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(54) Title: PROCESS FOR MAKING γ–METHYLENE–10–DEAZAAMINOPTERIN (MDAM)

(57) Abstract

A process to synthesize the dihydrofolate reductase inhibitor, 4–amino–4–deoxy–10–deazaaminopterin (MDAM). The process synthesizes the key intermediate, pteroic acid in 5 steps, and uses commonly available starting materials. The steps include the formation of a 6–substituted pteridine, followed by alkylation, hydrogenation, and hydrolysis steps to form pteroic acid from the starting material which is tetraamino pyrimidine, or a salt thereof.

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PROCESS FOR MAKING γ-METHYLENE-10-DEAZAAMINOPTERIN (MDAM)

FIELD OF THE INVENTION

This invention relates to a novel and improved process for synthesizing the useful antitumor agent γ-methylene-10-deazaaminopterin (MDAM).

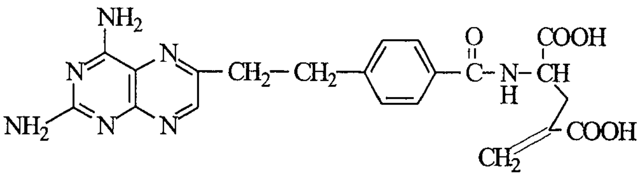
BACKGROUND OF THE INVENTION

A. Brief History of MDAM

MDAM is a potent inhibitor of dihydrofolate reductase (DHFR). The therapeutic value of MDAM as an antitumor agent has been well documented in recent literature, as have its increased specificity and reduced toxicity, particularly when compared to analogs, such as methotrexate (MTX). MDAM is currently undergoing Phase I clinical trials at Johns Hopkins University as an antitumor compound.

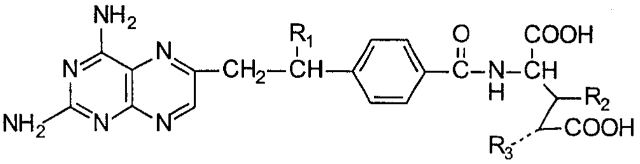
MDAM and certain derivatives thereof, are the subject of United States Patents 4,996,207 and 5,073,554, and is also disclosed and claimed in several overseas patents in Europe, Canada, Mexico and Japan. MDAM has the following structure I:

(I)



The general formula for known active derivatives of MDAM is seen below as Formula II:

(II)



wherein R_1 is hydrogen or lower alkyl;

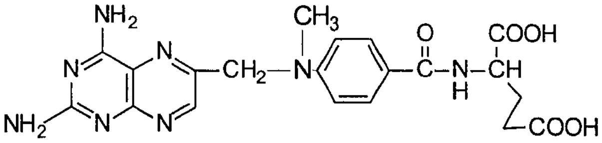
R_2 is hydrogen or hydroxy;

R_3 is hydrogen or methylene; and

the dashed line indicates a single bond or a double bond.

MDAM is similar in structure to the known antitumor and anti-inflammatory drug methotrexate seen below as Formula III:

(III)



