -CH₂-, Ar = 2,5-thienyl, and R₁ and R₂ = H, was synthesized by the following process.

a) 5-Bromothiophene-2-carboxylic acid

To a solution of 5-bromothiophene-2-carboxaldehyde (12ml, 19.28g, 0.1mol) in acetone (400ml), was added, portionwise, KMnO₄ (19.57g, 0.12mol) over a 10 minute interval. The mildly exothermic reaction was left stirring, with no external temperature control, overnight. The crude reaction mixture was filtered and the filter-cake extracted, alternately, with 1N NaOH (3 x 150ml) and warm water (2 x 200ml). The combined aqueous extracts were washed with ether (100ml), then acidified by addition of conc. HCl. The precipitate that formed was collected by filtration to yield the product as a yellow solid (17.3lg, 83% yield) which melted at a 140°C.

The following analyses indicate that the product obtained was 5-bromothiophene-2-carboxylic acid:

NMR(CDCl₃) δ = 7.64(1H, d, J = 4.0Hz), 7.12(1H, d, J = 4.0Hz)

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Anal. ($C_5H_3O_2SBr$) C,H,S,Br

b) Methyl 5-bromothiophene-2-carboxylate

A solution of 5-bromothiophene-2-carboxylic acid (17.6g, 85 mmol) in ether (200ml) was combined with an ether solution containing excess diazomethane. The excess CH_2N_2 was consumed by addition of glacial acetic acid and the resultant solution was dried over K_2CO_3 and $MgSO_4$, then concentrated, *in vacuo*, to provide the product as a yellow solid (18.72g, 100% yield) mp 58°-59°

The following analyses indicate that the product was methyl 5-bromothiophene-2-carboxylate:

NMR($CDCl_3$) δ = 7.55(1H, d, J = 4.0Hz), 7.07(1H, d, J = 4.0Hz), 3.87(3H, s)

Anal. ($C_6H_5O_2SBr$) C,H,S,Br

c) Methyl 5-(3-hydroxypropynyl)thiophene-2-carboxylate

This product was prepared using the procedure described in Example 2(b). From 11.05 grams (50 mmol) of methyl 5-bromothiophene-2-carboxylate there was obtained 7.41 grams (76% yield) of a yellow solid melting at 66 to 68°C.

The following analyses indicate that the product was methyl 5-(3-hydroxypropynyl)thiophene-2-carboxylate:

NMR($CDCl_3$) δ = 7.64(1H, d, J = 3.9Hz), 7.15(1H, d, J = 3.9Hz), 4.52(2H, broad), 3.88(3H, s), 1.84(1H, broad)

Anal. ($C_9H_8O_3S$) C,H,S

d) Methyl 5-(3-hydroxypropyl)thiophene-2-carboxylate

To a solution of methyl 5-(3-hydroxypropynyl)thiophene-2-carboxylate, (7.41g, 38 mmol) in THF (140ml) was added 2,4,6-triisopropylbenzenesulfonyl hydrazide (96.13g, 0.32mol) in four portions at 90 minute intervals. After the reaction had been heated at reflux for a total of 6.5 hours, the solvent was removed by concentration, *in vacuo*. The residue obtained was partitioned between 0.5N NaOH (700ml) and ether (500ml). The layers were separated and the aqueous phase extracted with ether (250ml). The combined organic extracts

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were washed with 0.5N NaOH (2 x 150 ml), dried over Na_2SO_4 and concentrated, *invacuo*, to give an oil which was purified by flash chromatography. Elution with hexane/EtOAc (2:1) yielded the product as a yellow oil (3.67g.48% yield).

The following analyses indicate that the product was methyl 5-(3-hydroxypropyl)thiophene-2-carboxylate:

NMR(CDCl_3) δ = 7.64(1H, d, J = 3.8Hz), 6.82(1H, d, J = 3.8Hz), 3.86(3H, s), 3.71(2H, t, J = 6.2Hz), 2.96(2H, t, J = 7.6Hz), 1.96(2H, tt, J = 6.2, 7.6Hz)

Anal. ($\text{C}_9\text{H}_{12}\text{O}_3\text{S}$) C,H,S

e) Methyl 5-(3-bromopropyl)thiophene-2-carboxylate

This product was prepared using the procedure described for Example 2(d). From 3.67 grams (18.3 mmol) of methyl 5-(3-hydroxypropyl)thiophene-2-carboxylate there was obtained 4.56 grams (95% yield) of a yellow oil.

The following analyses indicate that that product was methyl 5-(3-bromopropyl)thiophene-2-carboxylate:

NMR(CDCl_3) δ = 7.64(1H, d, J = 3.7Hz), 6.85(1H, d, J = 3.7Hz), 3.86(3H, s), 3.43(2H, t, J = 6.4Hz), 3.03 (2H, t, J = 7.2Hz), 2.22 (2H, tt, J = 6.4, 7.2Hz)

Anal. ($\text{C}_9\text{H}_{11}\text{O}_2\text{SBr}$) C,H,S,Br

f) Methyl 5-[3-(acetylthio)propyl]thiophene-2-carboxylate

This product was prepared using the procedure described for Example 2(e). From 5.01 grams (19mmol) of methyl 5-(3-bromopropyl)thiophene-2-carboxylate there was obtained 4.54 grams (92% yield) of a yellow oil.

The following analyses indicate that that product was methyl 5-[3-(acetylthio)propyl]thiophene-2-carboxylate:

NMR(CDCl_3) δ = 7.63(1H, d, J = 3.7Hz), 6.81(1H, d, J = 3.7Hz), 3.86(3H, s), 2.91 (4H, t, J = 7.3 Hz), 2.34 (3H, s), 1.97 (2H, pentet, J = 7.3Hz)

Anal. ($\text{C}_{11}\text{H}_{14}\text{O}_3\text{S}_2$) C,H,S

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g) Methyl 5-(3-thiopropyl)thiophene-2-carboxylate

This product was prepared using the procedure described for example 2 (f). From 4.29 grams (16.6 mmol) of methyl 5-[3-(acetylthio)propyl]thiophene-2-carboxylate there was obtained 3.35 grams (93% yield) of an orange-colored oil.

The following analyses indicate that that product was methyl 5-(3-thiopropyl)thiophene-2-carboxylate:

NMR(CDCl₃) δ = 7.63(1H, d, J = 3.7Hz), 6.81(1H, d, J = 3.7Hz), 3.86(3H, s), 2.98(2H, t, J = 7.4Hz), 2.57(2H, dt, J = 8.0, 7.1Hz), 1.99(2H, tt, J = 7.1, 7.4Hz), 1.36(1H, t, J = 8.0Hz)

Anal. (C₉H₁₂O₂S₂) C,H,S

h) Methyl 5-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)thiophene-2-carboxylate

This material was prepared using the procedure described in example 2(g). From 3.03 grams (14 mmol) of methyl-5-(3-thiopropyl)thiophene-2-carboxylate there were obtained 1.88 grams (44% yield) of an off-white solid melting at 196°C (dec.).

The following analyses indicate that the product was methyl 5-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)thiophene-2-carboxylate.

NMR(DMSO-d₆) δ = 9.96(1H, br s), 7.61(1H, d, J = 3.8Hz), 6.93(1H, d, J = 3.8Hz), 6.29(4H, broad), 3.77(3H, s), 2.97(2H, t, J = 7.5Hz), 2.45(2H, t, J = 6.8Hz), 1.74(2H, tt, J = 6.8, 7.5Hz)

Anal. (C₁₃H₁₆N₄O₃S₂) C,H,N,S

i) 5-(3-[(2,6-Diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl thiophene-2-carboxylic acid

This material was prepared using the procedure described for example 2 (h). By saponification of 1.7 grams (5 mmol) of methyl 5-(3-[(2,6-diamino-4-(3H)-oxopyrimidin-5-yl)thio]propyl)thiophene-2-carboxylate there was obtained

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1.53 grams (94% yield) 5-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)thiophene-2-carboxylic acid as a yellow powder melting at 254°C (dec.).

The following analysis indicate that that product was the 5-(3-[(2,6-diamino-4(3H)-oxopyrimidine-5yl)thio]propyl)thiophene-2-carboxylic acid:

NMR(DMSO-d₆) δ = 12.86(1H, broad), 10.02(1H, broad), 7.53(1H, d, J = 3.7Hz), 6.89(1H, d, J = 3.7Hz), 6.30(4H, br, s), 2.95(2H, t, J = 7.4Hz), 2.46(2H, t, J = 6.9Hz), (1.73(2H, tt, J = 6.9, 7.4 Hz)

Anal. (C₁₂H₁₄N₄O₃S₂·0.5H₂O) C,H,N,S

j) Diethyl N-[2-(5-[3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)propyl]thienoyl)]-S-glutamate

This product was prepared using the procedure described for example 2(i). From 1.31 grams (4 mmol) of 5-(3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)thiophene-2-carboxylic there was obtained 879 mg (43% yield) of diethyl N-[2-(5-[3-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)propyl]thienoyl)-S-glutamate as an off-white solid melting at 136-138°C.

The following analyses indicate that that product was diethyl N-[2-(5-[3-[(2,6-diamino-4(3H)-oxopyrimidine-5-yl]thio)propyl]thienoyl)]-S-glutamate.

NMR (CDCl₃) δ = 11.24 (1H, brs) 7.36 (1H, d, J = 3.7Hz), 7.02 (1H, d, J = 7.8 Hz), 6.72 (1H, d, J = 3.7 Hz), 6.29 (2H, broad), 5.55 (2H, broad), 4.72 (1H, ddd, J = 4.8, 7.8, 12.6 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.10 (2H, q, J = 7.1 Hz), 2.89 (2H, t, J = 7.2Hz), 2.55 (2H, t, J = 7.0Hz), 2.45 (2H, t, J = 7.2Hz), 2.34 - 2.22(1H, m), 2.18 - 2.08 (1H, m), 1.89 (2H, pentet, J = 7.2Hz), 1.29 (3H, t, J = 7.1Hz).

Anal. (C₂₁H₂₉N₅O₆S₂) C,H,N,S

k) N-[2-(5-[3-[(2,6-diamino-4(3H)oxopyrimidin-5-yl]thio)propyl]thienoyl)]-S-glutamic acid

This product was prepared using the procedure described for example 2(j). Saponification of 716 mg (1.4 mmol) of

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diethyl N-[2-(5-[3-([2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)propyl] thienoyl)]-S-glutamate yielded 558 mg (87% yield) of a yellow powder melting at 171 to 173°C.

The following analyses indicate that that product was N-[2-(5-[3-([2,6-diamino, 4(3H)-oxypyrimidine-5-yl]thio)-propyl] thionoyl)]-S-glutamic acid:

NMR(DMSO-d₆) δ = 12.41(2H, broad), 10.09(1H, broad), 8.49(1H, d, J = 7.7Hz), 7.66(1H, d, J = 3.6Hz), 6.85(1H, d, J = 3.6Hz), 6.39(4H, broad), 4.36-4.28(1H, m), 2.92(2H, t, J = 7.2Hz), 2.46(2H, t, J = 6.8Hz), 2.32(2H, t, J = 7.3Hz), 2.11-2.00(1H, m), 1.95-1.83(1H, m), 1.73(2H, tt, J = 6.8, 7.2Hz)

Anal. (C₁₇H₂₁N₅O₆S₂·0.3H₂O) C,H,N,S

EXAMPLE 4

Synthesis of N-[(4-[2-([2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]thio)benzoyl]-S-glutamic acid

This compound of formula III, wherein A is sulfur, n = 2, x = S, Ar = 1,4-phenylene-, and R₁ and R₂ = H, was synthesized via the following process.

a) 4-[2[(t-butyldimethylsilyloxy)ethyl]thio]benzoic acid and methyl 4-[(2-hydroxyethyl)thio]benzoate

A solution of 4-mercaptopbenzoic acid (5.09g, 33mmol) in DMF (60ml) was added to a slurry of 60% NaOH (2.64g, 66mmol) in DMF (60ml) under argon at 0°C. This mixture was stirred at 0°C for 90 minutes prior to the dropwise addition of a solution of 1-(tert.-butyldimethylsilyloxy)-2-iodoethane (8.59g, 30mmol) in DMF (30ml). The resultant reaction mixture was stirred for 3 hours at room temperature, then poured over a mixture of 0.5N HCl (70ml) and ice (200g) and diluted with water (500ml). The precipitate that formed was collected by filtration to give a peach-colored solid (9.28g, 99% yield) which was used without further purification.

The above product 4-[2[(t-butyldimethylsilyloxy)-ethyl]thio] benzoic acid (8.75g, 28mmol) was dissolved in

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CH₃OH (300ml) containing conc. H₂SO₄ (3ml) and this solution was refluxed overnight. The solvent was removed by concentration, *in vacuo*, and the residue was partitioned between saturated NaHCO₃ (300ml) and ether (300ml). The layers were separated and the aqueous phase extracted with EtOAc (200ml). The combined organic extracts were dried over Na₂SO₄ and concentrated, *in vacuo*, to give an orange gum which was purified by flash chromatography. Elution with hexane/EtOAc (2:1) yielded the product as a white solid (3.53g, 59% yield) which melted at 58°C.

The following analyses indicate that that product was methyl 4-(2-hydroxyethyl)thio benzoate:

NMR(CDCl₃) δ = 7.94(2H, d, J = 8.5Hz), 7.36(2H, d, J = 8.5Hz), 3.90(3H, s), 3.83(2H, q, J = 6.1Hz), 3.21 (2H, t, J = 6.1Hz), 1.96(1H, t, J = 6.1Hz)

Anal. (C₁₀H₁₂O₃S) C,H,S

b) Methyl 4-[(2-bromoethyl)thio]benzoate

This product was prepared according to the procedure as described for example 2(d). From 1.06 grams (5 mmol) of methyl 4-[(2-hydroxyethyl)thio]benzoate, there was obtained 1.34 grams (97% yield) of a white solid melting at 77-78°C.

The following analyses indicate that that product was methyl 4-[(2-bromoethyl)thio]benzoate:

NMR(CDCl₃) δ = 7.97(2H, d, J = 8.5Hz), 7.35(2H, d, J = 8.5Hz), 3.91(3H, s), 3.53-3.46(2H, m), 3.42-3.36(2H, m)

Anal. (C₁₀H₁₁O₂SBr) C,H,S,Br

c) Methyl 4-[(2-(acetylthio)ethyl]thio]benzoate

This product was prepared according to the procedure described for example 2(e). From 1.24 grams (45 mmol) of methyl 4-[(2-bromoethyl)thio]benzoate there was obtained 1.17g (96% yield) of a yellow solid melting at 62-63°C.

The following analyses indicates that the product was methyl 4-[(2-(acetylthio)ethyl]thio]benzoate:

NMR(CDCl₃) δ = 7.96(2H, d, J = 8.6Hz), 7.39(2H, d, J = 8.6Hz), 3.90(3H, s), 3.19-3.09(4H, m), 2.36(3H, s)

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Anal. ($C_{12}H_{14}O_3S_2$) C,H,S

d) Methyl 4-[(2-mercaptoethyl)thio]benzoate

This product was prepared according to the procedure described for example 2(f). From 2.35 grams (8.7 mmol) of methyl-4-[(2-(acetylthio)ethyl]thio)benzoate there was obtained 1.92 grams (97% yield) of a yellow solid melting at 51°C.

The following analyses indicate that that product was methyl 4-[(2-mercaptoethyl)thio]benzoate:

NMR ($CDCl_3$) δ = 7.94(2H, d, J = 8.5Hz), 7.32(2H, d, J = 8.5Hz), 3.90(3H, s), 3.22(2H, t, J = 7.2Hz), 2.79 (2H, dt, J = 8.3, 7.2Hz), 1.73 (1H, t, J = 8.3Hz)

Anal. ($C_{10}H_{12}O_2S_2$) C,H,S

e) Methyl 4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thio]benzoate

This product was prepared according to the procedure described for example 2(g). From 2.51 grams (11 mmol) of methyl-4-[(2-(acetylthio)ethyl]thio)benzoate there was obtained 1.96 grams (56% yield) of a white solid melting at 219-221°C.

The following analysis indicate that that product was methyl 4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thio]benzoate:

NMR(DMSO- d_6) δ = 10.08(1H, br, s), 7.81(2H, d, J = 8.5Hz), 7.29(2H, d, J = 8.5Hz), 6.39(4H, broad), 3.82(3H, s), 3.16(2H, t, J = 7.9Hz), 2.66(2H, t, J = 7.9Hz)

Anal. ($C_{14}H_{16}N_4O_3S_2 \cdot 0.9H_2O$) C,H,N,S

f) 4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thio]benzoic acid

This product was prepared using the procedure described for example 2(h). Saponification of 1.76 grams (5mmol) of methyl-4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thio]benzoate yielded 1.57 grams (93% yield) of a white solid melting at 273-275°C (decomposed).

-29-

The following analyses indicate that that product was 4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5yl) thio]ethyl)thio]benzoic acid:

NMR(DMSO-d₆) δ = 10.11(1H, br, s), 7.79(2H, d, J = 8.5Hz), 7.26(2H, d, J = 8.5Hz), 6.40(4H, broad), 3.15(2H, t, J = 7.8Hz), 2.66(2H, t, J = 7.8Hz)

Anal. (C₁₃H₁₄N₄O₃S₂) C,H,N,S

g) Diethyl N-[(4-[2-[(2,6-diamino-4(3H)-oxypyrimidin-5yl]thio)ethyl]thio)benzoyl]-S-glutamate

This product was prepared using the procedure described for example 2 (i). From 1.36 grams (4 mmol) of 4-[(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thio]benzoic acid there was obtained 905 mg (43% yield) of an off-white solid melting at 87 to 89°C.