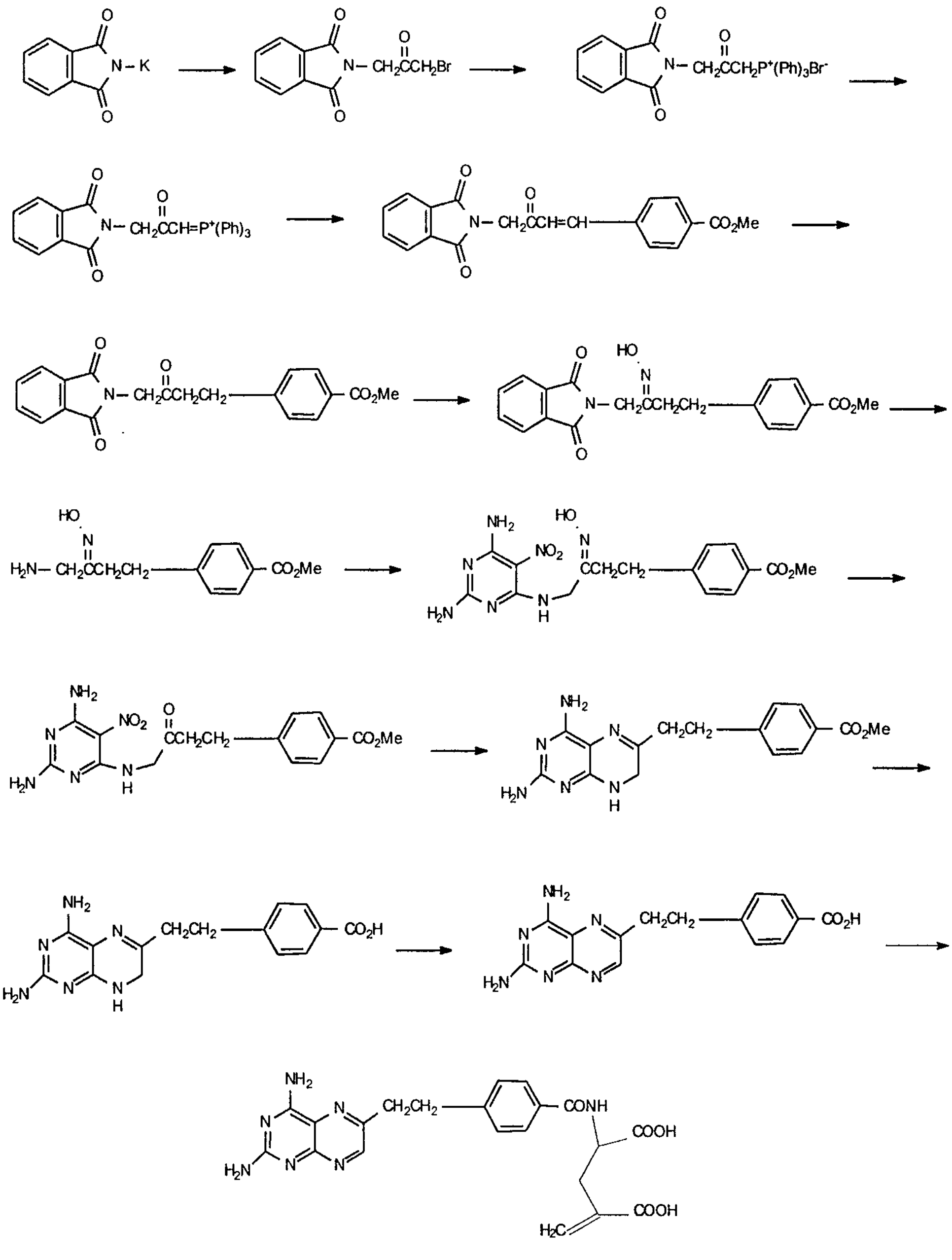
MDAM specifically inhibits dihydrofolate reductase to a far greater extent than MTX. Further, MDAM does not undergo the polyglutamylation common to MTX and derivatives, thereby reducing the toxicity of MDAM far below that of MTX. The greater efficacy and reduced toxicity of MDAM (compared to MTX and derivatives thereof) has been well established for many years now.

B. Current Synthetic Procedures For Making MDAM

The current process for making MDAM involves a complex 11 step procedure to make the key intermediate, plus an additional three steps to produce the key intermediate diethylγ-methylene glutamate), and a final coupling step, followed by the hydrolysis of the ester to MDAM. The prior processes to make the key intermediate, and/or MDAM or derivatives are outlined in the above mentioned U.S. Patents, and in several publications known in the literature, the most notable being Nair, *J. Org. Chem.*, 50:1879, 1985, which publication recites the process shown below as Scheme I.

Further, the previous process for preparing MDAM required the nitration of 2,4-diamino-6-chloro-pyrimidine (a necessary reagent), which was a difficult, time-consuming, step in the process. A schematic depiction of the prior process is shown below as Scheme 1, and is intended to illustrate the complexity and difficulty previously associated with synthesizing MDAM.

Scheme 1



As shown in Scheme 1, the prior art process for synthesizing MDAM requires a number of awkward and time-consuming maneuvers to produce the desired product. The most awkward and time-consuming step is the required nitration of 2,4-diamino-6-chloro-pyrimidine, which is necessary for the eighth step in the process, the addition of the pyrimidine ring with the 5- and 6-positions having attached nitrogen groups to enable its later conversion to the required pteridine fused ring group.

The prior art processes to synthesize MDAM were further limited in their adaptability to bulk manufacturing. Cost effectiveness, yield and purity are significant considerations in the bulk manufacture of any pharmaceutical product, and the prior processes employed to synthesize MDAM were neither cost effective nor did they generate acceptable yields of product.

SUMMARY OF THE INVENTION

The process described by this invention delivers a highly efficient, convergent process for the synthesis of MDAM and useful derivatives thereof. The inventive process reduces the number of steps to produce the pteroic acid intermediate from 11 to 5, and further, significantly increases the yield and purity of the end product when compared with prior processes employed to synthesize these useful compounds.

As stated above, the inventive process involves a five step process to produce the pteroic acid intermediate, which is then coupled to an ester of γ-methylene glutamic acid, and finally hydrolyzed to form the desired end product in a highly pure form.

The process begins with the conversion of 2,4,5,6-tetraaminopyrimidine to the corresponding pteridine intermediate. After bromination and alkylation of this 6-hydroxymethyl pteridine intermediate to form a 9,10-dehydro pteroic acid ester, reduction of the 9,10-double bond produces pteroic acid ester, and then hydrolyzing the ester to form pteroic acid (the key intermediate), coupling with γ-methylene glutamic acid ester and hydrolyzing the resulting compound (the diester of MDAM) yields MDAM in very high yield.

The schemes and specific examples for carrying out the inventive process are illustrated in the detailed description set forth below.

It is an object of this invention to provide for a highly efficient and convergent process for synthesizing MDAM and useful derivatives thereof.

Another object is to provide for a process for synthesizing MDAM and derivatives, which process is readily adaptable to GMP conditions.

Another object is to provide for a process for synthesizing MDAM which is time efficient, cost effective, and produces MDAM in sufficiently high yield.

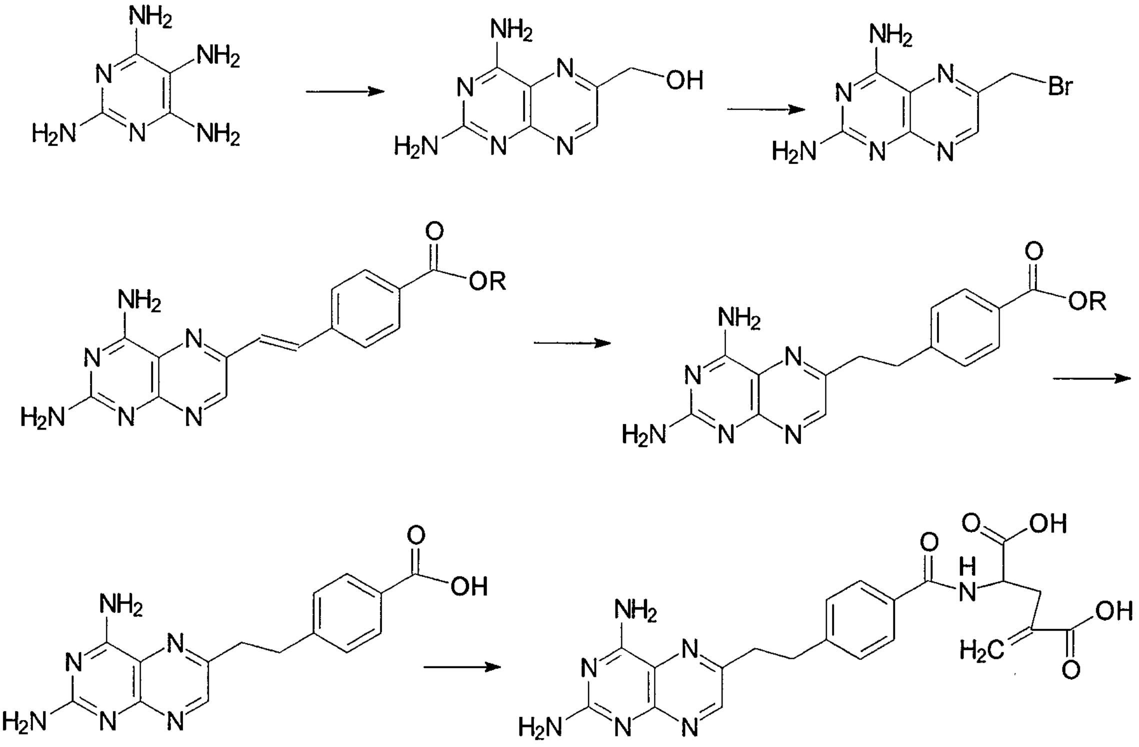
Other objects will become apparent from a reading of the following detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The preferred embodiments herein described are not intended to be exhaustive or to limit the invention to the precise details disclosed. They have been chosen and described to explain the principles of the invention, and the application and practical use thereof, so that those skilled in the art may understand its teachings.