The following analyses indicate that that product was diethyl N-[(4-[2-[(2,6-diamino-4(3H)-oxypyrimidin-5yl]thio)-ethyl]thio)benzoyl]-S-glutamate:

NMR(CDCl₃) δ = 11.18(1H, br, s), 7.65(2H, d, J = 8.4Hz), 7.38(1H, d, J = 7.9Hz), 7.22(2H, d, J = 8.4Hz), 6.54(2H, broad), 5.67(2H, broad), 4.77 (1H, ddd, J = 4.8, 7.9, 12.7Hz), 4.21(2H, q, J = 7.1Hz), 4.08(2H, q, J = 7.1Hz), 3.10(2H, t, J = 7.5Hz), 2.70(2H, t, J = 7.5Hz), 2.62-2.40(2H, m), 2.37-2.24(1H, m), 2.21-2.09(1H, m), 1.29(3H, t, J = 7.1Hz), 1.21(3H, t, J = 7.1Hz)

Anal. (C₂₂H₂₉N₅O₆S₂) C,H,N,S

h) N-[(4-[2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]thio)benzoyl]-S-glutamic acid

This product was prepared using the procedure described for example 2 (j). From 649 mg (1.2 mmol) of the corresponding diethyl ester, there was obtained 529 mg (91% yield) of a white solid melting at 161-162°C (dec.).

The following analyses indicate that that product was N-[(4-[2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]-thio)benzoyl]-S-glutamic acid:

-30-

NMR(DMSO-d₆) δ = 12.39(2H, broad), 10.22(1H, broad), 8.58(1H, d, J = 7.7Hz), 7.78(2H, d, J = 8.4Hz), 7.28(2H, d, J = 8.4Hz), 6.56(2H, broad), 6.44(2H, broad), 4.37(1H, ddd, J = 4.9, 7.7, 12.6Hz), 3.14(2H, t, J = 7.8Hz), 2.66(2H, t, J = 7.8Hz), 2.34(2H, t, J = 7.4 Hz), 2.13 - 2.03 (1H, m), 2.01 - 1.87 (1H, m)

Anal. (C₁₈H₂₁N₅O₆S₂·1.8HCl) C, H, N, S

EXAMPLE 5

Synthesis of N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)benzoyl]-S-glutamic acid

This compound of formula III, wherein A is sulfur, n = 2, x = -NH-, Ar = 1,4-phenylene-, and R₁ and R₂ = H, was synthesized via the following process.

(a) methyl 4-(N-[2-(tert.-butyldimethylsilyloxy)ethyl]-amino) benzoate

To a solution of 1-(tert.-butyldimethylsilyloxy)-2-iodoethane (45.10gm, 160mmol) in DMF (50mL) was added methyl 4-aminobenzoate (4.54gm, 30mmol) and diisopropylethylamine (6mL, 34mmol). This solution was heated at 95° for approximately 21 hours. The solvent was removed by concentration, *in vacuo*, and the residue obtained was partitioned between CH₂Cl₂ (150mL) and water (150mL). The layers were separated and the aqueous phase extracted with CH₂Cl₂ (150mL). The combined organic extracts were washed with 10% Na₂S₂O₃ (120mL), dried over Na₂SO₄ and concentrated, *in vacuo*, to give an orange oil which was purified by flash chromatography. Elution with hexane: EtOAc (9:1) provided the product as a colorless oil (3.85gm, 41% yield).

The following analyses indicated that the product was methyl 4-(N-[2-(tert.-butyldimethylsilyloxy)ethyl]-amino)benzoate:

-31-

NMR(CDCL₃) δ = 7.86(2H, d, J = 8.8Hz), 6.57(2H, d, J = 8.8Hz), 4.48(1H, broad), 3.85(3H, s), 3.82(2H, t, J = 5.2Hz), 3.27(2H, q, J = 5.2Hz), 0.90(9H, s), 0.06(6H, s)

Anal. (C₁₆H₂₇NO₃Si) C,H,N

(b) methyl 4-[N-(2-hydroxyethyl)amino]benzoate

A 1.0M solution of tetrabutylammonium fluoride in THF (30mL, 30mmol) was added to a solution of methyl 4-(N-[2-(tert.-butyldimethylsilyloxy)ethyl]amino)benzoate (3.67gm, 12mmol) in THF (50mL). This mixture was stirred for 18 hours at ambient temperature, then diluted with water (100mL) and extracted with ether (2 x 75mL) and EtOAc (75mL). The combined organic extracts were washed with brine (90mL), dried over Na₂SO₄ and concentrated, *in vacuo*, to give a yellow gum which was purified by flash chromatography. Elution with hexane: EtOAc (1:1) yielded 2.10gm (91%) of product as a white solid melting at 116° to 117°.

The following analyses indicated that the product was methyl 4-[N-(2-hydroxyethyl)amino]benzoate:

NMR(CDCL₃) δ = 7.85(2H, d, J = 8.8Hz), 6.58(2H, d, J = 8.8Hz), 4.52(1H, broad), 3.89-3.83(5H, m), 3.34(2H, q, J = 5.2Hz), 1.98(1H, t, J = 5.1Hz)

Anal. (C₁₀H₁₃NO₃) C,H,N

(c) methyl 4-(N-[2-(acetylthio)ethyl]amino)benzoate

A solution of triphenylphosphine (7.34gm, 28mmole) in THF (60mL) was cooled to 0°. Diethyl azidodicarboxylate (4.4mL, 28mmol) was added to this solution which was stirred at 0° for 30 minutes. To this mixture was added a solution of methyl 4-[N-(2-hydroxyethyl)amino]benzoate (2.73gm, 14mmol) and thiolacetic acid (2.0mL, 28mmol) in THF (30mL). The resultant mixture was stirred at 0° for 45 minutes, then for an additional 2 hours at ambient temperature. The solvent was then removed by concentration, *in vacuo*, and the residue obtained was purified by flash chromatography. Elution with hexane: EtOAc(4:1) yielded 1.87gm (53%) of product as a white solid melting at 90° to 91°.

-32-

The following analyses indicated that the product was methyl 4-(N-[2-(acetylthio)ethyl]amino)benzoate:

NMR (CDCL₃) δ = 7.86(2H, d, J = 8.8Hz), 6.58(2H, d, J = 8.8Hz), 4.47(1H, t, J = 6.5Hz), 3.84(3H, s), 3.39(2H, q, J = 6.5Hz), 3.10(2H, t, J = 6.5Hz), 2.37(3H, s)

Anal. (C₁₂H₁₅NO₃S) C,H,N,S

(d) methyl 4-[N-(2-thioethyl)amino]benzoate

To a solution of methyl 4-(N-[2-(acetylthio)ethyl]amino)-benzoate (1.95gm, 7.7mmol) in methanol (40mL) was added 15mL of 2N NaCl. This mixture was heated at reflux for 18 hours, then diluted with saturated NaHCO₃ (75mL) and ether (75mL). The layers were separated and the aqueous phase was extracted with ether (2 x 60mL). The combined organic extracts were dried over Na₂SO₄ and concentrated, *in vacuo*, to give a colorless oil which was purified by flash chromatography. Elution with CH₂Cl₂: hexane (4:1) yielded 1.17gm (72%) of product as a white solid melting at 46° to 47°

The following analyses indicated that the product was methyl 4-[N-2(-thioethyl)amino]benzoate:

NMR(CDCL₃) δ = 7.87(2H, d, J = 8.8Hz), 6.59(2H, d, J = 8.8Hz), 3.85(3H, s), 3.41(2H, t, J = 6.4Hz), 2.78(2H, dt, J = 8.3, 6.4Hz), 1.42(1H, t, J = 8.3Hz)

Anal. (C₁₀H₁₃NO₂S) C,H,N,S

(e) methyl 4-[N-(2-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)amino]benzoate

This material was prepared using the procedure described in example 2(g). From 1.96gm (9.3mmol) of methyl 4-[N-(2-thioethyl)amino] benzoate there were obtained 916mg (29%) of a beige solid melting at 253° (dec.)

The following analyses indicated that the product was methyl 4-[N-(2-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)amino]benzoate:

-33-

NMR(DMSO-d₆) δ = 10.11(1H, s), 7.66(2H, d, J = 8.7Hz), 6.55(2H, d, J = 8.7Hz), 6.43(2H, broad), 6.36(2H, br s), 3.72(3H, s), 3.16(2H, t, J = 6.4Hz), 2.59(2H, t, J = 6.4Hz)

Anal. (C₁₄H₁₇N₅O₃S·0.1H₂O·0.6H₃OH) C, H, N, S

(f) 4-[N-(2-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino]benzoic acid

This material was prepared using the procedure described in example 2(h). From 838mg (2.5mmol) of methyl 4-[N-(2-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino]benzoate there were obtained 786mg (98%) of an off-white solid melting at 263° (dec.)

The following analyses indicated that the product was 4-[N-(2-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino]benzoic acid:

NMR(DMSO-d₆) δ = 11.34(1H, broad), 7.93(2H, broad), 7.65(2H, d, J = 8.7Hz), 7.31(2H, broad), 6.55(2H, d, J = 8.7Hz), 3.20(2H, t, J = 6.7Hz), 2.65(2H, t, J = 6.7Hz)

Anal. (C₁₃H₁₅N₅O₃S·1.1 HCl) C, H, N, S

(g) diethyl N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)benzoyl]-S-glutamate

This material was prepared using the procedure described for example 2(i). From 707mg (2.2mmol) of 4-[N-(2-[2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino] benzoic acid there were obtained 215mg (19%) of a white solid melting at 173°-174°

The following analyses indicated that the product was diethyl N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)benzoyl]-S-glutamate:

NMR(DMSO-d₆) δ = 10.11(1H, s), 8.23(1H, d, J = 7.3Hz), 7.63(2H, d, J = 8.6Hz), 7.08(1H, broad), 6.54(2H, d, J = 8.6Hz), 6.42(2H, br s), 6.36(2H, br s), 4.35(1H, ddd, J = 5.6, 7.3, 12.8Hz), 4.07(2H, q, J = 7.0Hz), 4.03(2H, q, J = 7.0Hz), 3.16(2H, t, J = 6.4Hz), 2.59(2H, t, J = 6.4Hz), 2.40(2H, t, J = 7.4Hz), 2.09-1.91(2H, m), 1.17(3H, t, J = 7.0Hz), 1.15(3H, t, J = 7.0Hz)

-34-

Anal. ($C_{22}H_{30}N_6O_6S \cdot 0.6 CH_3OH$) C, H, N, S

(h) N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)benzoyl]-S-glutamic acid

This material was prepared using the procedure described for example 2(j). From 175mg (0.35mmol) of diethyl N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]amino)-benzoyl]-S-glutamate there were obtained 115mg (75%) of a white solid melting at 227° to 228° (dec.)

The following analyses indicated that the product was N-[4-(N-[2-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]-amino)benzoyl]-S-glutamic acid:

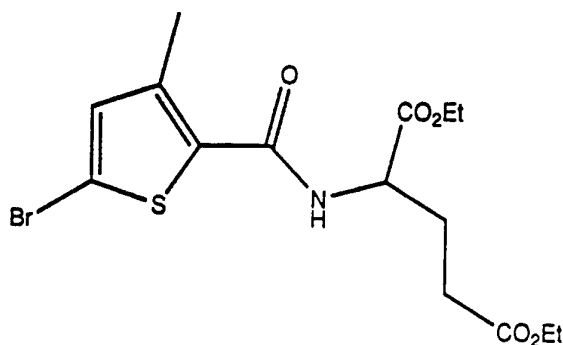
NMR(DMSO-d₆) δ = 12.25(2H, broad), 10.09(1H, broad), 8.09(1H, d, J = 7.7Hz), 7.61(2H, d, J = 8.6Hz), 6.51(2H, d, J = 8.6Hz), 6.42(2H, br s), 6.35(2H, br s), 4.30(1H, ddd, J = 5.3, 7.7, 12.6Hz), 3.13(2H, t, J = 6.4Hz), 2.56(2H, t, J = 6.4Hz), 2.29(2H, t, J = 7.3Hz), 2.05-1.95(1H, m), 1.93-1.83(1H, m)

Anal. ($C_{18}H_{22}N_6O_6S$) C, H, N, S

EXAMPLE 6

N-[5-(3-[2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio]propyl)-3-methyl-thieno-2-yl]-L-glutamic acid

(a) 2-[(5-Bromo-3-methyl-thiophene-2-carbonyl)-amino]-glutamic acid diethyl ester



-35-

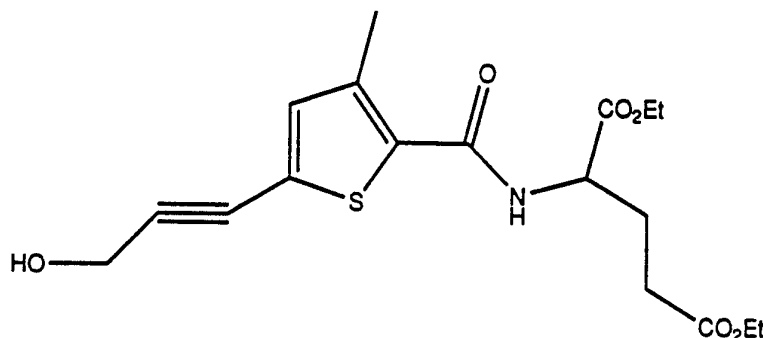
To a stirred solution of 10.86 g (49.1 mmol) of 5-Bromo-3-methyl-thiophene-2-carboxylic acid (prepared according to D. Spinelli, JCS Perkin Trans. II, 1972, 1866), 6.97 g (51.6 mmol) of 1-Hydroxybenzotriazole hydrate, 9.0 mL (51.7 mmol) of diisopropylethylamine and 12.36 g (51.6 mmol) of L-glutamic acid diethyl ester hydrochloride in 70 mL of DMF was added 9.89 g (51.6 mmol) of 1-(3-Dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride. The reaction mixture was stirred under Argon for 18 hours, poured into H₂O and extracted with ethyl acetate. The organic layer was washed sequentially with 0.5 N HCl, saturated NaHCO₃ soln and saturated NaCl soln, dried over MgSO₄, then concentrated under reduced pressure. This residue was purified by flash chromatography on silica gel eluting methylene chloride-ethyl acetate (20:1). In this manner, there was obtained 19.70 g (99%) of the desired product as a colorless oil.

IR (neat) 3329, 2982, 1738, 1651, 1545, 1514, 1417, 1377, 1258, 1206 cm⁻¹.

¹H NMR (CDCl₃) δ 1.24 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.04-2.45 (4H, m), 2.48 (3H, s), 4.12 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.71 (1H, ddd, J = 12.3, 7.2, 4.8 Hz), 6.56 (1H, d, J = 7.3 Hz), 6.87 (1H, s).

Anal. (calc. for C₁₅H₂₀BrNO₅S); C, H, Br, N, S.

(b) 2-([5-(3-Hydroxy-prop-1-ynyl)-3-methyl-thiophene-2-carbonyl]-amino)-glutamic acid diethyl ester



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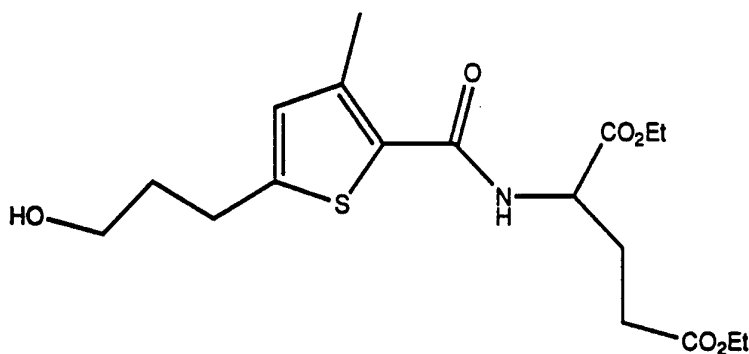
To a stirred solution of 13.73 g (33.8 mmol) of bromide 6(a) and 2.36 mL (40.5 mmol) of propargyl alcohol in 170 mL of diethylamine was added 0.47 g (0.7 mmol) of bis(triphenylphosphine) palladium (II) chloride and 0.13 g (0.7 mmol) of cuprous iodide. The reaction mixture was stirred under Argon for 18 hours. The volatiles were evaporated under reduced pressure and the brown residue was purified by flash chromatography on silica gel eluting methylene chloride-ethyl acetate (9:1). In this manner, there was obtained 12.36 g (96%) of the desired product as a yellow oil.

IR (neat) 3366, 2982, 2250, 1738, 1640, 1545, 1516, 1445, 1377 cm^{-1} .

^1H NMR (CDCl_3) δ 1.24 (3H, t, $J = 7.1$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.80 (1H, broad t), 2.04-2.46 (4H, m), 2.47 (3H, s), 4.12 (2H, q, $J = 7.0$ Hz), 4.24 (2H, q, $J = 7.1$ Hz), 4.50 (2H, d, $J = 5.2$ Hz), 4.73 (1H, m), 6.63 (1H, d, $J = 7.3$ Hz), 6.96 (1H, s).

Anal. (calc. for $\text{C}_{18}\text{H}_{23}\text{NO}_6\text{S}$); C, H, N, S.

(c) 2-([5-(3-Hydroxy-propyl)-3-methyl-thio-
phene-2-carbonyl]-amino)-glutamic acid diethyl ester



A Parr flask containing 10.32 g (27.1 mmol) of alkyne 6(b), 4.00 g of 5% Pd/C and 150 mL of ethanol was shaken under 45 psi of hydrogen for 2 hours. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. In this manner, there was obtained 9.80 g (94%) of the desired product as a colorless oil.