The process of this invention is designed to produce, in substantially pure form, the antitumor agent γ-methylene-10-deazaaminopterin (MDAM). The general process described by this invention is shown below as Scheme II:

Scheme II



As shown in Scheme II, the starting material is 2,4,5,6-tetraamino pyrimidine (a). The starting material is preferably obtained as a salt of the actual compound. Most preferred is the sulfate salt, which compound is available commercially from Aldrich Chemical Company and others.

This initial step in the process is carried out according to published conditions in Baugh, et al, *The Journal of Organic Chemistry,* Volume 29: p 3610 (1969).

The intermediate 6-hydroxymethyl pteridine (b) is the resultant compound, which is formed by a closure of the fused 'B' ring of the pteridine molecule by condensation of 2,4,5,6-tetraamino pyrimidine and dihydroxy acetone. The preferred reagents are those which support these conditions and the ring formation, preferably ketols, and in the most preferred case, 1,3-dihydroxy-2-propanone.

The 6-hydroxymethyl pteridine (b) is then brominated to facilitate the alkylation step of the process. The bromination is achieved by reacting the 6-hydroxymethyl intermediate (b) with an excess of a brominating reagent which replaces the terminal hydroxy moiety with a bromine atom. Preferred reagents for the brominating step are identified in Piper, et al, *The Journal of Organic Chemistry,* Volume 42:208 (1977). The most preferred reagent is dibromotriphenylphosphine, and subsequent work up and recrystallization from acetic acid and isopropanol yields the hydrogen bromide isopropanol salt of 6-bromomethyl pteridine (c) in high yield.

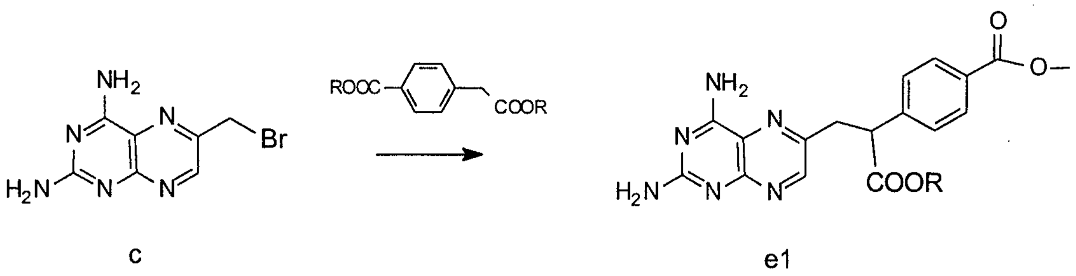
This intermediate salt (c) is then reacted with carbonyl compounds under Wittig reaction conditions to form the 9,10-dehydro pteroate ester intermediate (d). The most preferred intermediate (d) is shown as the methyl ester of 9,10-dehydro pteroic acid. Preferred reagents used in the reaction are triphenylphosphine and methyl-4-formyl benzoate, in a strong base such as sodium methoxide, to form the intermediate (d).