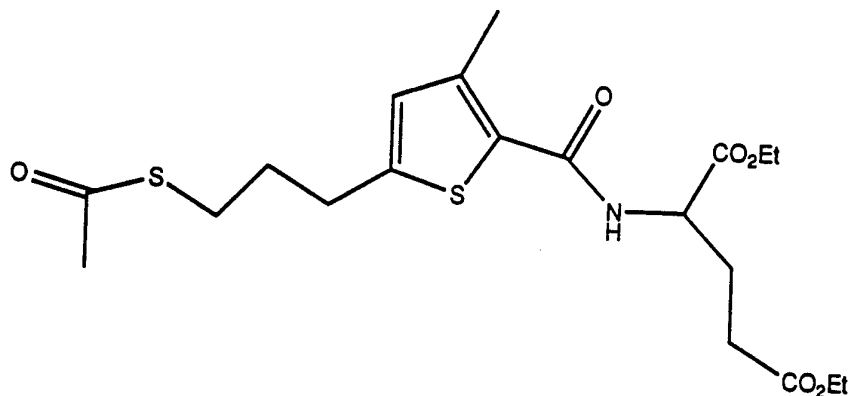
-37-

IR (neat) 3354, 2980, 2930, 1732, 1634, 1514, 1447, 1377 cm^{-1}

^1H NMR (CDCl_3) δ 1.23 (3H, t, $J = 7.1$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.93 (2H, pentet, $J = 7.2$ Hz), 2.08-2.46 (4H, m), 2.47 (3H, s), 2.88 (2H, t, $J = 7.6$ Hz), 3.70 (2H, t, $J = 6.3$ Hz), 4.11 (2H, q, $J = 7.1$ Hz), 4.23 (2H, q, $J = 7.1$ Hz), 4.75 (1H, m), 6.47 (1H, d, $J = 7.3$ Hz), 6.62 (1H, s).

Anal. (calc. for $\text{C}_{18}\text{H}_{27}\text{NO}_6\text{S}$); C, H, N, S.

(d) 2-[[5-(3-Acetylsulfanyl-propyl)-3-methyl-thio-phenene-2-carbonyl]-amino]-glutamic acid diethyl ester



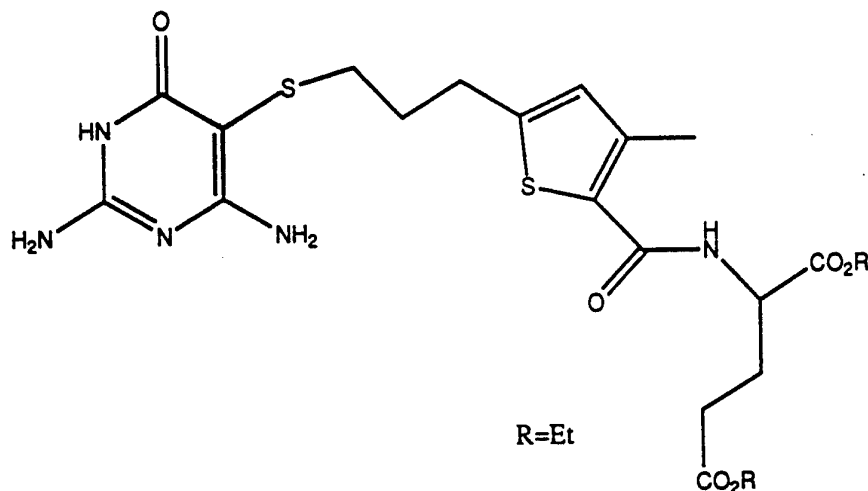
This material was prepared according to the procedure described in example 5(c) using alcohol 6(c). The product (67%) was isolated as a yellow oil by flash chromatography on silica gel eluting methylene chloride-ethyl acetate (20:1).

IR (neat) 3366, 2980, 2936, 1736, 1692, 1649, 1510, 1447, 1377 cm^{-1} .

^1H NMR (CDCl_3) δ 1.23 (3H, t, $J = 7.2$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.95 (2H, pentet, 7.3 Hz), 2.04-2.48 (4H, m), 2.34 (3H, s), 2.47 (3H, s), 2.83 (2H, t, $J = 7.4$ Hz), 2.91 (2H, t, $J = 7.2$ Hz), 4.11 (2H, q, $J = 7.1$ Hz), 4.23 (2H, q, $J = 7.1$ Hz), 4.75 (1H, m), 6.47 (1H, d, $J = 7.4$ Hz), 6.61 (1H, s).

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(e) N-[5-(3-[(2,6-Diamino-4(3H)-oxypyrimidin-5-yl)thiol propyl]-3-methyl-thieno-2-yl]-L-glutamic acid diethyl ester



A stirred solution of 0.40 g (0.90 mmol) of thioacetate 6(d) in 10 mL of ethanol saturated with dry HCl gas was heated at 50°C under Argon for 2 hours. The volatiles were removed under reduced pressure. The residue was dissolved in methylene chloride and re-concentrated to remove traces of HCl. This procedure was repeated twice. The resulting crude thiol and 0.18 g (0.90 mmol) of 5-Bromo-2,6-diamino-4(3H) pyrimidinone were dissolved in degassed DMF. To this solution was added 0.15 mL (0.90 mmol) of diisopropyl-ethylamine. After heating under Argon at 100°C for 2.5 hours, the cooled reaction mixture was poured into H₂O and extracted with ethyl acetate. The organic layer was washed twice with sat. NaCl soln, dried over MgSO₄ and concentrated at reduced pressure. The residue was flash chromatographed on silica gel eluting methylene chloride-methanol (9:1). In this manner, there was obtained 0.17 g (38%) of the desired product as a white solid.

mp 164-165 C

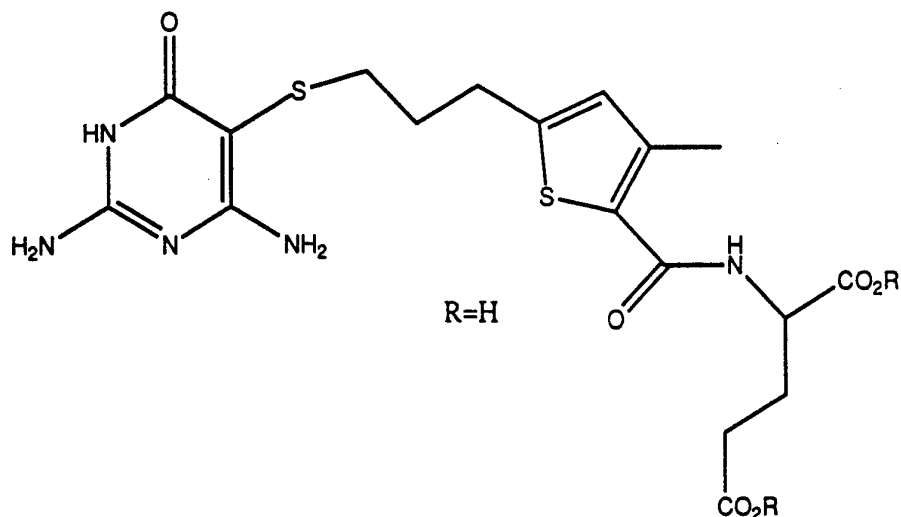
IR (KBr) 3329, 2930, 1734, 1636, 1597, 1518, 1441cm⁻¹.

¹H NMR (dmso-d₆) δ 1.16 (3H, t, J = 7.1 Hz), 1.18 (3H, t, J = 7.0 Hz), 1.71 (2H, m), 1.90-2.10 (2H, m), 2.32 (3H, s), 2.40 (4H, m), 2.86 (2H, t, J = 7.4 Hz), 4.07 (4H, m), 4.32 (1H, m), 6.30 (4H, broad s), 6.67 (1H, s), 8.13 (1H, d, J = 7.5 Hz), 9.95 (1H, s)

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Anal. (calc. for $C_{22}H_{31}N_5O_6S_2 \cdot 1.0 H_2O$); C, H, N, S.

(f) N-[5-(3-[2,6-Diamino-4(3H)-oxypyrimidin-5-yl]thio]propyl)-3-methyl-thieno-2-yl]-L-glutamic acid



This product was prepared according to the general procedure described in example 2(j) using diethyl ester 6(e). The product (82%) was collected as a white solid:

mp 217-220 C.

IR (KBr) 3341, 3200, 2922, 1709, 1620, 1516, 1468, 1263 cm^{-1} .

1H NMR (dmso- d_6) δ 1.71 (2H, pentet, $J = 7.2$ Hz), 1.74-2.05 (2H, m), 2.31 (2H, t, $J = 7.5$ Hz), 2.33 (3H, s), 2.46 (2H, t, $J = 6.9$ Hz), 2.86 (2H, t, $J = 6.9$ Hz), 4.28 (1H, m), 6.33 (4H, broad s), 6.66 (1H, s), 7.96 (1H, d, $J = 7.7$ Hz), 9.96 (1H, broad s).

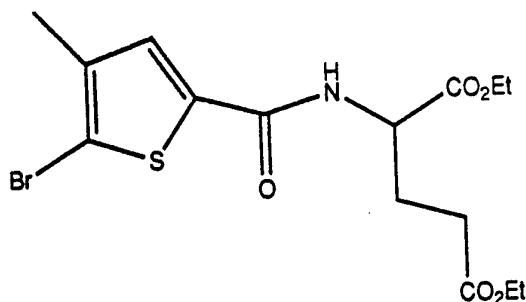
Anal. (calc. for $C_{18}H_{23}N_5O_6S_2 \cdot 1.3 H_2O$); C, H, N, S.

EXAMPLE 7

N-[5-(3-[2,6-Diamino-4(3H)-oxypyrimidin-5-yl]thio]propyl)-4-methyl-thieno-2-yl]-L-glutamic acid

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(a) 2-[(5-Bromo-4-methyl-thiophene-2-carbonyl)-amino]-glutamic acid diethyl ester



The starting 5-Bromo-4-methyl-thiophene-2-carboxylic acid was prepared according to M. Nemec, Collection Czechoslov. Chem. Commun. 39, 3527, (1974).

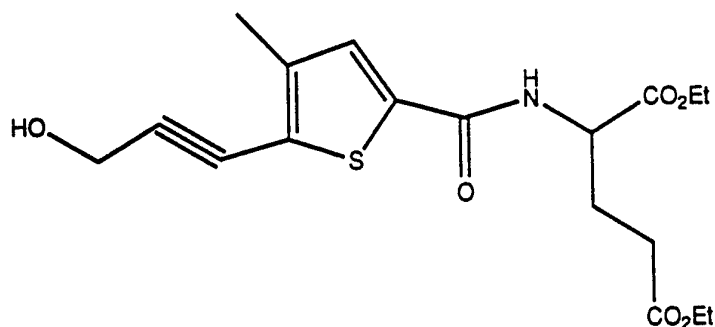
The titled compound was prepared according to the general procedure described in example 6(a) and isolated (89%) as a lt. yellow oil by flash chromatography on silica gel eluting methylene chloride-ethyl acetate (25:1).

IR(neat) 3339, 2984, 1738, 1634, 1562, 1527, 1425, 1209 cm^{-1} .

^1H NMR (CDCl_3) δ 1.24 (3H, t, $J = 7.1$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 2.04-2.52 (4H, m), 2.20 (3H, s), 4.12 (2H, q, $J = 7.3$ Hz), 4.23 (2H, q, $J = 7.3$ Hz), 4.70 (1H, ddd, $J = 12.3, 7.7, 4.8$ Hz), 6.84 (1H, d, $J = 7.4$ Hz), 7.22 (1H, s).

Anal. (calc. for $\text{C}_{15}\text{H}_{20}\text{BrNO}_5\text{S}$); C, H, Br, N, S.

(b) 2-[(5-(3-Hydroxy-prop-1-ynyl)-4-methyl-thiophene-2-carbonyl)-amino]-glutamic acid diethyl ester



This material was prepared according to the general procedure described in example 6(b) using the bromo compound 7(a). The product (94%) was isolated as a lt. yellow oil by

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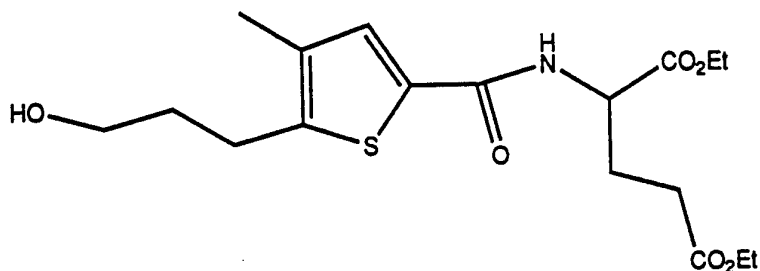
flash chromatography on silica gel eluting a gradient of 8-11% ethyl acetate in methylene chloride.

IR(neat) 3329, 2980, 2222, 1738, 1634, 1557, 1532, 1447 cm^{-1} .

^1H NMR (CDCl_3) δ 1.23 (3H, t, $J = 7.1$ Hz), 1.29 (3H, t, $J = 7.2$ Hz), 2.04 (3H, s), 2.07-2.50 (4H, m), 4.14 (2H, q, $J = 7.1$ Hz), 4.24 (2H, q, $J = 7.1$ Hz), 4.54 (2H, s), 4.71 (1H, m), 6.88 (1H, d, $J = 7.4$ Hz), 7.27 (1H, s).

Anal. (calc. for $\text{C}_{18}\text{H}_{23}\text{NO}_6\text{S}$); C, H, N, S.

(c) 2-([5-(3-Hydroxy-propyl)-4-methyl-thio-phenene-2-carbonyl]-amino)-glutamic acid diethyl ester



This material was prepared according to the general procedure described in example 6(c) using alkyne 7(b). The product (100%) was isolated as a colorless oil.

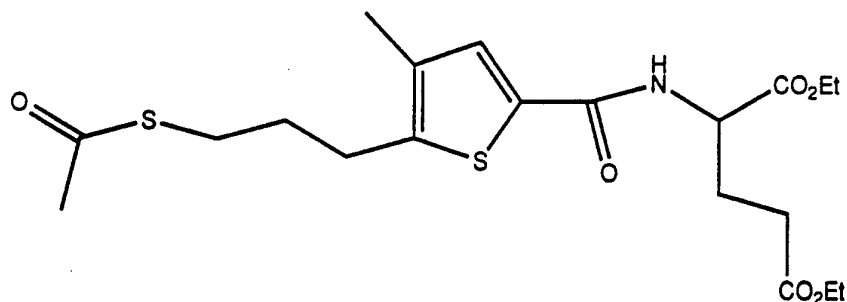
IR (neat) 3337, 2980, 2938, 1738, 1632, 1560, 1530, 1449 cm^{-1} .

^1H NMR (CDCl_3) δ 1.23 (3H, t, $J = 7.2$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.89 ((2H, pentet, $J = 7.2$ Hz), 2.06-2.49 (4H, m) 2.16 (3H, s), 2.85 (2H, t, $J = 7.5$ Hz), 3.70 (2H, t, $J = 6.3$ Hz), 4.11 (2H, q, $J = 7.1$ Hz), 4.23 (2H, q, $J = 7.2$ Hz), 4.74 (1H, m), 6.67 (1H, d, $J = 7.6$ Hz), 7.26 (1H, s).

Anal. (calc. for $\text{C}_{18}\text{H}_{27}\text{NO}_6\text{S}$); C, H, N, S.

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(d) 2-{[5-(3-Acetylsulfanyl-propyl)-4-methyl-thio-phenene-2-carbonyl]-amino}-glutamic acid diethyl ester



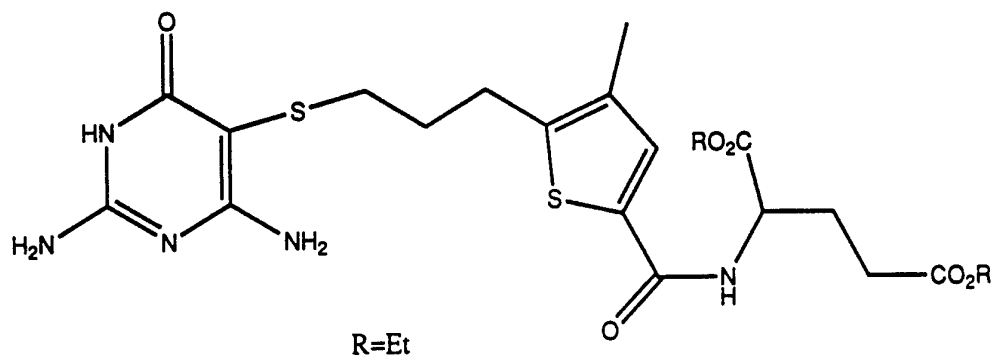
This material was prepared according to the general procedure described in example 5(c) using alcohol 7(c). The product (56%) was isolated as a yellow oil by flash chromatography on silica gel eluting ether-hexanes (2:1).

IR (neat) 3337, 2982, 2938, 1736, 1694, 1634, 1526, 1449, 1206 cm^{-1} .

^1H NMR (CDCl_3) δ 1.20 (3H, t, $J = 7.1$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.91 (2H, pentet, $J = 7.4$ Hz), 2.08-2.49 (4H, m), 2.15 (3H, s), 2.34 (3H, s), 2.80 (2H, t, $J = 7.4$ Hz), 2.91 (2H, t, $J = 7.2$ Hz), 4.13 (2H, q, $J = 7.1$ Hz), 4.24 (2H, q, $J = 7.2$ Hz), 4.74 (1H, m), 6.67 (1H, d, $J = 7.6$ Hz), 7.26 (1H, s).

Anal. (calc. for $\text{C}_{20}\text{H}_{29}\text{NO}_6\text{S}_2$); C, H, N, S.

(e) N-[5-(3-[(2,6-Diamino-4(3H)-oxopyrimidin-5-yl)thio]propyl)-4-methyl-thieno-2-yl]-L-glutamic acid diethyl ester



This material was prepared according to the general procedure described in example 6(e) using thio acetate 7(d).

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The product (48%) was isolated as a white solid by flash chromatography eluting methylene chloride-methanol (9:1).

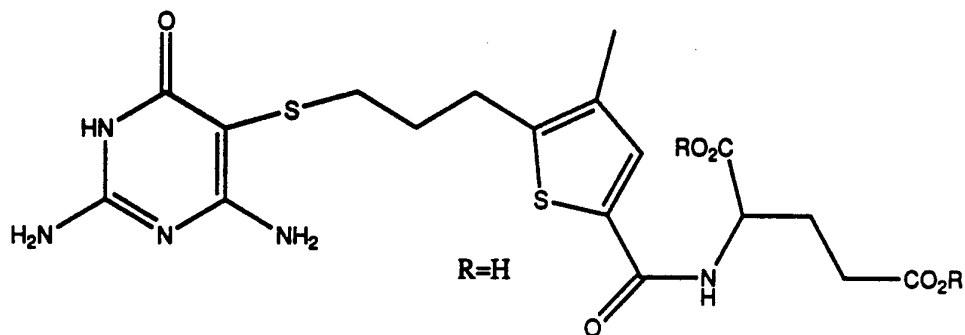
mp 159-160 C.

IR (KBr) 3324, 2980, 1734, 1657, 1632, 1603, 1466, 1206 cm^{-1} .

^1H NMR ($\text{dmsO}-d_6$) δ 1.15 (3H, t, $J = 7.1$ Hz), 1.17 (3H, t, $J = 7.1$ Hz), 1.68 (2H, m), 2.01 (2H, m), 2.11 (3H, s), 2.43 (4H, m), 2.85 (2H, t, $J = 7.3$ Hz), 4.04 (2H, q, $J = 7.1$ Hz), 4.12 (2H, q, $J = 7.1$ Hz), 4.34 (1H, m), 6.31 (4H, broad s), 7.55 (1H, s), 8.52 (1H, d, $J = 7.5$ Hz), 9.95 (1H, s).

Anal. (calc. for $\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_6\text{S}$); C, H, N, S.

(f) N-[5-(3-[2,6-Diamino-4(3H)-oxopyrimidin-5-yl] thio] propyl)-4-methyl-thieno-2-yl]-L-glutamic acid



This product was prepared according to the general procedure described in example 2(j) using diethyl ester 7(e). The product (87%) was collected by filtration as a white solid.

mp 154-158 C.

IR (KBr) 3322, 3179, 2922, 1705, 1632, 1564, 1445 cm^{-1} .

^1H NMR ($\text{dmsO}-d_6$) δ 1.68 (2H, m), 1.92-2.01 (2H, m), 2.10 (3H, s), 2.31 (2H, t, $J = 7.4$ Hz), 2.47 (2H, t, $J = 7.4$ Hz), 2.82 (2H, t, $J = 7.4$ Hz), 4.29 (1H, m), 6.29 (4H, broad s), 7.53 (1H, s), 8.36 (1H, d, $J = 7.7$ Hz), 9.90 (1H, broad s).

Anal. (calc. for $\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_6\text{S}_2 \cdot 0.40\text{H}_2\text{O}$); C, H, N, S.

EXAMPLE 8Synthesis of N-(6-[[[2,6-diamino-4(3H)oxopyrimidino-5-yl]-thio)methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl)-S-glutamic acid

This compound of Formula II, wherein A = S, group = CH₂, ring = 6,2-(4,5,6,7-tetrahydrobenzothienyl) and R₁, R₂ and R₃ = H, was synthesized via the following process.

(a). bromomethyl 3-thienyl ketone

To a chilled solution of 3-acetylthiophene (12.62 g, 100 mmol) in THF (350 mL) was added phenyltrimethylammonium tribromide (39.10 g, 104 mmol). This mixture was left standing, with occasional swirling, for 2 hours at 0°. The precipitate was removed by filtration and washed with ether (2x75 mL). The combined filtrates were poured into a mixture of saturated NaHCO₃ (200 mL) and 10% Na₂S₂O₃ (200 mL). The layers were separated and the aqueous phase extracted with EtOAc (150 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated, *in vacuo*, to give a brown oil which was purified by flash chromatography. Elution with hexane: CH₂Cl₂ (2:1) provided the product as a white solid (15.54 g, 76% yield) melting at 60°-61°.

The following analyses indicated that the product was bromomethyl 3-thienyl ketone.

NMR (CDCl₃) δ = 8.18 (1H, dd, J = 1.3, 2.9 Hz), 7.58 (1H, dd, J = 1.3, 5.1 Hz), 7.37 (1H, dd, J = 2.9, 5.1 Hz), 4.34 (2H, s)

Anal. (C₆H₅OSBr) C, H, S, Br

(b). diethyl 2-[2-oxo-2-(3-thienyl)ethyl] malonate

Diethyl malonate (6.5 mL, 6.86 g, 42.8 mmol) was added dropwise to a suspension of sodium hydride (1.66 g, 41.5 mmol) in THF (15 mL) under argon at 0°. After stirring at 0° for 15 minutes, a solution of bromomethyl 3-thienyl ketone 8(a) (5.20 g, 25.4 mmol) in THF (60 mL) was added to this mixture.

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The resultant, yellow slurry was stirred at ambient temperature for 1 hour, then diluted with EtOAc (100 mL) and poured into water (150 mL). The layers were separated and the aqueous phase extracted with EtOAc (2x100 mL). The combined organic extracts were washed with brine (100 mL), dried over MgSO_4 and concentrated, *in vacuo*, to give a yellow oil which was purified by flash chromatography. Elution with hexane:EtOAc (5:1) provided the product as a pale yellow oil (5.63 g, 78%).

The following analyses indicated that the product was diethyl 2-[2-oxo-2-(3-thienyl)ethyl] malonate.

NMR (CDCl_3) δ = 8.12 (1H, dd, J = 1.2, 2.9 Hz), 7.55 (1H, dd, J = 1.2, 5.1 Hz), 7.33 (1H, dd, J = 2.9, 5.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.04 (1H, t, J = 7.2 Hz), 3.54 (2H, d, J = 7.2 Hz), 1.29 (6H, t, J = 7.1 Hz)

Anal. ($\text{C}_{13}\text{H}_{16}\text{O}_5\text{S}$) C, H, S

(c). 2-[2-oxo-2-(3-thienyl)ethyl] malonic acid

Diethyl 2-[2-oxo-2-(3-thienyl) ethyl] malonate 8(b) (5.39 g, 19 mmol) was suspended in 10% KOH (50 mL) and left stirring at ambient temperature for 18 hours. The resultant solution was acidified to pH 1 by addition of 6N HCL and the precipitate that formed was collected by filtration to provide the product (4.22 g, 98%) as an off-white solid melting at 161°-162° (dec.).

The following analyses indicated that the product was 2-[2-oxo-2-(3-thienyl)ethyl] malonic acid.

NMR (DMSO-d_6) δ = 12.85 (2H, broad), 8.59 (1H, dd, J = 1.2, 2.8 Hz), 7.62 (1H, dd, J = 2.8, 5.1 Hz), 7.49 (1H, dd, J = 1.2, 5.1 Hz), 3.75 (1H, t, J = 7.2 Hz), 3.44 (2H, d, J = 7.2 Hz)

(d). 4-(3-thienyl) butyric acid

Hydrazine hydrate (1.3 mL, 1.34 g, 26.8 mmol) was added dropwise to a solution of KOH (3.54 g, 63 mmol) and 2-[2-oxo-2-(3-thienyl) ethyl] malonic acid 8(c) (4.00 g, 17.5

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mmol) in ethylene glycol (30 mL). This solution was heated at reflux for 6 hours. After cooling to room temperature, the crude reaction mixture was poured into a mixture of 6N HCl (50 mL) and ice (200 g). This aqueous mixture was saturated with NaCl, then extracted with ether (3x70 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated, *in vacuo*, to provide an orange oil (2.61 g) of sufficient purity to be employed in the subsequent reaction without any further purification.

(e). 7-oxo-4,5,6,7-tetrahydrobenzothiophene

To a solution (8% by wt) of P_2O_5 in methanesulfonic acid (80 mL) was added the crude 4(3-thienyl) butyric acid 8(d) (2.61 g, 15.3 mmol). The resultant reaction mixture was stirred at ambient temperature for 90 minutes, then cautiously poured into water (450 mL). After cooling to room temperature, this aqueous mixture was extracted with EtOAc (3x200 mL). The combined organic extracts were washed with saturated NaHCO_3 (2x150 mL) and brine (150 mL), dried over MgSO_4 and concentrated, *in vacuo*, to give a brown oil which was purified by flash chromatography. Elution with hexane:EtOAc (4:1) provided the product as a yellow oil (1.65 g, 71%).

The following analyses indicated that the product was 7-oxo-4,5,6,7-tetrahydrobenzothiophene.

NMR (CDCl_3) δ = 7.61 (1H, d, J = 4.9 Hz), 6.97 (1H, d, J = 4.9 Hz), 2.88 (2H, t, J = 6.1 Hz), 2.61 (2H, t, J = 6.5 Hz), 2.18 (2H, tt, J = 6.5 Hz)

Anal. ($\text{C}_8\text{H}_8\text{OS}$) C, H, S

(f). methyl-7-oxo-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate

A solution of 7-oxo-4,5,6,7-tetrahydrobenzothiophene 8(e) (1.37 g, 9 mmol) in DMF (10 mL) was added dropwise, under an argon atmosphere, to a suspension of NaH (800 mg, 20 mmol) in DMF (6 mL). The resultant, purple solution was stirred at ambient temperature for 15 minutes, then cooled to 0° prior

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to the dropwise addition of dimethyl carbonate (5 mL, 5.35 g, 59 mmol). The resultant reaction mixture was stirred at ambient temperature for 90 minutes, then poured into water (150 mL) and extracted with ether (3x50 mL) and EtOAc (50 mL). The combined organic extracts were dried over MgSO_4 and concentrated, *in vacuo*, to give an orange oil which was purified by flash chromatography. Elution with hexane:EtOAc (4:1) provided the product as a yellow oil (1.28 g, 68%)

The following analyses indicated that the product was methyl 7-oxo-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate.

NMR (CDCl_3) δ = 7.66 (1H, d, J = 4.8 Hz), 6.97 (1H, d, J = 4.8, Hz), 3.77 (3H, s), 3.61 (1H, dd, J = 4.8, 9.0 Hz), 3.05 (1H, ddd, J = 4.9, 6.5, 17.1 Hz), 2.87 (1H, ddd, J = 4.9, 8.2, 17.1 Hz), 2.62-2.50 (1H, m), 2.43-2.33 (1H, m)

Anal. ($\text{C}_{10}\text{H}_{10}\text{O}_3\text{S}$) C, H, S

(g). methyl 7-hydroxy-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate and 7-hydroxy-6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzothiophene

To a solution of methyl 7-oxo-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate 8(f) (2.57 g, 12.2 mmol) in THF (15 mL) and CH_3OH (10 mL) at 0° was added, portionwise, NaBH_4 (465 mg, 12.2 mmol). The resultant reaction mixture was stirred for 2 hours, gradually warming to 15° , then poured into saturated NH_4Cl (30 mL). The layers were separated and the aqueous phase extracted with EtOAc (30 mL). The combined organic extracts were dried over MgSO_4 and concentrated, *in vacuo*, to give a yellow oil which was purified by flash chromatography. Elution with hexane:EtOAc (3:2) provided two separate products. The faster-eluting product was a yellow oil (0.91 g, 35%).

The following analyses indicated that this product was methyl 7-hydroxy-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate.

NMR (CDCl_3) δ = 7.25 (1H, d, J = 5.1 Hz), 6.78 (1H, d, J = 5.1 Hz), 5.23-5.16 (1H, m), 3.78 (3H, s), 3.09 (1H, broad), 2.90-2.57 (3H, m), 2.38-2.05 (2H, m)

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Anal. ($C_{10}H_{12}O_3S$) C, H, S

The slower-eluting product was a milky-white gum (1.28 g, 57%)

The following analyses indicated that this second product was 7-hydroxy-6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzo-thiophene.

NMR ($CDCl_3$) δ = 7.24 (1H, d, J = 5.1 Hz), 6.79 (1H, d, J = 5.1 Hz), 5.03-4.85 (1H, m), 3.96-3.79 (2H, m), 2.86-2.54 (3H, m), 2.07-1.94 (1H, m), 1.91-1.80 (1H, m), 1.77-1.71 (1H, m), 1.63-1.48 (1H, m)

Anal. ($C_9H_{12}O_2S$) C, H, S

(h). methyl 4,5,6,7-tetrahydrobenzothiophene-6-carboxylate

To a solution of methyl 7-hydroxy-4,5,6,7-tetrahydrobenzo-thiophene-6-carboxylate 8(g) (459 mg, 2.2 mmol) and Et_3SiH (0.7 mL, 510 mg, 4.4 mmol) in CH_2Cl_2 (10 mL), under argon at -5° , was added $BF_3 \cdot Et_2O$ (0.55 mL, 635 mg, 4.5 mmol). The resultant reaction mixture was stirred for 3 hours, gradually warming to 15° , then poured into saturated $NaHCO_3$ (30 mL). After addition of K_2CO_3 (~1 g), the layers were separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL) and ether (2x15 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, *in vacuo*, to give a yellow oil which was purified by flash chromatography. Elution with hexane:EtOAc (95:5) provided the product as a colourless oil (300 mg, 71%).

The following analyses indicated that the product was methyl 4,5,6,7-tetrahydrobenzothiophene-6-carboxylate.

NMR ($CDCl_3$) δ = 7.07 (1H, d, J = 5.1 Hz), 6.75 (1H, d, J = 5.1 Hz), 3.73 (3H, s), 3.11-2.94 (2H, m), 2.85-2.59 (3H, m), 2.27-2.18 (1H, m), 1.94-1.81 (1H, m)

Anal. ($C_{10}H_{12}O_2S$) C, H, S

(i). 6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzothiophene

Method A:

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A solution of methyl 4,5,6,7-tetrahydrobenzothio-
phene-6-carboxylate 8(h) (209 mg, 1.1 mmol) in THF (6 mL) was
added to a slurry of LiAlH_4 (50 mg, 1.3 mmol) in THF (3 mL).

The resultant reaction mixture was heated at reflux for
3 hours. After cooling to room temperature, the crude
reaction mixture was diluted with saturated NH_4Cl (20 mL).
The layers were separated and the aqueous phase extracted
with ether (10 mL), then with EtOAc (2x10 mL). The combined
organic extracts were dried over MgSO_4 and concentrated, *in vacuo*,
to provide the product as a colourless oil (167 mg,
93%).

Method B:

To a solution of 7-hydroxy-6-(hydroxymethyl)-
4,5,6,7-tetrahydrobenzothiophene (988 mg, 5.4 mmol) and
 Et_3SiH (1.8 mL, 1.31 g, 11.3 mmol) in CH_2Cl_2 (25 mL), under
argon at -5° , was added $\text{BF}_3\text{-Et}_2\text{O}$ (1.4 mL, 1.62 g, 11.3 mmol).
The resultant reaction mixture was stirred for 3 hours,
gradually warming to 10° , then poured into saturated NaHCO_3
(50 mL). After addition of K_2CO_3 (1.5 g), the layers were
separated and the aqueous phase extracted with ether (2x40
mL). The combined organic extracts were dried over MgSO_4 and
concentrated, *in vacuo*, to give a yellow oil which was
purified by flash chromatography. Elution with hexane:EtOAc
(2:1) provided the product as a colourless oil (593 mg, 66%).

The following analyses indicated that the product was
6-(hydroxymethyl)-5,5,6,7-tetrahydrobenzothiophene.

NMR (CDCl_3) δ = 7.06 (1H, d, J = 5.1 Hz), 6.76 (1H, d,
 J = 5.1 Hz), 3.66 (2H, d, J = 6.4 Hz), 2.94 (1H, dd, J = 5.2,
16.2 Hz), 2.79-2.71 (1H, m), 2.68-2.46 (2H, m), 2.11-1.96
(2H, m), 1.54-1.46 (1H, m)

Anal. ($\text{C}_9\text{H}_{12}\text{OS}$) C, H, S

(j). 6-[(t.-butyldimethylsilyloxy) methyl]-4,5,6,7-
tetra-hydrobenzothiophene

To a solution of t-butyldimethylsilyl chloride (4.38 g,
29.1 mmol) in CH_2Cl_2 (25 mL) was added Et_3N (4.1 mL, 2.98 g,

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29.4 mmol) followed by a solution of 6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzothiophene 8(i) (4.44 g, 26.4 mmol) in CH_2Cl_2 (50 mL) and 4-(dimethylamino) pyridine (100 mg). The resultant reaction mixture was stirred at ambient temperature for 18 hours, then poured into water (100 mL). The layers were separated and the organic phase was washed with 0.5 N HCl (100 mL) and brine (100 mL), then dried over MgSO_4 and concentrated, *in vacuo*, to give a yellow oil which was purified by flash chromatography. Elution with hexane:EtOAc (95:5) provided the product as a colourless oil (6.49 g, 86%).

The following analyses indicated that the product was 6-[(t-butyldimethylsilyloxy)methyl]-4,5,6,7-tetrahydrobenzothiophene.