The ester (d) is then reduced to saturate the C9-C10 bond. The preferred process involves reacting the ester intermediate (d) with palladium/carbon palladium on alumina in an organic solvent to produce intermediate methyl pteroate ester (e).

The methyl pteroate ester (e) is then converted to the key pteroic acid intermediate (f), 4-amino-4-deoxy-10-deaza pteroic acid. Preferably the hydrolysis is conducted in a strong base, most preferably in aqueous sodium hydroxide solution along with an organic solvent 2-methoxyethanol.

The pteroic acid intermediate (f) is then activated, and coupled (as the mixed anhydride) to an ester of γ-methylene glutamic acid to form the esterified form of MDAM, and then hydrolyzed to the active compound (g) of Formula I.

Scheme IIa



In an alternative alkylation of intermediate (c), the bromomethyl pteridine salt (c) is pH neutralized, then reacted with a diester of homoterephthallic acid (4-carboxybenzoic acid) to form the esterified form of the 10-carboxy intermediate (e1). Intermediate (e1) is then decarboxylated and hydrolyzed the other ester group with a strong nucleophile to form the key pteroic acid intermediate (f). Preferred nucleophilic agents include sodium cyanide, most preferably dissolved in dimethylsulfoxide (DMSO).

The following specific examples illustrate the best mode for carrying out the inventive process. The examples are disclosed for illustrative purposes only, and are not to be considered as limiting the invention to the precise details set forth.

All NMR data are expressed in parts per million (ppm), and the resonances expressed as follows: s, singlet; d, doublet; t, triplet; br.s., broad singlet; sep, septet; c, complex set of signals, the center of which is provided.

Example 1

2,4-diamino-6-hydroxymethyl pteridine

Tetraaminopyrimidine sulfate (10 mmoles) was taken up in 40 mL of water and barium chloride (10 mmoles) in 10 mL of water was added. The mixture was placed in a boiling water bath for 10 min. After cooling, the barium chloride was removed by filtration and the solid was washed on the filter with about 10 mL of water. The combined washings and filtrate were made up to 50 mL with water. The solution was added to a solution of 150 mL of 4 M sodium acetate containing dihydroxy acetone (30 mmoles) and cysteine hydrochloride monohydrate (10 mmoles in a 1 L Erlenmeyer flask and was placed on a rotary shaker at room temperature for 24 hours. After this period, the flask was placed in the cold for several hours, the precipitate was then collected by suction filtration and washed with cold water. The precipitate was resuspended in 100 mL of water and heated to boiling; if necessary drops of 1N sodium hydroxide solution were added to effect the solution. Norit (0.5 g) was added and the hot solution thoroughly mixed, filtered hot through heated funnel. After cooling to room temperature, the pH was adjusted to 6.0 with 1 N HCl and the flask was placed in the cold for several hours. The precipitate was collected by filtration, washed with cold water, ethanol, (50:50) ethanol-ether, and finally with ether, then dried in vacuo. The yield after an additional recrystallization from water is 55%. The compound exhibited following proton NMR signals in DMSO-d6; (ppm) 8.8 (s, 2H, aromatic), 7.6 and 6.6 (broad singlets, 4H, Amino), 5.5 (broad singlet, 1H, hydroxy) and 4.65 (s, 2H, benzylic).