NMR (CDCl_3) δ = 7.05 (1H, d, J = 5.1 Hz), 6.75 (1H, d, J = 5.1 Hz), 3.60 (2H, d, J = 6.2 Hz), 2.89 (1H, dd, J = 5.2, 16.2 Hz), 2.76-2.68 (1H, m), 2.65-2.42 (2H, m), 2.03-1.92 (2H, m), 1.53-1.41 (1H, m), 0.91 (9H, s), 0.06 (6H, s)

Anal. ($\text{C}_{15}\text{H}_{26}\text{OSSi}$) C, H, S

(k). diethyl N-(6-[(t-butyldimethylsilyloxy)methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate

To a solution of 6-[(t-butyldimethylsilyloxy)methyl]-4,5,6,7-tetrahydrobenzothiophene 8(j) (7.69 g, 27.2 mmol) in THF (100 mL), under argon at -70° , was added 2.5 M n-butyllithium in hexane (12 mL, 30 mmol). The resultant reaction mixture was stirred for an additional 40 minutes at -70° , then at -10° for 45 minutes while dry CO_2 was bubbled through the solution. The crude reaction mixture was subsequently poured into saturated NH_4Cl (300 mL). The layers were separated and the aqueous phase was extracted with ether (2x150 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated, *in vacuo*, to give a yellow solid (8.24 g). This intermediate was employed in the subsequent reaction without any further purification.

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To a solution of the aforementioned crude 6-[(t.-butyldimethylsilyloxy) methyl]-4,5,6,7-tetrahydrobenzo-thiophene-2-carboxylic acid (8.24 g), 1-hydroxybenzo-triazole (4.05 g, 30 mmol) and glutamic acid diethyl ester hydrochloride (7.19 g, 30 mmol) in DMF (65 mL) were added diisopropyl-ethylamine (5.2 mL, 3.86 g, 30 mmol) and 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (5.75 g, 30 mmol). The resultant solution was stirred at ambient temperature for 16 hours, then poured into brine (400 mL), diluted with water (150 mL) and extracted with ether (3x250 mL). The combined organic extracts were dried over MgSO_4 and concentrated, *in vacuo*, to give a yellow oil which was purified by flash chromatography. Elution with hexane:EtOAc (4:1) provided the product as a yellow oil (6.63 g, 48%).

The following analyses indicated that the product was diethyl N-(6-[(t.-butyldimethylsilyloxy) methyl]-4,5,6,7-tetra-hydrobenzothieno-2-yl) glutamate.

NMR (CDCl_3) δ = 7.23 (1H, s), 6.67 (1H, d, J = 7.7 Hz), 4.73 (1H, ddd, J = 4.8, 7.7, 12.6 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.59 (2H, d, J = 6.2 Hz), 2.89 (1H, dd, J = 5.1, 16.7 Hz), 2.75-2.23 (6H, m), 2.15-1.91 (3H, m), 1.56-1.42 (1H, m), 1.29 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz), 0.90 (9H, s), 0.06 (6H, s)

Anal. ($\text{C}_{25}\text{H}_{41}\text{NO}_6\text{SSi}$) C, H, N, S

(1). diethyl N-[6-(hydroxymethyl)-4,5,6,7-tetra-hydrobenzothieno-2-yl] glutamate

This material was prepared using the procedure described in example 5(b). From diethyl N-(6-[(t.-butyldimethylsilyloxy) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate 8(k) (6.03 g, 11.8 mmol) there was obtained a yellow gum (3.62 g, 77%).

The following analyses indicated that the product was diethyl N-[6-(hydroxymethyl)-4,5,6,7-tetrahydro-benzothieno-2-yl] glutamate.

-52-

NMR (CDCl_3) δ = 7.23 (1H, s), 6.70 (1H, d, J = 7.6 Hz), 4.73 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.66 (2H, d, J = 6.3 Hz), 2.94 (1H, dd, J = 5.0, 16.9 Hz), 2.77-2.22 (6H, m), 2.15-1.97 (3H, m), 1.57-1.44 (1H, m), 1.29 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz)

Anal. ($\text{C}_{19}\text{H}_{27}\text{NO}_6\text{S}\cdot 0.5\text{H}_2\text{O}$) C, H, N, S

(m). diethyl N-[6-(bromomethyl)-4,5,6,7-tetrahydro-benzothieno-2-yl] glutamate

This material was prepared using the procedure described in example 2(d). From diethyl N-[6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate 8(l) (3.45 g, 8.7 mmol) there was obtained a colourless gum (3.68 g, 92%).

The following analyses indicated that the product was diethyl N-[6-(bromomethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate.

NMR (CDCl_3) δ = 7.23 (1H, s), 6.73 (1H, d, J = 7.6 Hz), 4.73 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 4.23 (2H, q, J = 7.2 Hz), 4.10 (2H, q, J = 7.2 Hz), 3.49-3.41 (2H, m), 3.04 (1H, dd, J = 5.0, 16.8 Hz), 2.79-2.03 (9H, m), 1.67-1.53 (1H, m), 1.29 (3H, t, J = 7.2 Hz), 1.23 (3H, t, J = 7.2 Hz)

Anal. ($\text{C}_{19}\text{H}_{26}\text{NO}_5\text{SBr}$) C, H, N, S, Br

(n). diethyl N-(6-[(acetylthio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate

This material was prepared by using the procedure described in example 2(e). From diethyl N-[6-(bromomethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate 8(m) (3.68 g, 8.0 mmol) there was obtained an orange oil (3.46 g, 95%).

The following analyses indicated that the product was diethyl N-(6-[(acetylthio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate.

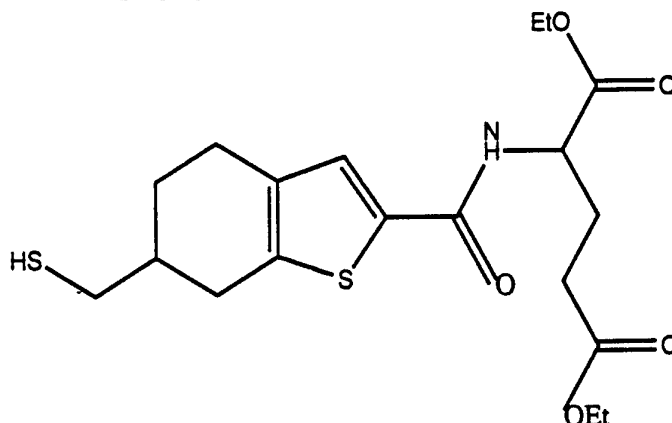
NMR (CDCl_3) δ = 7.21 (1H, s), 6.69 (1H, d, J = 7.6 Hz), 4.72 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.02-2.92 (3H, m), 2.74-2.38

-53-

(5H, m), 2.36 (3H, s), 2.35-2.22 (1H, m), 2.15-1.96 (3H, m), 1.57-1.46 (1H, m), 1.29 (3H, t, $J = 7.1$ Hz), 1.23 (3H, t, $J = 7.1$ Hz)

Anal. ($C_{21}H_{29}NO_6S_2 \cdot 0.5H_2O$) C, H, N, S

(o). diethyl N-[6-(thiomethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate



A solution of diethyl N-(6-[(acetylthio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate 8(n) (3.52 g, 7.7 mmol) in 0.5 N ethanolic HCl (60 mL) was heated at reflux for 3 hours. The reaction was diluted with water (25 mL) and the ethanol was removed by concentration, *in vacuo*. The aqueous residue was extracted with ether (2x50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated, *in vacuo*, to provide the product as an orange syrup (3.09 g, 97%).

The following analyses indicated that the product was diethyl N-[6-(thiomethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate.

NMR ($CDCl_3$) δ = 7.23 (1H, s), 6.69 (1H, d, $J = 7.6$ Hz), 4.73 (1H, ddd, $J = 4.8, 7.6, 12.6$ Hz), 4.23 (2H, q, $J = 7.1$ Hz), 4.12 (2H, q, $J = 7.1$ Hz), 3.04 (1H, dd, $J = 5.0, 16.6$ Hz), 2.77-2.23 (8H, m), 2.15-1.92 (3H, m), 1.59-1.48 (1H, m), 1.38 (1H, t, $J = 8.3$ Hz), 1.30 (3H, t, $J = 7.1$ Hz), 1.23 (3H, t, $J = 7.1$ Hz)

Anal. ($C_{19}H_{27}NO_5S_2 \cdot 0.25H_2O$) C, H, N, S

-54-

(p). diethyl N-(6-([2,6-diamino-4(3H)-oxopyrimidin-5-yl]thio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate

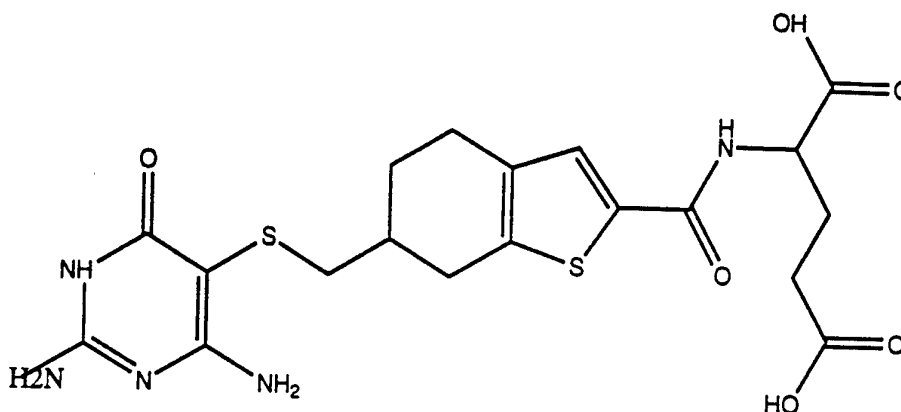
This material was prepared using the procedure described in example 2(g). From diethyl N-[6-(thiomethyl)-4,5,6,7-tetrahydrobenzothieno-2-yl] glutamate 8(o) (3.00 g, 7.3 mmol) and 5-bromo-2,6-diamino-4(3H)-pyrimidinone (1.44 g, 7.0 mmol) there was obtained a yellow solid (712 mg, 19%) melting at 122°-128°.

The following analyses indicated that the product was diethyl N-(6-([2,6-diamino-4(3H)-oxopyrimidin-5-yl] thio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate.

NMR (DMSO-d₆) δ = 9.92 (1H, s), 8.53 (1H, d, J = 7.5 Hz), 7.54 (1H, s), 6.28 (4H, br s), 4.34 (1H, ddd, J = 5.3, 7.5, 9.4 Hz), 4.08 (2H, q, J = 7.1 Hz), 4.03 (2H, q, J = 7.1 Hz), 3.07 (1H, dd, J = 4.4, 16.6 Hz), 2.66-2.43 (5H, m), 2.40 (2H, t, J = 7.4 Hz), 2.09-1.89 (3H, m), 1.83-1.73 (1H, m), 1.48-1.37 (1H, m), 1.17 (3H, t, J = 7.1 Hz), 1.15 (3H, t, J = 7.1 Hz)

Anal. (C₂₃H₃₁N₅O₆S₂) C, H, N, S

(q). N-(6-([2,6-diamino-4(3H)-oxopyrimidin-5-yl] thio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamic acid



This material was prepared using the procedure described in example 2(j). From diethyl N-(6-([2,6-diamino-4(3H)-oxopyrimidin-5-yl] thio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamate 8(p) (594 mg, 1.1 mmol) there was

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obtained a white solid (493 mg, 93%) melting at 227°-230° (dec.).

The following analyses indicated that the product was N-(6-[[[2,6-diamino-4(3H)-oxypyrimidin-5-yl] thio) methyl]-4,5,6,7-tetrahydrobenzothieno-2-yl) glutamic acid.

NMR (DMSO-d₆) δ = 12.38 (2H, broad), 10.08 (1H, broad), 8.42 (1H, d, J = 7.8 Hz), 7.53 (1H, s), 6.44 (4H, br s), 4.30 (1H, ddd, J = 4.6, 7.8, 12.6 Hz), 3.07 (1H, dd, J = 4.3, 16.6 Hz), 2.66-2.36 (5H, m), 2.31 (2H, t, J = 7.3 Hz), 2.10-1.77 (4H, m), 1.46-1.37 (1H, m)

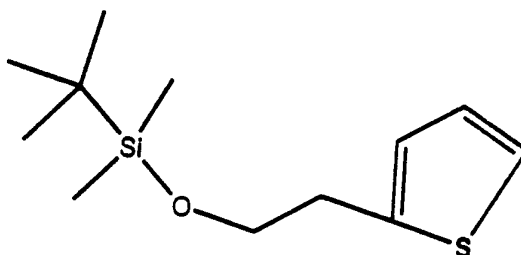
Anal. (C₁₉H₂₃N₅O₆S₂·0.7H₂O) C, H, N, S

EXAMPLE 9

Synthesis of N-(5-[2-([2,6-Diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]thieno-2-yl)-L-glutamic acid

This compound of formula X, wherein Ar is unsubstituted thienylene, A is sulfur and R₁, R₂ and R₃ are all hydrogen, was synthesized by the following process.

9a 2-[2-(t-Butyldimethylsilyloxy)ethyl]thiophene



To a solution of t-butyldimethylsilyl chloride (26.38 g, 0.18 mol), triethylamine (25 mL, 0.18 mol) and 4-(dimethylamino)pyridine (300 mg, 2.5 mmol) in CH₂Cl₂ (200 mL) under an argon atmosphere at -5°C was added, dropwise, 2-(2-thienyl)ethanol (18 mL, 0.16 mol). The resultant reaction mixture was stirred at 0°C for 30 minutes, then at room temperature overnight. The crude reaction mixture was poured into water (300 mL), and the layers were separated.

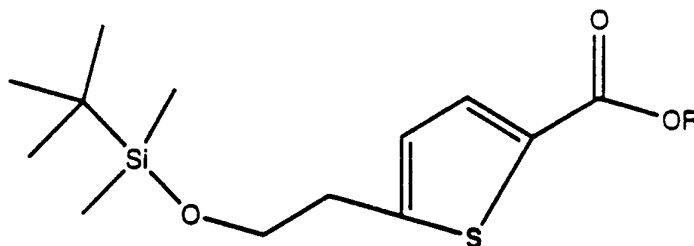
-56-

The organic phase was washed with 0.5N HCl (200 mL), then with brine (200 mL), dried over Na_2SO_4 and concentrated, in vacuo, to give a yellow oil, contaminated with a white solid, which was purified by flash chromatography. Elution with hexane/ethyl acetate (95.:5) yielded the product as a yellow oil (38.73 g, 99% yield).

NMR (CDCl_3) δ = 7.13 (1H, d, J = 5.1 Hz), 6.92 (1H, dd, J = 3.3, 5.1 Hz), 6.83 (1H, d, J = 3.3 Hz), 3.82 (2H, t, J = 6.7 Hz), 3.03 (2H, t, J = 6.7 Hz), 0.89 (9H, s), 0.03 (6H, s)

Anal. ($\text{C}_{12}\text{H}_{22}\text{O SSi}$)C,H,S

9b) 5-[2(t-Butyldimethylsilyloxy)ethyl]thiophene-2-carboxylic acid and Methyl 5-(2-hydroxyethyl)thiophene-2-carboxylate



A 1.6M solution of n-butyllithium in hexane (140 mL, 0.22 mol) was added to a solution of 36.16 g (0.15 mol) of 9a in 350 mL of THF under argon at -75°C . The resultant reaction mixture was stirred for 1 hour at -70°C . Dry CO_2 was then bubbled through this solution for 40 minutes at -65°C , then for 60 minutes at -5°C and finally for an additional 75 minutes while warming to room temperature. The crude reaction mixture was poured into a mixture of saturated NH_4Cl (600 mL) and ice (600 g) and extracted with 300 mL of ether, then twice with 300 mL portions of ethyl acetate. The combined organic extracts were dried over Na_2SO_4 and concentrated, in vacuo, to give 47 g of a yellow solid which was used without further purification.

The above product (47 g) was dissolved in methanol (1L) containing conc. H_2SO_4 (10 mL), and this solution was refluxed overnight. The solvent was removed by

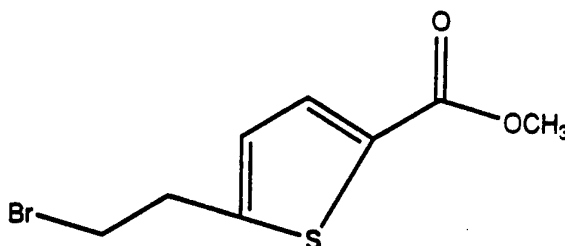
-57-

concentration, in vacuo, and the residue was partitioned between saturated NaHCO_3 (300 mL) and ether (300 mL). The layers were separated and the aqueous phase extracted twice with 300 mL of ethyl acetate. The combined organic extracts were dried over Na_2SO_4 and concentrated, in vacuo, yielding an orange oil which was purified by flash chromatography. Elution with 2:1 hexane/ethyl acetate provided the product as a yellow oil (12.31 g, 44% yield).