Example 2

2,4-diamino-6-bromomethyl pteridine

Solid hydroxymethyl pteridine (110 mmoles) was added to a mixture of Ph_3PBr_2 (363 mmoles) and dimethylacetamide

(360 mL) in a 2L three necked flask. The mixture was stirred at 20-25° C for 3.5 hours. The solution that formed was treated with drop wise during 15 minutes with 7.2 mL of ethanol and stirred for 15 minutes longer before benzene (1.17 L) was added. A dark oil precipitated, and the mixture was stirred for 30 minutes longer and left to stand overnight. The clear supernatant liquid was decanted from the semi solid precipitate, which was dissolved with stirring in hot glacial acetic acid (600 mL, pre heated to 100°C). The solution was filtered while hot, and the beige, crystalline material that separated from the cooled filtrate was collected after 4 hours at 20-25°C. The ether washed solid (solvated by acetic acid) was recrystallized from isopropanol to give yellow orange platelets, which were washed with ether and dried to yield 50% of the theoretical yield. The compound exhibited following proton NMR signals in DMSO-d6; (ppm) 9.3 and 9.2 (broad singlet, 4H, amino groups), 9.0 (s, 1H, aromatic), 4.9 (s, 2H, benzylic), 3.8 (septet, 1H, CH of isopropanol), and 1.0 (d, 6H, methyls of isopropanol).

Example 3

Methyl-9,10-dehydro-4-amino-4-deoxy pteroate

100 grams of the intermediate from Example 2 above was stirred in a solution of 3.0 liters of N,N-dimethylacetamide and one molar equivalent of triphenylphosphine for 4 hours at 80° C. The solution was allowed to cool to room temperature, after which two molar equivalents of sodium methoxide and one molar equivalent of methyl-4-formyl benzoate were added, and stirred for 18 more hours. The solution was then diluted with 12.0 liters of distilled water and the precipitate was filtered under suction and then washed successively with 200 mL of toluene and diethyl ether. The solid was then dried under a vacuum, to yield 62.0 grams of the title compound (75% yield).

^{1}H NMR: 8.85δ (s,1H); 8.0δ-7.65δ (d,4H); 7.85δ-7.5δ (d,2H); 3.8δ (s,3H).

Example 4

Methyl-4-amino-4-deoxy pteroate

40 grams of the intermediate from Example 3 above was dissolved in 10.0 liters of glacial acetic acid, and 20.0 grams of 10% palladium on carbon catalyst added and hydrogenated at ambient temperature and pressure. The reaction was stopped after 24 hours and the palladium filtered out of the solution. The filtrate was then heated to 45° C and purged with air for four hours and evaporated the solvent or in alternate method the filtrate was diluted with 3.0 liters of 3% hydrogen peroxide and stirred four more hours at room temperature. Evaporation of the solvent at reduced pressure yielded 32.0 grams of the title compound representing a yield of 80%.

^{1}H NMR: 8.5δ (s,1H); 7.82δ-7.4δ (d,4H); 7.45δ-6.5δ (br.s 4H); 3.8δ (s,3H); 3.1δ (s,4H).

Example 5

4-Amino-4-deoxy pteroic Acid

20.0 grams of the intermediate from Example 4, above, was dissolved and stirred in 500 mL of 0.5 N sodium hydroxide and 500 mL of 2-methoxyethanol at room temperature for 24 hours. The solution was concentrated to 100 mL, filtered, and the filtrate acidified with glacial acetic acid to pH 4.5. A copious yellow precipitate formed and was refrigerated overnight, then filtered, washed with distilled water and dried. The crude product was determined to be 90% pure by HPLC, and resulted in the recovery of 17.0 grams of the title compound (90% yield).

^{1}H NMR: 8.45δ (s,1H); 7.8δ-7.3δ (d,4H); 7.5δ-6.7δ (br.s, 4H); 3.1δ (s,4H).

MS(FAB): 310

Example 6

Methyl-4-amino-4-deoxy-10-methoxycarbonyl-pteroate

3.0 grams of dimethyl homoterephthallate is slowly added to a stirred suspension of 1.64 grams of 35% potassium hydride in mineral oil and 12 mL of dry N,N-dimethyl formamide which has been cooled to 0° C. After 30 minutes the yellow solution was cooled to -40° C, and 1.42 grams of 6-bromomethyl pteridine hydrogen bromide (0.33 molar equivalents) in 5 mL of dry DMF was added over a period of 10 minutes. Alternatively, one molar equivalent of 6-bromomethyl pteridine may be used. The mixture was brought to room temperature and stirred for two more hours. The solvent was then removed by vacuum, and the residue was extracted with chloroform and then dried over anhydrous magnesium sulfate, then concentrated in vacuo to yield 2.0 grams (~60% yield) of the title compound.