NMR (CDCl_3) δ = 7.66 (1H, d, J = 3.7 Hz), 6.88 (1H, d, J = 3.7 Hz), 3.89 (2H, t, J = 6.2 Hz), 3.86 (3H, s), 3.09 (2H, t, J = 6.2 Hz)

Anal. ($\text{C}_8\text{H}_{10}\text{O}_3\text{S}$) C, H, S

9c) Methyl 5-(2-bromoethyl)thiophene-2-carboxylate



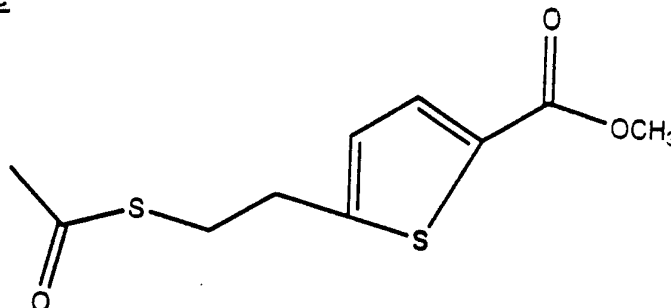
A solution of 11.2 g (42 mmol) of triphenylphosphine in 50 mL of methylene chloride was added dropwise to a solution of 6.52 g, (35 mmol) of 9b) and 13.93 g (42 mmol) of CBr_4 in 100 mL of methylene chloride at 0°C . The resultant reaction mixture was stirred for 60 minutes at 0° , then overnight at room temperature. The solvent was removed by concentration, in vacuo, and the residue obtained was purified by flash chromatography. Elution with hexane/ethylene acetate (9:1) yielded the product as a yellow oil (7.74 g, 89% yield).

NMR (CDCl_3) δ = 7.66 (1H, d, J = 3.8 Hz), 6.89 (1H, d, J = 3.8 Hz), 3.87 (3H, s), 3.58 (2H, t, J = 7.1 Hz), 3.38 (2H, t, J = 7.1 Hz)

Anal. ($\text{C}_8\text{H}_9\text{O}_2\text{S Br}$) C, H, S, Br

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9d) Methyl 5-[2-(acetylthio)ethyl]thiophene-2-carboxylate

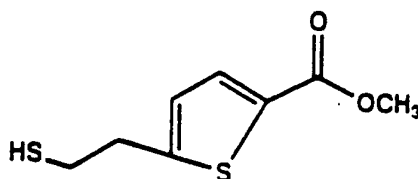


A mixture of 6.23 g (25 mmol) of 9c and 5.71 g (50 mmol) of potassium thiolacetate in 100 mL of acetone was heated at reflux for 30 minutes. After cooling to room temperature, the crude reaction mixture was filtered, and the filtrate was concentrated, in vacuo. The residue obtained was partitioned between ether and water (150 mL each). The layers were separated and the aqueous phase was extracted with 100 mL of ether and 100 mL of ethyl acetate. The combined organic extracts were dried over Na_2SO_4 and concentrated, in vacuo, yielding a red oil which was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) yielded the product as a yellow solid (5.61 g, 92% yield) mp $67^\circ\text{--}68^\circ\text{C}$.

NMR (CDCl_3) δ = 7.64 (1H, d, J = 3.7 Hz), 6.85 (1H, d, J = 3.7 Hz), 3.86 (3H, s), 3.19–3.06 (4H, m), 2.35 (3H, s)

Anal. ($\text{C}_{10}\text{H}_{12}\text{O}_3\text{S}_2$) C, H, S

9e) Methyl 5-(2-thioethyl)thiophene-2-carboxylate



A solution of 4.89 g (20 mmol) of 9d in 1N methanolic HCl (75 mL) was heated at reflux for 2 hours. The reaction

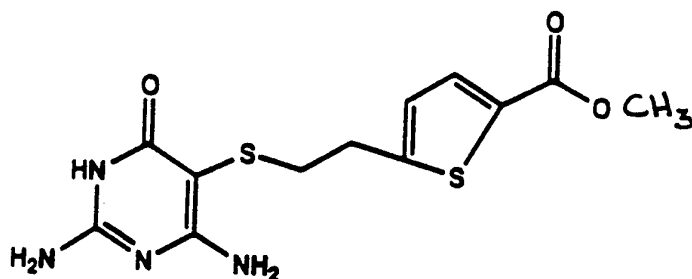
-59-

was diluted with water (50 mL) and the methanol was removed by concentration, in vacuo. The aqueous residue was extracted with ether (2 x 75 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated, in vacuo, to provide the product as a yellow oil (3.93 g, 97% yield).

NMR (CDCl_3) δ = 7.66 (1H, d, J = 3.7 Hz), 6.86 (1H, d, J = 3.7 Hz), 3.87 (3H, s), 3.14 (2H, t, J = 7.1 Hz), 2.83 (2H, dt, J = 8.2, 7.1 Hz), 1.50 (1H, t, J = 8.2 Hz)

Anal. ($\text{C}_8\text{H}_{10}\text{O}_2\text{S}_2$) C, H, S

9f) Methyl 5-(2-[(2,6-diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thiophene-2-carboxylate



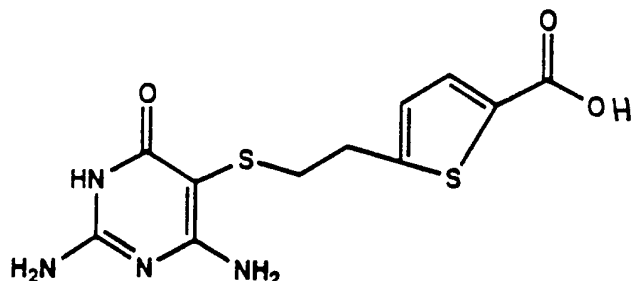
Argon was bubbled through a slurry of 3.00 g (14.6 mmol) of 5-bromo-2,6-diamino-4(3H)-pyrimidinone in 25 mL of DMF. To this slurry was added a solution of 3.24 g (16.0 mmol) of 9e in 30mL of DMF and 5 mL (3.71 g, 28.7 mmol) of diisopropylethylamine. The resultant reaction mixture was heated at 100°C for 90 minutes, then poured into water (350 mL). The precipitate that formed was collected by filtration, washed twice with 75 mL of water then twice with 75 mL of ether to provide the product as an off-white solid (3.22 g, 67% yield) mp 228°-229°C.

NMR ($\text{DMSO}-d_6$) δ = 9.98 (1H, s), 7.62 (1H, d, J = 3.6 Hz), 7.02 (1H, d, J = 3.6 Hz), 6.33 (4H, br s), 3.77 (3H, s), 2.98 (2H, t, J = 7.1 Hz), 2.72 (2H, t, J = 7.1 Hz)

Anal. ($\text{C}_{12}\text{H}_{14}\text{N}_4\text{O}_3\text{S}_2$) C, H, N, S

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9g) 5-(2-[(2,6-Diamino-4(3H)-oxypyrimidin-5-yl)thio]ethyl)thiophene-2-carboxylic acid

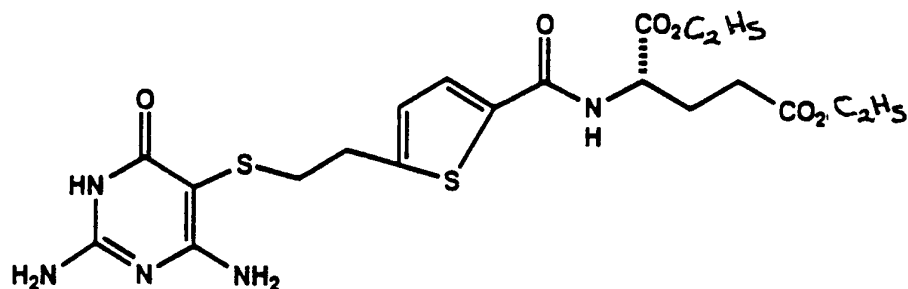


A suspension of 2.94 g (9.0mmol) of 9f in 125 mL of 1N NaOH was stirred overnight at room temperature, then filtered. The filtrate was acidified to ~pH5 by addition of ~30 mL of 6N HCl. The precipitate that formed was collected by filtration and washed twice with 25 mL of water to provide the product as a yellow powder (2.65 g, 94% yield mp 273° (dec)).

NMR (DMSO-d₆) δ = 12.86 (1H, broad), 9.99 (1H, br s), 7.53 (1H, d, J = 3.7 Hz), 6.97 (1H, d, J = 3.7 Hz), 6.34 (4H, br s), 2.97 (2H, t, J = 7.3 Hz), 2.72 (2H, t, J = 7.3 Hz)

Anal. (C₁₁H₁₂N₄O₃S₂) C, H, N, S

9h) Diethyl N-(5-[2-[(2,6-diamino-4(3H)- oxypyrimidin-5-yl]thio)ethyl]thieno-2-yl]-L-glutamate



A solution of 1.87 g (6.0 mmol) of 9g, 3.3 mL (3.04g, 30mmol) of 4-methylmorpholine and 2.41 g (9.0 mmol) of phenyl N-phenylphosphoramidochloridate in 75 mL of 1-methyl-2-pyrrolidinone was stirred for one hour prior to addition of 2.88 g (12.0 mmol) of L-glutamic acid diethyl ester

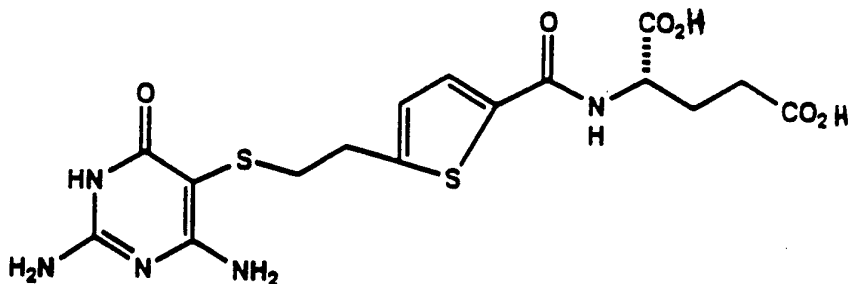
-61-

hydrochloride. The resultant reaction mixture was stirred overnight at room temperature, then concentrated, in vacuo. The residue obtained was partitioned between chloroform and water (100 mL each). The layers were separated and the aqueous phase extracted with 100 mL of chloroform. The combined organic extracts were dried over Na_2SO_4 and concentrated, in vacuo, yielding an orange gum which was purified by flash chromatography. Elution with 4% methanol in chloroform yielded the product as a pale yellow solid (1.41 g, 47% yield, mp $95^\circ\text{-}96^\circ\text{C}$).

NMR (DMSO- d_6) δ = 9.98 (1H, s), 8.61 (1H, d, J = 7.5 Hz), 7.67 (1H, d, J = 3.0 Hz), 6.94 (1H, d, J = 3.0 Hz), 6.32 (4H, br s), 4.40-4.33 (1H, m), 4.09 (2H, q, J = 7.1 Hz), 4.03 (2H, q, J = 7.1 Hz), 2.94 (2H, t, J = 7.3 Hz), 2.70 (2H, t, J = 7.3 Hz), 2.40 (2H, t, J = 7.4 Hz), 2.13-2.01 (1H, m), 2.00-1.91 (1H, m), 1.17 (3H, t, J = 7.1 Hz), 1.15 (3H, t, J = 7.1 Hz)

Anal. ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_6\text{S}_2$: 0.5 H_2O) C, H, N, S

9i) N-(5-[2-([2,6-Diamino-4(3H)-oxopyrimidin-5-yl]thio)ethyl]thieno-2-yl)-L-glutamic acid



A solution of 1.16 g (2.3 mmol) of 9h 90 mL of 1N NaOH was stirred at room temperature for 70 hours, then acidified to ~pH5 by addition of ~20 mL of 6N HCl. The precipitate that formed was collected by filtration and washed three times with 10 mL of water to yield the product as an off-white powder (878 mg, 85% yield, mp $228^\circ\text{-}230^\circ\text{C}$ (dec)).

NMR (DMSO- d_6) δ = 12.41 (2H, broad), 10.02 (1H, br s), 8.51 (1H, d, J = 7.7 Hz), 7.67 (1H, d, J = 3.3 Hz), 6.94 (1H, d, J = 3.3 Hz), 6.36 (4H, br s), 4.36-4.28 (1H, m), 2.94 (2H,

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t, J = 7.3 Hz), 2.70 (2H, t, J = 7.3 Hz), 2.32 (2H, t, J = 7.1 Hz), 2.12-2.00 (1H, m), 1.95-1.85 (1H, m)

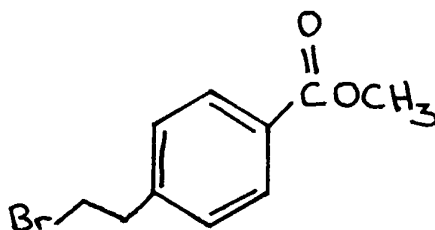
Anal (C₁₆H₁₉N₅O₆S₂) C, H, N, S.

EXAMPLE 10

Synthesis of N-(4-[4-[2-([2,6-Diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]benzoyl]-L-glutamic acid

This compound of formula X, wherein A is sulfur, Ar is unsubstituted phenylene and R₁, R₂ and R₃ are all hydrogen, was synthesized by the following process.

10a) Methyl 4-(2-bromoethyl)benzoate

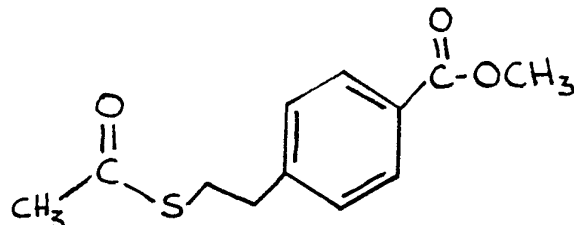


A solution of 9.16 g (40 mmol) of 4-(2-bromoethyl)-benzoic acid in 100 mL of THF was combined with an ether solution containing excess diazomethane. The excess diazomethane was consumed by addition of glacial acetic acid and the resultant solution was concentrated, in vacuo. The residue obtained was partitioned between saturated NaHCO₃ (150 mL) and ethyl acetate (150 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (70 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated in vacuo, yielding the product as a yellow oil (9.65 g, 99% yield).

NMR (CDCl₃) δ = 8.01 (2H, d, J = 8.3 Hz), 7.30 (2H, d, J = 8.3 Hz), 3.92 (3H, s), 3.59 (2H, t, J = 7.4 Hz), 3.23 (2H, t, J = 7.4 Hz)

Anal. (C₁₀H₁₁O₂Br) C, H, Br

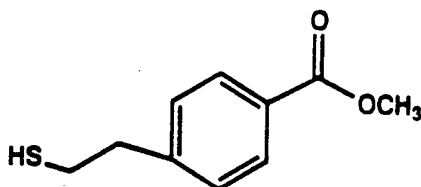
-63-

10b) Methyl 4-[2-(acetylthio)ethyl]benzoate

Methyl 4-[2-(acetylthio)ethyl]benzoate was prepared by following the procedure described for 9d. Thus from 1.22 g (5.0 mmol) of 10a 1.14 g of 10b was obtained (96% yield) as a yellow solid (mp 68°C).

NMR (CDCl₃) δ = 7.97 (2H, d, J = 8.2 Hz), 7.29 (2H, d, J = 8.2 Hz), 3.91 (3H, s), 3.13 (2H, t, J = 7.3 Hz), 2.92 (2H, t, J = 7.3 Hz), 2.33 (3H, s)

Anal. (C₁₂H₁₄O₃S) C, H, S

10c) Methyl 4-(2-thioethyl)benzoate

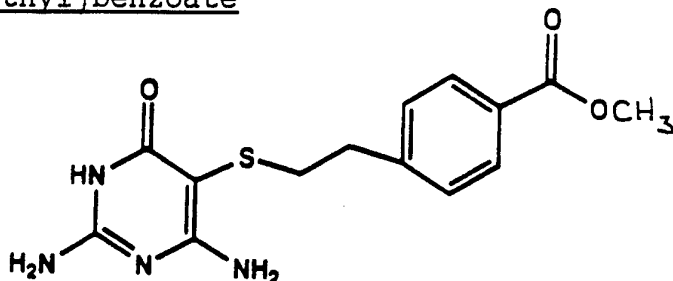
Methyl 4-(2-thioethyl)benzoate was prepared by following the procedure described for 9e. Thus from 1.05 g (4.4 mmol) of 10b there was obtained 8.53 mg of 10c as a yellow oil (99% yield).

NMR (CDCl₃) δ = 7.98 (2H, d, J = 8.2 Hz), 7.27 (2H, d, J = 8.2 Hz), 3.91 (3H, s), 2.98 (2H, t, J = 7.3 Hz), 2.81 (2H, dt, J = 7.9, 7.3 Hz), 1.37 (1H, t, J = 7.9 Hz)

Anal. (C₁₀H₁₂O₂S) C, H, S

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10d) Methyl 4-(2-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)benzoate

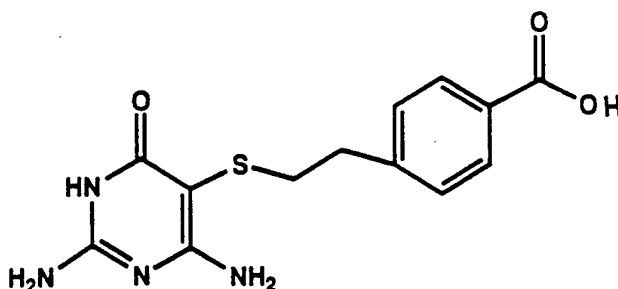


Methyl 4-(2-[(2,6-diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)benzoate was prepared by following the procedure described for 9f. Thus, from 2.45 g (12.5 mmol) of 10c there was obtained 1.58 g of 10d as a white solid (43% yield, mp 286°-288°C (dec)).

NMR (DMSO-d₆) δ 9.98 (1H, br s), 7.85 (2H, d, J = 8.2 Hz), 7.36 (2H, d, J = 8.2 Hz), 6.33 (4H, broad), 3.82 (3H, s), 2.81 (2H, t, J = 7.3 Hz), 2.70 (2H, t, J = 7.3 Hz)

Anal. (C₁₄H₁₆N₄O₃S) C, H, N, S

10e) 4-(2-[(2,6-Diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)benzoic acid



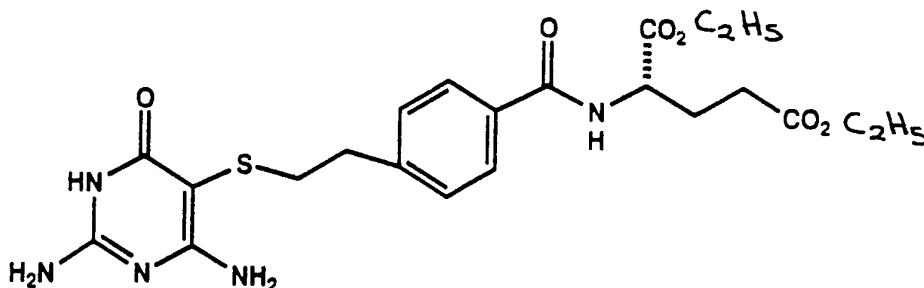
4-(2-[(2,6-Diamino-4(3H)-oxopyrimidin-5-yl)thio]ethyl)benzoic acid was prepared by following the procedure described for 9g. Thus from 1.44 g (4.5 mmol) of 10d, there was obtained 1.27 g of 10e as a white solid (93% yield, mp 291°-292°).

NMR (DMSO-d₆) δ = 12.78 (1H, br s), 9.98 (1H, br s), 7.82 (2H, d, J = 8.2 Hz), 7.32 (2H, d, J = 8.2 Hz), 6.31 (4H, broad), 2.80 (2H, t, J = 7.3 Hz), 2.69 (2H, t, J = 7.3 Hz)

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Anal. ($C_{13}H_{14}N_4O_3S : 0.5 H_2O$) C, H, N, S

10f) Diethyl N-(4-[2-([2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]benzoyl)-L-glutamate

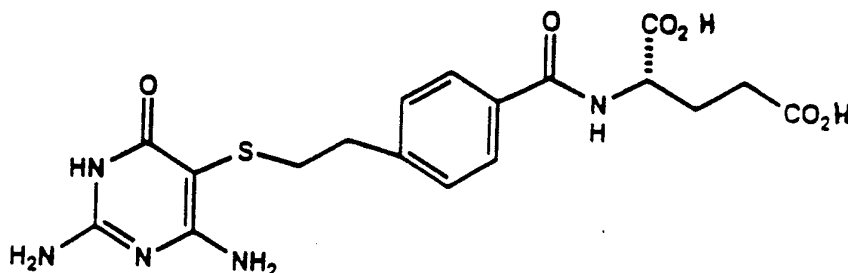


Diethyl N-(4-[2-([2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]benzoyl)-L-glutamate was prepared by following the procedure described for 9h. Thus, from 919 mg (3.0 mmol) of 10e, there was obtained 660 mg of 10f as a white solid (45% yield, mp 105°-107°C).

NMR (acetone- d_6) δ = 10.72 (1H, s), 7.87 (1H, d, J = 8.0 Hz), 7.80 (2H, d, J = 7.8 Hz), 7.31 (2H, d, J = 7.8 Hz), 6.62 (2H, s), 6.11 (2H, s), 4.65 (1H, ddd, J = 5.3, 8.0, 13.3 Hz), 4.15 (2H, q, J = 7.1 Hz), 4.07 (2H, q, J = 7.1 Hz), 2.93-2.78 (4H, m), 2.50 (2H, t, J = 7.3 Hz), 2.29-2.17 (1H, m), 2.15-2.05 (1H, m), 1.23 (3H, t, J = 7.1 Hz), 1.18 (3H, t, J = 7.1 Hz)

Anal. ($C_{22}H_{29}N_5O_6S$) C, H, N, S

10g N-(4-[2-([2,6-diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]benzoyl)-L-glutamic acid



N-(4-[2-([2,6-Diamino-4(3H)-oxypyrimidin-5-yl]thio)ethyl]benzoyl)-L-glutamic acid was prepared by

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following the procedure described for 9i. Thus from 246 mg (0.5 mmol) of 10f there was obtained 76 mg of 10g (35% yield, mp 177-181°C.

NMR (DMSO-d₆) δ = 10.01 (1H, br s), 8.49 (1H, d, J = 7.6 Hz), 7.77 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 6.33 (4H, broad), 4.35 (1H, ddd, J = 5.3, 7.6, 13.0 Hz), 2.78 (2H, t, J = 6.8 Hz), 2.69 (2H, t, J = 6.8 Hz), 2.33 (2H, t, J = 7.3 Hz), 2.09- 1.89 (2H, m)

Anal. (C₁₈H₂₁N₅O₆·1.05 H₂O) C, H, N, S

EXAMPLE 11

Biological and Biochemical Evaluation

In Vitro Testing

Cellular growth in the presence of the compounds according to the present invention was assessed using two cell lines: the L1210 murine leukemia (ATCC CCL 219) and CCRF-CEM, a human lymphoblastic leukemia line of T-cell origin (ATCC CCL 119). Both lines were maintained in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum without antibiotics.

IC₅₀ values were determined in 160 microliter microcultures each containing 1500 (L1210) or 10,000 (CCRF-CEM) cells established in 96 well plates in growth medium supplemented with 50 IU/mL penicillin and 50 mcg/mL streptomycin. Growth was measured over 3 days (L1210) or 5 days (CCRF-CEM) of continuous exposure to varying concentrations of each test compound added 4 hours after initial cell plating by the MTT-tetrazolium reduction assay of Mosmann T.J. (Immunol. meth. 65, 55-63 (1983)), modified according to Alley et al. (Cancer Res. 48, 589-601 (1988)). Water insoluble derivatives were dissolved in DMSO and diluted to a final concentration of 0.5% solvent in cell cultures.

Determination of Inhibition Constants for GARFT

Method I (used for examples 2-5 and 10):

GARFT inhibition constants were measured by the method of Cleland (*Biochim. Biophys. Acta* 67, 173-187 (1963)). Assays were done at 22°C and initiated by addition of enzyme using the spectrophotometric assay of Young et al. (*Biochemistry* 23, 3979-3986 (1984)) and monitoring the reaction at 295 nm. The GARFT domain of the human enzyme was used. The variable substrate was 10-formyl-5,8-dideazafolate at concentrations of 0.83 μM , 1.25 μM , 2.5 μM and 5 μM while the other substrate, GAR (glycinamide ribonucleotide), was held constant at 20 μM . The assay mix contained 20 mM Hepes pH 7.5, 20 μM GAR, and variable amounts of 10-formyl-5, 8-dideazafolate and inhibitor. For each inhibitor five concentrations were used ranging from 0 to approximately 3Ki. The data were plotted as the velocity of the reaction versus the reciprocal of the 10-formyl-5, 8-dideaza-folate concentration. The inhibition constants were measured from a replot of the slopes of these lines obtained for each concentration of inhibitor versus the inhibitor concentration.

Method II (used for examples 6, 7, 8 and 9):

The GARFT assay method of Young, et al. (*Biochemistry* 23, 3979-3986(1984)) was modified and used as described below. Reaction mixtures contained the catalytic domain of the human GARFT, inhibitor, 20 μM glycinamide ribonucleotide (GAR), 10 or 20 μM N¹⁰-formyl-5,8-dideazafolate (FDDF), and 50 mM Tris-Cl at pH 7.5. The reaction was initiated with the addition of enzyme to a final concentration of 11 nM and followed by monitoring the increase in absorbance at 294 nm at 20°C ($\epsilon_{294} = 18.9 \text{ mM}^{-1}\text{cm}^{-1}$).

GARFT constants (K_i) were determined from the dependence of the steady-state catalytic rate upon inhibitor and substrate concentration. The type of

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inhibition observed was determined to be competitive with respect to FDDF by the dependence of the apparent K_i ($K_{i,app}$) on the concentration of FDDF and was shown to be described by $K_{i,app} = K_i + (K_i/K_m)[FDDF]$. The Michaelis constant K_m , was determined independently by the dependence of the catalytic rate upon FDDF concentration. Data for both the K_m and K_i determinations were fitted by non-linear methods to the Michaelis equation or the Michaelis equation for competitive inhibition as appropriate. Data resulting from the tight-binding inhibition was analyzed, and the K_i was determined by fitting the data to the tight-binding equation of Morrison (*Biochem Biophys Acta* 185, 269-286 (1969)) by non-linear methods.

GARFT Inhibition and Cell Culture Data

Ex.	GARFT	Cell Line, IC ₅₀ (μM)		
	K_i μM	L1210	L1210-C192	CCRF-CEM
2	0.11	0.079	5.0	0.065
4	0.11	0.11	10.0	0.13
3	0.035	0.04	10.5	0.049
5	0.088	0.09	4.2	0.08
6	0.008	0.12	>25	0.068
7	0.032	0.089	28	0.099
8	0.030	0.01	9.0	0.008
9	2.00	0.25	22	0.62
10	30	1.5	12	1.2

Determination of Inhibition Constants for AICARFT

The assay method of Black et al. (*Anal Biochem* 90, 397-401 (1978)) was modified and used as described below. Buffers were degassed under vacuum prior to the preparation

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of substrate solutions and degassed buffer was used in the reaction mixtures. Reaction mixtures contained partially purified AICARFT from cultured CEM cells, inhibitor, 100 μM AICAR (5-aminoimidazole-4-carboxamide-ribonucleotide), 50 μM of a racemic mixture of N_{10} -formyl-tetrahydrofolate (FTHF), 25 mM KCl, 50 mM 2-mercaptoethanol and 50 mM Tris-Cl pH 7.4. The reaction was initiated with the addition of the enzyme solution to a final concentration of 0.1 mg/mL and the reaction followed by monitoring the increase in absorbance at 298 nm at 37°C ($\epsilon_{298} = 28 \text{ mM}^{-1} \text{ cm}^{-1}$).

AICARFT inhibition constants (K_i) were determined from the dependence of the steady-state catalytic rate upon inhibitor and substrate concentration. The type of inhibition observed and the determination of K_i was performed as described above for inhibitors of GARFT with the appropriate substrates and the AICARFT assay. Treatment of experimental data was essentially the same.

AICARFT K_i values for examples 9 and 10 were determined to be 35 and 60 μM respectively.

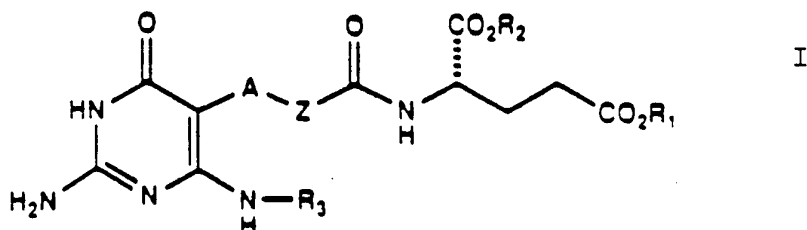
It will be apparent to those skilled in the art that various modifications and variations can be made in the processes and products of the present invention. Thus, it is intended that the present invention cover modifications and variations of this invention which fall within the scope of the appended and equivalent claims.

Specifically, it is recognized that no specific example has been given of making the polymeric polyglutamate form of the compounds of the present invention. One skilled in the art, however, could do this synthetically in accord with the literature methods. Normally, moreover, as explained above, polyglutamation, preferably with the addition of one to five glutamate units to the compounds of the present invention, more preferably, one to four glutamate units, will occur inside the cells.

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CLAIMS

1. A compound having the formula I



wherein:

A represents sulfur or selenium;

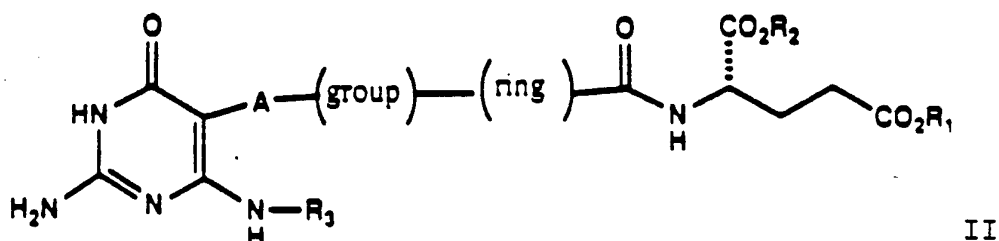
Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein when said non-cyclic spacer is bonded to A, said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms and further wherein when said non-cyclic spacer is bonded to -C(O)-, said non-cyclic spacer separates -C(O)- from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R₁ and R₂ represent, independently, H or C₁ to C₆ alkyl or other readily lyzable groups; and

R₃ represents H or a straight, branched or cyclic C₁ to C₆ alkyl group optionally carrying one or more halogen, hydroxyl or amine groups; or a pharmaceutically acceptable salt thereof.

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2. A compound according to claim 1 having the formula
II



wherein:

A is sulfur or selenium;

Z is -(group)-(ring)-,

wherein (group) represents a non-cyclic spacer which separates A from (ring) by 1 to 5 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous and optionally carrying one or more substituents independently selected from C₁ to C₆ alkyl or C₂ to C₆ alkenyl groups, C₁ to C₆ alkoxy or C₁ to C₆ alkoxy(C₁ to C₆)alkyl groups, C₂ to C₆ alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings;

and wherein (ring) represents one or more of a substituted or unsubstituted monocyclic carbo- or heterocyclic ring or a fused or non-fused polycarbocyclic or heterocyclic ring optionally substituted with one or more substituents selected from those recited for (group);

R₁ and R₂ represent, independently, H, C₁ to C₆ alkyl or other readily lyzable groups; and

R₃ represents hydrogen or a straight, branched or cyclic C₁ to C₆ alkyl group optionally carrying halogen, hydroxyl or amine substitution; or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 1 wherein the moiety Z is represented by Q-X-Ar wherein:

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Q represents a C₁-C₅ alkylene, or a C₂-C₅ alkenylene or alkynylene radical optionally carrying one or more substituents independently selected from C₁ to C₆ alkyl or C₂ to C₆ alkenyl groups, C₁ to C₆ alkoxy or C₁ to C₆ alkoxy(C₁ to C₆)alkyl groups, C₂ to C₆ alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings;

X represents a methylene, monocyclic carbo- or heterocyclic ring, sulfur, oxygen or amino radical, optionally carrying one or more substituents independently selected from C₁ to C₆ alkyl or C₂ to C₆ alkenyl groups, C₁ to C₆ alkoxy or C₁ to C₆ alkoxy(C₁ to C₆)alkyl groups, C₂ to C₆ alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings; and

Ar represents a monocyclic carbo- or heterocyclic aromatic ring or a bicyclic carbo- or heterocyclic ring, all or a portion of which may be aromatic, and wherein the Ar may be fused to the monocyclic carbo- or heterocyclic ring of X, and wherein the Ar optionally carries one or more substituents independently selected from C₁ to C₆ alkyl or C₂ to C₆ alkenyl groups, C₁ to C₆ alkoxy or C₁ to C₆ alkoxy(C₁ to C₆)alkyl groups, C₂ to C₆ alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused poly-carbocyclic or poly-heterocyclic rings;

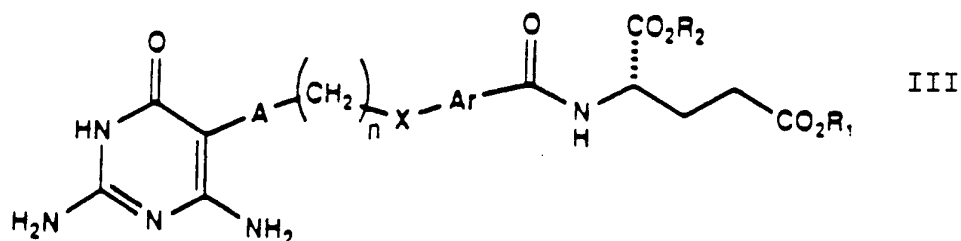
or a pharmaceutically acceptable salt thereof.

4. A compound or salt according to claim 2, wherein the moiety (group) represents a C₁ to C₄ alkylene group; and the moiety (ring) represents a substituted or unsubstituted, fused or non-fused carbocyclic or heterocyclic bicyclic ring system, or a substituted or unsubstituted, carbocyclic or heterocyclic monocyclic ring system, or at

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least two monocyclic ring systems linked by a single bond, said monocyclic ring systems being independently substituted or unsubstituted.

5. A compound having the formula III



wherein:

n represents an integer from 0 to 5;

A represents sulfur or selenium;

X is methylene, monocyclic carbo- or heterocyclic ring, O, S, or -NH-;

Ar is an aromatic radical, wherein Ar can form a fused bicyclic ring system with said ring of X; and

R₁ and R₂, which can be the same or different, are hydrogen or alkyl radicals having 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof.

6. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is methylene, Ar is phenylene and R₁ and R₂ are hydrogen.

7. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is methylene, Ar is 2,5-thienyl and R₁ and R₂ are hydrogen.

8. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is S, Ar is phenylene and R₁ and R₂ are hydrogen.

9. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is -NH-, Ar is phenylene and R₁ and R₂ are hydrogen.

10. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is methylene, Ar is phenylene and R₁ and R₂ are alkyl radicals having 1 to 6 carbon atoms.

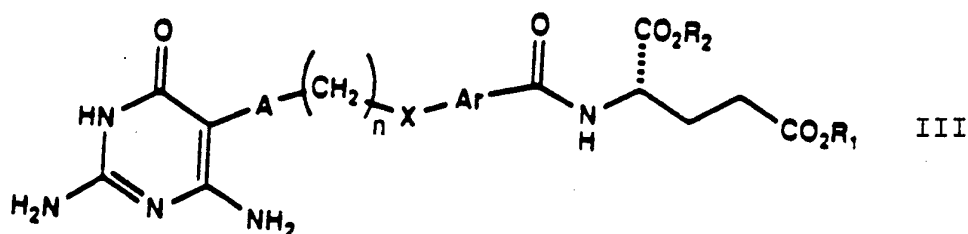
-74-

11. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is methylene, Ar is 2,5-thienyl and R₁ and R₂ are ethyl groups.