^{1}H NMR: 8.8δ (s,1H); 7.9δ-7.3δ (d,4H); 7.6δ-6.6δ (br.s,4H); 4.62δ (t,1H); 3.5δ-3.55δ (s,6H); 3.3δ-3.5δ (m,2H).

Example 7

4-amino-4-deoxy-pteroic acid

0.55 grams (1.44 mmoles) of the intermediate from Example 7 above, and 0.21 grams (4.32 mmoles) of sodium cyanide were dissolved in 10 mL of dimethylsulfoxide and stirred for 3 hours at 175° C-180° C for 3 hours. The dark mixture was cooled, and the solvent removed by vacuum. The residue was dissolved in 15 mL of distilled water, filtered, and the filtrate acidified with glacial acetic acid. The precipitate was collected and washed with water to obtain 0.41 grams of crude pteroic acid in quantitative yield.

^{1}H NMR: 8.45δ (s,1H); 7.8δ-7.3δ (d,4H); 7.5δ-6.7δ (br.s,4H); 3.1δ (s,4H).

MS(FAB): 310

The pteroic acid intermediate is coupled with γ-methylene glutamic acid (or an ester thereof) to form MDAM. The coupling process is carried out as described in any of the above referenced patents or publications.

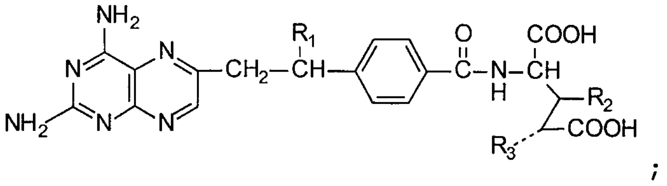
Further synthesis of Formula II compounds may be carried out as disclosed in PCT Publication WO 91/10666, published July 25, 1991.

The above description does not limit the invention to the details given above, but may be modified within the scope of the following claims.

What Is Claimed Is:

1. A process for synthesizing a compound of the formula:

(II)



;

wherein R_1 is hydrogen or lower alkyl;

R_2 is hydrogen or hydroxy;

R_3 is hydrogen or methylene; and

the dashed line indicates a single bond or a double bond;

said process comprising the steps of:

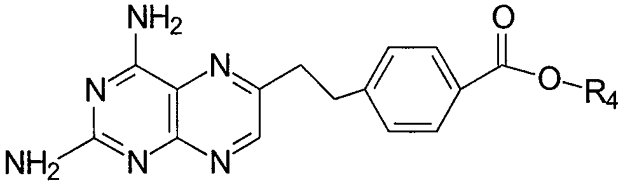
a) providing a starting reagent 2,4,5,6-tetraamino pyrimidine, or a salt thereof;

b) reacting the starting reagent with 1,3-dihydroxy-2-propanone to form the intermediate compound, 6-hydroxymethyl pteridine;

c) reacting the intermediate compound with a brominating agent to form 6-bromomethyl pteridine;

d) alkylating the 6-bromomethyl pteridine to form an esterified intermediate of the formula:

(IV)



;

wherein R_4 is hydrogen or lower alkyl;

e) hydrolyzing the formula IV compound to form the acid homologue thereof;

f) reacting the acid homologue of the formula IV compound with a γ-methylene glutamate ester to form an esterified form of the formula II compound; and

g) hydrolyzing the esterified compound of step f)) to form the formula II compound.

2. The process of Claim 1 wherein step d) includes alkylating the 6-bromomethyl pteridine with a homoterephthallic acid ester, then decarboxylation at the 10 position and hydrolysis of the resulting pteroate ester to form the formula IV compound.

3. The process of Claim 1 wherein step d) includes alkylating the 6-bromomethyl pteridine with a 4-acyl benzoic acid ester, then reducing the resulting pteroate ester under catalytic conditions followed by reoxidation to the formula IV compound.

4. The process of Claim 1 wherein the starting reagent is present as the sulfate salt thereof.

5. The process of Claim 1 wherein step c) includes brominating the hydroxymethyl intermediate with an excess of dibromotriphenylphosphine in an organic solvent to form 6-bromomethyl pteridine hydrogen bromide salt.

6. The process of Claim 5 wherein step c) further includes recrystallizing the hydrogen bromide salt in an alcohol and acid mixture to form the hydrogen bromide isopropanol salt of 6-bromomethyl pteridine.

7. A process for producing γ-methylene-10-deazaaminopterin comprising the steps of:

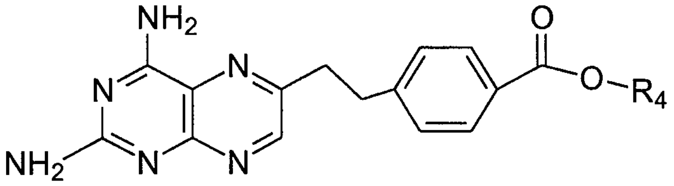
a) providing as a starting reagent 2,4,5,6-tetraamino pyrimidine, or a salt thereof;

b) reacting the starting reagent with 1,3-dihydroxy-2-propanone to form the intermediate compound, 6-hydroxymethyl pteridine;

c) reacting the intermediate compound with a brominating agent to form 6-bromomethyl pteridine;

d) alkylating the 6-bromomethyl pteridine to form an esterified intermediate of the formula:

(IV)



;

wherein R_4 is hydrogen or lower alkyl;

e) hydrolyzing the formula IV compound to form the acid analog thereof;

f) reacting the acid homologue of the formula IV compound with a diethyl γ-methylene glutamate to form an esterified form of γ-methylene-10-deazaaminopterin; and

g) hydrolyzing the esterified compound of step f)to form γ-methylene-10-deazaaminopterin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21743

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 475/08
US CL :544/258, 260
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. : 544/258, 260

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)

CA file, CA PLUS UNPATFULL
CAS REACT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,684,653 A (TAYLOR et al.) 1987, column 7, lines 1-11 and 32-48.	1-7
Y	US 5,374,726 A (DEGRAW et al.) 20 December 1994, columns 3-4 Scheme II, lines 13-68.	1-7
Y	US 4,369,319 A (DEGRAW et al.) 18 January 1983, columns 3-4 "STAGE 7", "STAGE 8" to column 6, line 11. line 65.	1-7
Y	US 4,753,939 A (DEGRAW et al.) 28 June 1988, columns 3-4 "STAGE 7" to "STAGE 9", line 65.	1-7
Y	US 5,073,554 A (M. G. NAIR) 17 December 1991, columns 7-8 "SCHEME 2" to column 10, line 11.	1-7

☒ 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 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
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"O"	document referring to an oral disclosure, use, exhibition or other means		
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,996,207 A (NAIR et al.) 26 February 1991, column 6, lines 30-68, column 11, lines 1-37.	1-7
Y	BAUGH et al. The Synthesis of 6 Hydroxymethylpteridines. J. Org. Chem. 1964, Vol. 23, pages 3610-3612, especially page 3610, column 1 through paragraph bridging column 2.	1-7
Y	PIPER et al. Preparation of 6-(bromomethyl)-2,4-pteridinediamine Hydrobromide and its use in improved synthesis of Methotrexate and Related Compounds. J. Org. Chem. 1977, Vol. 42, No. 2, pages 208-211, especially page 208, column 2 first full paragraph, page 209, column 2, paragraph bridging page 210 and page 210 column 1, first full paragraph.	1-7
Y	NAIR, M. G. Folate Analogues, 24. Syntheses of the antitumor agents 10-Deazaaminopterin (10-DAAM) and 10-ethyl-10-deazaaminopterin (10-EDAAM). J. Org. Chem. 1995, Vol. 50, No. 11, pages 1879-1884, especially paragraph bridging page 1883-1884.	1-7
A	US 5,550,128 A (NAIR et al.) 27 August 1996, column 6, lines 2-59.	1-7