12. A compound or salt according to claim 5 wherein n is 2, A is sulfur, X is sulfur, Ar is phenylene and R₁ and R₂ are ethyl groups.

13. A compound or salt according to claim 5, wherein n is 2, A is sulfur, X is -NH-, Ar is phenylene and R₁ and R₂ are ethyl groups.

14. An antiproliferative composition comprising a compound having the formula III



wherein n represents an integer from 0 to 5;

A is sulfur or selenium;

X is methylene, monocyclic carbo- or heterocyclic ring, O, S, or -NH-;

Ar is an aromatic radical, wherein Ar can form a fused bicyclic ring system with said ring of X; and

R₁ and R₂, which can be the same or different, are hydrogen or alkyl radicals having 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof in combination with a pharmaceutically acceptable carrier.

15. A composition according to claim 14 wherein n is 2, A is sulfur, X is methylene, and Ar is phenylene.

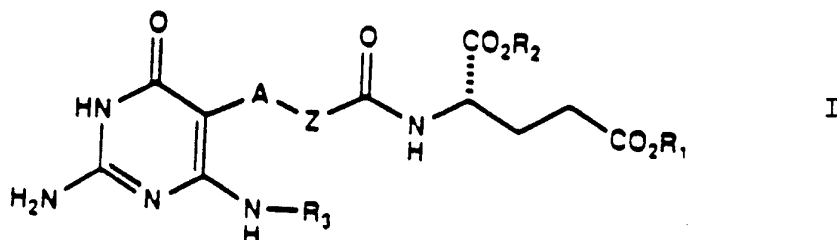
16. A composition according to claim 14 wherein n is 2, A is sulfur, X is methylene, and Ar is 2,5-thienyl.

17. A composition according to claim 14 wherein n is 2, A is sulfur, X is S, and Ar is phenylene.

18. A composition according to claim 14 wherein n is 2, A is sulfur, X is -NH-, and Ar is phenylene.

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19. An antiproliferative composition comprising a compound having the formula I:



wherein:

A represents sulfur or selenium;

Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein when said non-cyclic spacer is bonded to A, said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms and further wherein when said non-cyclic spacer is bonded to -C(O)-, said non-cyclic spacer separates -C(O)- from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

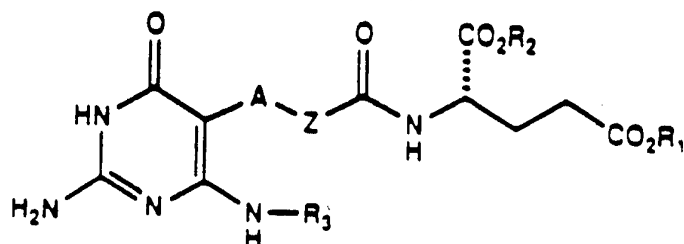
R₁ and R₂ represent, independently, H, C₁ to C₆ alkyl or other readily lyzable groups; and

R₃ represents H or straight, branched or cyclic C₁ to C₆ alkyl group optionally carrying one or more halogen, hydroxyl or amine groups; or a pharmaceutically acceptable salt thereof;

in combination with a pharmaceutically acceptable carrier.

20. A process for inhibiting the growth and proliferation of the cells of microorganisms and of higher organisms, which process comprises administering to a host in need of such treatment an effective amount of a compound having the structural formula I

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I

wherein:

A represents sulfur or selenium;

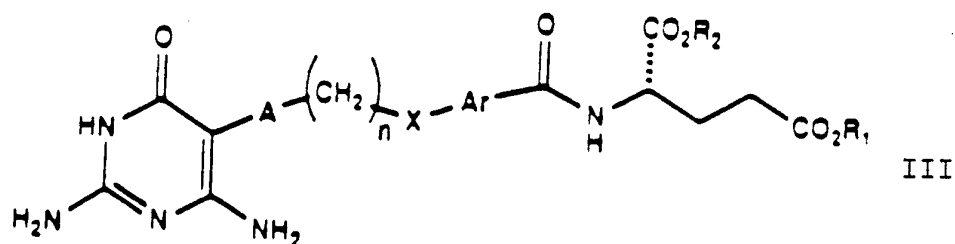
Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein when said non-cyclic spacer is bonded to A, said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms and further wherein when said non-cyclic spacer is bonded to -C(O)-, said non-cyclic spacer separates -C(O)- from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R₁ and R₂ represent, independently, H, C₁ to C₆ alkyl or other readily lyzable groups; and

R₃ represents H or straight, branched or cyclic C₁ to C₆ alkyl group optionally carrying one or more halogen, hydroxyl or amine groups; or a pharmaceutically acceptable salt thereof.

21. A process for inhibiting the growth and proliferation of the cells of microorganisms and higher organisms, which process comprises administering to a host in need of such treatment an effective amount of a compound having the structural formula III

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wherein:

n represents an integer from 0 to 5;

A represents sulfur or selenium;

X is methylene, monocyclic carbo- or heterocyclic ring, O, S, or -NH-;

Ar is an aromatic radical, wherein Ar can form a fused bicyclic ring system with said ring of X; and

R₁ and R₂, which can be the same or different, are hydrogen or alkyl radicals having 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof.

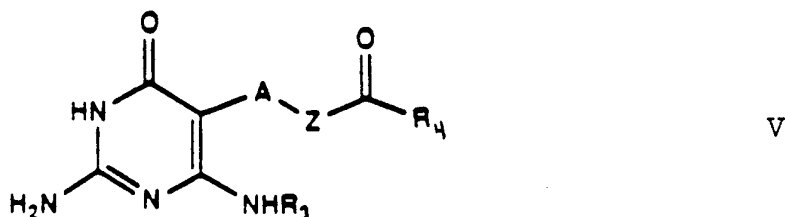
22. A process according to claim 21 wherein n is 2, A is sulfur, X is methylene and Ar is phenylene.

23. A process according to claim 21 wherein n is 2, A is sulfur, X is methylene and Ar is 2,5-thienyl.

24. A process according to claim 21 wherein n is 2, A is sulfur, X is sulfur and Ar is phenylene.

25. A process according to claim 21 wherein n is 2, A is sulfur, X is -NH-, and Ar is phenylene.

26. A process for preparing a 5-substituted pyrimidinone compound having the formula V



wherein:

A represents sulfur or selenium;

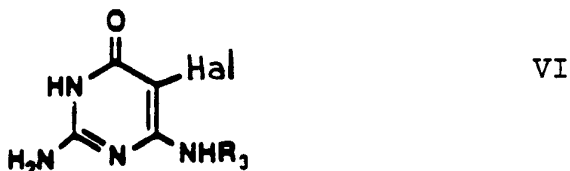
-78-

Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R₃ represents H or a straight, branched or cyclic (C₁ to C₆) alkyl group, optionally carrying one or more hydroxyl or amine groups; and

R₄ represents hydroxy, (C₁ to C₆) alkyloxy group optionally carrying one or more hydroxyl or amine groups, or a protected or unprotected amino acid linked to the acyl group of formula V by the amine portion of the amino acid; or a pharmaceutically acceptable salt thereof;

which process comprises reacting a compound having the formula VI



wherein hal is bromine, chlorine, iodine, or fluorine, and R₃ is as defined above, with a compound having the formula IV



wherein A, Z, and R₄ are as defined above, in the presence of a nonnucleophilic auxiliary base in a solvent in which at least one of said reactants is at least partially soluble under conditions sufficient to obtain the compound of formula V.

27. A process according to claim 26 wherein the non-nucleophilic auxiliary base is selected from alkali or earth metal carbonates and trialkyl amines.

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28. A process according to claim 27 wherein the solvent is a dipolar aprotic solvent.

29. A process according to claim 28 wherein the solvent is selected from dimethylsulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide, and N-methyl-2-pyrrolidinone.

30. A process according to claim 26 wherein A represents sulfur and Z represents $-(CH_2)_n-X-Ar-$ wherein n is an integer from 0 to 5,

X represents a methylene, monocyclic carbo- or heterocyclic ring, sulfur, oxygen or amino radical, optionally carrying one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused polycarbocyclic or poly-heterocyclic rings; and

Ar represents a monocyclic carbo- or heterocyclic aromatic ring or a bicyclic carbo- or heterocyclic ring, all or a portion of which may be aromatic, and wherein the Ar may be fused to the monocyclic carbo- or heterocyclic ring of X, and wherein the Ar optionally carries one or more substituents independently selected from C_1 to C_6 alkyl or C_2 to C_6 alkenyl groups, C_1 to C_6 alkoxy or C_1 to C_6 alkoxy(C_1 to C_6)alkyl groups, C_2 to C_6 alkynyl groups, acyl groups, halogen, amino groups, hydroxyl groups, nitro groups or mercapto groups, monocyclic carbo- or heterocyclic rings, and fused or non-fused polycarbocyclic or poly-heterocyclic rings.

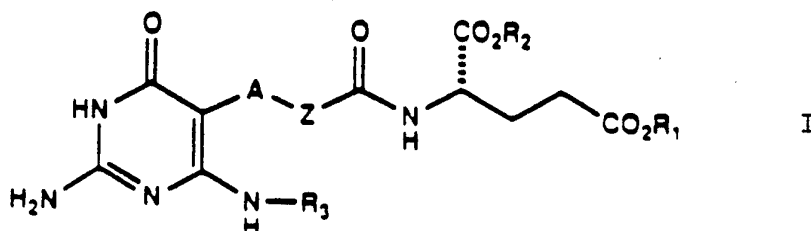
31. A process according to claim 30 wherein the non-nucleophilic auxiliary base is selected from alkali or earth metal carbonates and trialkylamines.

32. A process according to claim 31 wherein the solvent is a dipolar aprotic solvent.

33. A process according to claim 32 wherein the solvent is selected from dimethylsulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide, and N-methyl-2-pyrrolidinone.

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34. A process for inhibiting GARFT comprising the step of administering to a host in need of such inhibition an effective amount of a compound having the formula I:



wherein:

A represents sulfur or selenium;

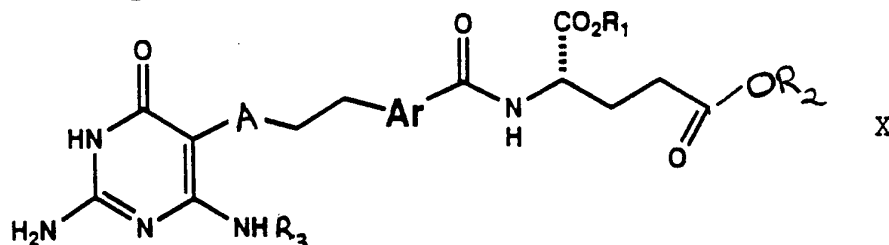
Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical, wherein when said non-cyclic spacer is bonded to A, said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms and further wherein when said non-cyclic spacer is bonded to -C(O)-, said non-cyclic spacer separates -C(O)- from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R₁ and R₂ represent, independently, H or C₁ to C₆ alkyl or other readily lyzable groups; and

R₃ represents H or straight, branched or cyclic C₁ to C₆ alkyl group optionally carrying one or more halogen, hydroxyl or amine groups; or a pharmaceutically acceptable salt thereof.

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35. A compound of the formula X



wherein:

A represents sulfur or selenium;

Ar represents an unsubstituted phenylene or thienylene radical;

R₁ and R₂ represent, individually, hydrogen or C₁ to C₆ alkyl or other readily lyzable groups;

R₃ represents hydrogen or a straight, branched or cyclic C₁-C₆ alkyl group, optionally carrying one or more halogen, hydroxyl or amine groups; or

a pharmaceutically acceptable salt thereof.

36. A compound according to claim 35 wherein A is sulfur and Ar represents an unsubstituted phenylene radical.

37. A compound according to claim 35 wherein A is sulfur and Ar represents an unsubstituted thienylene radical.

38. A compound according to claim 35 wherein A is sulfur, Ar is unsubstituted thienylene; and R₁, R₂ and R₃ are hydrogen.

39. A compound according to claim 35 wherein A is sulfur, Ar is unsubstituted phenylene; and R₁, R₂ and R₃ are hydrogen.

40. A method for inhibiting the growth and proliferation of the cells of microorganisms and higher organisms, which comprises administering to a host in need of such treatment an effective amount of the compound having the structural formula X as defined in claim 35, or a pharmaceutically acceptable salt thereof.

41. A method according to claim 40 wherein A is sulfur and Ar represents an unsubstituted phenylene radical.

42. A method according to claim 40 wherein A is sulfur and Ar represents an unsubstituted thienylene radical.

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43. A method according to claim 40 wherein A is sulfur, Ar is an unsubstituted thienylene radical; and R_1 , R_2 and R_3 are hydrogen.

44. A method according to claim 40 wherein A is sulfur, Ar is an unsubstituted phenylene radical; and R_1 , R_2 and R_3 are hydrogen.

45. An antiproliferative composition comprising the compound having the formula X as defined in claim 35 or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

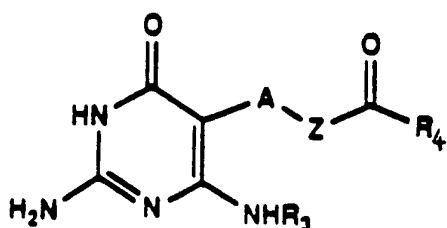
46. A composition according to claim 45 wherein A is sulfur and Ar represents an unsubstituted phenylene radical.

47. A composition according to claim 45 wherein A is sulfur and Ar represents an unsubstituted thienylene radical.

48. A composition according to claim 45 wherein A is sulfur, Ar is an unsubstituted thienylene radical; and R_1 , R_2 and R_3 are hydrogen.

49. A composition according to claim 45 wherein A is sulfur, Ar is an unsubstituted phenylene; and R_1 , R_2 and R_3 are hydrogen.

50. A compound having the formula V



V

wherein:

A represents sulfur or selenium;

Z represents 1) a substituted or unsubstituted non-cyclic spacer which separates A from the carbonyl carbon of the amido group by 1 to 10 atoms, said atoms being independently selected from carbon, oxygen, sulfur, nitrogen and phosphorous; 2) a substituted or unsubstituted mono- or fused or nonfused poly-carbocyclic or heterocyclic radical; or 3) a combination of at least one of said non-cyclic spacer and at least one of said carbocyclic or heterocyclic radical,

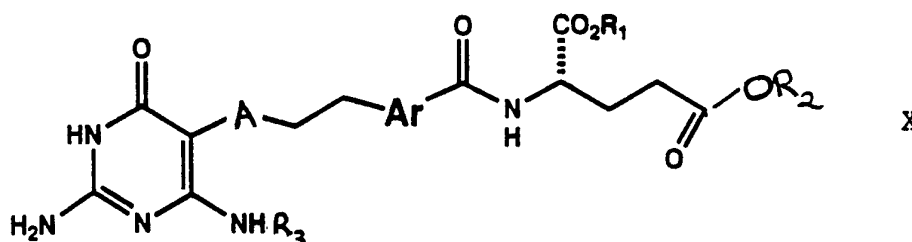
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wherein said non-cyclic spacer separates A from one of said carbocyclic or heterocyclic radicals by 1 to 10 atoms;

R_3 represents H or a straight, branched or cyclic (C_1 to C_6) alkyl group, optionally carrying one or more hydroxyl or amine groups; and

R_4 represents hydroxy, (C_1 to C_6) alkyloxy group optionally carrying one or more hydroxyl or amine groups, or a protected or unprotected amino acid linked to the acyl group of formula V by the amine portion of the amino acid; or a pharmaceutically acceptable salt thereof.

51. A process for inhibiting AICARFT comprising the step of administering to a host in need of such inhibition an effective amount of a compound having the formula X:



wherein:

A represents sulfur or selenium;

Ar represents an unsubstituted phenylene or thienylene radical;

R_1 and R_2 represent, individually, hydrogen or C_1 to C_6 alkyl or other readily lyzable groups;

R_3 represents hydrogen or a straight, branched or cyclic C_1 - C_6 alkyl group, optionally carrying one or more halogen, hydroxyl or amine groups; or

a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11795

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/505; C07D 239/49, 239/36

US CL : 514/272; 544/320, 321, 243

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/272; 544/320, 321, 243

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,920,125 (Taylor et al) 24 April 1990 see columns 1 and 3, claims 1-7.	1-25, 34-51
A	US, A, 4,880,812 (Kelley et al), 14 November 1989 see columns 1 and 2, claims 1-13.	1-25, 34-51
A	US, A, 4,871,743 (Taylor et al), 03 October 1989 see columns 1 and 2, claims 1-15.	1-25, 34-51
A	US, A, 4,686,223 (Cohnen et al) 11 August 1987 see column 3, Example 1.	26-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A" document defining the general state of the art which is not considered to be part of particular relevance	*X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E" earlier document published on or after the international filing date	*Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z"	document member of the same patent family
*O" document referring to an oral disclosure, use, exhibition or other means		
*P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 MARCH 1994	Date of mailing of the international search report APR 06 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Y.N. Gupta jd Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11795

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Telephone Practice
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/11795

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Group I. Claims 1-25, 34-49, and 51, drawn to compounds, composition and method of use.

Group II. Claims 26-33 and 50 drawn to an intermediate and process of making.

The Group II invention is drawn to an intermediate (Formula V) as claimed is deemed, due to its structural difference from Formula I, to be useful as an antibacterial or antifungal agent and is therefore distinct from Group I under PCT Rule 13.2