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(54) Title: A PROCESS FOR PREPARING INTERMEDIATES OF 10-PROPARGYL-10-DEAZAAMINOPTERIN (PRALATREXATE) SYNTHESIS AND THE INTERMEDIATES THEREOF

(57) Abstract: A process for preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid and other key intermediates in synthesis of 10-propargyl-10-deazaaminopterin (Pralatrexate) and the intermediates thereof. The 10-propargyl-10-deazaaminopterin (Pralatrexate) is obtained by peptide formation and ester hydrolysis of the intermediate compound 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid by methods known in the art.

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A PROCESS FOR PREPARING INTERMEDIATES OF 10-PROPARGYL-10-DEAZAAMINOPTERIN (PRALATREXATE) SYNTHESIS AND THE INTERMEDIATES THEREOF

FIELD OF INVENTION

The invention relates to a process for preparing intermediates of 10-propargyl-10-deazaaminopterin (Pralatrexate) synthesis and the intermediates thereof. More particularly, the invention relates to a novel process for preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14) a key intermediate in synthesis of Pralatrexate. The invention also relates to novel intermediates of the above process represented by compounds of formulae 2-16 in the graphical schemes 1, 2, 3 and 4 and a process of preparing such intermediates.

BACKGROUND

Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of T-cell neoplasms. PTCL is a rare form of blood cancer that has a poor prognosis and a high relapse rate. Most patients with PTCL have a poor outcome with conventional therapies and are not cured without stem-cell transplantation.

Pralatrexate, is a 10-deazaaminopterin derivative which has been developed for the potential treatment of malignancies. Pralatrexate is an antifolate, structurally a folate analog inhibitor of dihydrofolate reductase (DHFR) exhibiting high affinity for reduced folate carrier-1 (RFC-1) and folylpolyglutamate synthetase (FPGS), with antineoplastic and immunosuppressive activities, resulting in extensive internalization and accumulation in tumour cells. Pralatrexate selectively enters cells expressing RFC-1. Intracellularly, this agent is highly polyglutamylated and competes for the folate binding site of DHFR, blocking tetrahydrofolate synthesis, which may result in depletion of nucleotide precursors; inhibition of DNA, RNA and protein synthesis; and apoptotic tumor cell death. Efficient intracellular polyglutamylation of pralatrexate results in higher intracellular concentrations compared to non-polyglutamylated pralatrexate, which is more readily effuxed by the MRP (multidrug resistance protein) drug efflux pump.

RFC-1, an oncofetal protein expressed at highest levels during embryonic development, may be over expressed on the cell surfaces of various cancer cell types. Pralatrexate is the first and only drug approved by the Food and Drug Administration as a treatment for relapsed or refractory peripheral T-cell lymphoma, demonstrating the ability to reduce tumor size, but not to prolong life.

Pralatrexate is a folate analog metabolic inhibitor that competitively inhibits dihydrofolate reductase. It is also a competitive inhibitor for polyglutamylation by the enzyme folylpolyglutamyl synthetase. This inhibition results in the depletion of thymidine and other biological molecules the synthesis of which depends on single carbon transfer.

US 2005102671 17 discloses that T cell lymphoma is treated by administering to a patient suffering from T cell lymphoma a therapeutically effective amount of 10-propargyl-10-deazaaminopterin. Remission is observed in human patients, even with drug resistant T cell lymphoma at weekly dosages levels as low as 30 mg/m2. In general, the 10-propargyl-10-deazaaminopterin is administered in an amount of from 30 to 275 mg/m2 per dose.

US 2011/0190305 discloses diastereomers of 10-propargyl-10-deazaminopterin, compositions comprising optically pure diastereomers of 10-propargyl-10-deazaminopterin, in particular the two (R,S) diastereomers about the C10 position, method of preparation of the diastereomers and method of treatment of conditions related to inflammatory disorders and cancer.

US005354751 discloses heteroaroyl-10-deazaaminopterins and 10-alkenyl or 10-alkynyl-10-deazaaminopterins having pronounced anti-inflammatory activity, anti-leukemic and anti-tumorigenic activity, as well as a method for treatment of inflammatory diseases, leukemia and tumors. Pharmaceutical compositions containing these heteroaroyl-10-deazaaminopterin compounds are also disclosed. The invention further concerns a process for preparation of these compounds. A method for preparation of 10-propargyl-10-deazaaminopterin compound is also disclosed in this document.

Journal publication Bioorganic and Medicinal Chemistry (19) 2011, page 1151, synthetic approaches to the 2009 new drugs, also discloses a method for synthesis of Pralatrexate. The method comprises alkylating dimethyl homotrephthalate with propargyl bromide in the presence of KH in THF and then with 2,4-diamino-6-(bromomethyl)pteridine hydrobromide in the presence of KH in DMF to afford crude product. Subsequent hydrolysis of the diester with aqueous NaOH, followed by acidification with acetic acid to give crude carboxylic acid, followed by thermally induced decarboxylation in DMSO to give 10-deazapteroic acid derivative. Activation of carboxylic acid as a mixed anhydride using t-butyl chloroformate prior to coupling with diethyl L-glutamate hydrochloride in the presence of Et_3N in DMF to give 10-propargyl-10-deaza-aminopterin diethyl ester. Finally, saponification of diethyl ester with aqueous NaOH in 2-methoxyethanol, followed by acidifying with AcOH giving Pralatrexate.

Methods of preparing Pralatrexate known in the prior art are not only complicated but preparation of Pralatrexate using the methods disclosed in the prior art also result in very high manufacturing cost. Therefore, there is a need for an improved, simple and cost effective method for preparation of Pralatrexate which can be used for industrial scale preparation of this compound.

OBJECT OF THE INVENTION

The primary object of the invention is to provide a novel process for preparing 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid, a key intermediate in the synthesis of 10-propargyl-10-deazaaminopterin (Pralatrexate).

Another object of the invention is to provide novel intermediates 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid or alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate which are building blocks of the process of synthesizing 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid.

Another object of the invention is to provide process for preparation of such novel intermediates.

SUMMARY OF THE INVENTION

Accordingly, the invention discloses a novel process for preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14) which is a key intermediate in synthesis of 10-propargyl-10-deazaaminopterin (Pralatrexate). The 10-propargyl-10-deazaaminopterin (Pralatrexate) can be prepared from compound 14 through peptide formation and ester hydrolysis using several methods known in the art.

The invention also provides novel intermediates 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid or alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate represented by Figure-1, wherein the ester group can be selected from the group consisting of methyl, ethyl, isopropyl, butyl, phenyl and any alkyl or aryl functionality. The aldehyde function could be masked by an N-oxime, an acetal, a thioacetal, 3-alkylthiazolidine or any other group which could generate an aldehyde. These novel intermediates are building blocks of the novel process of synthesizing 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14)

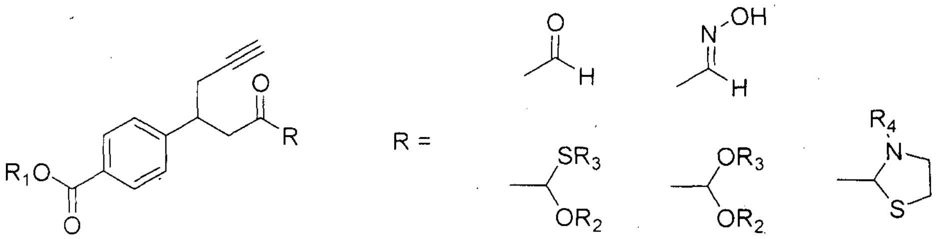


Figure-1

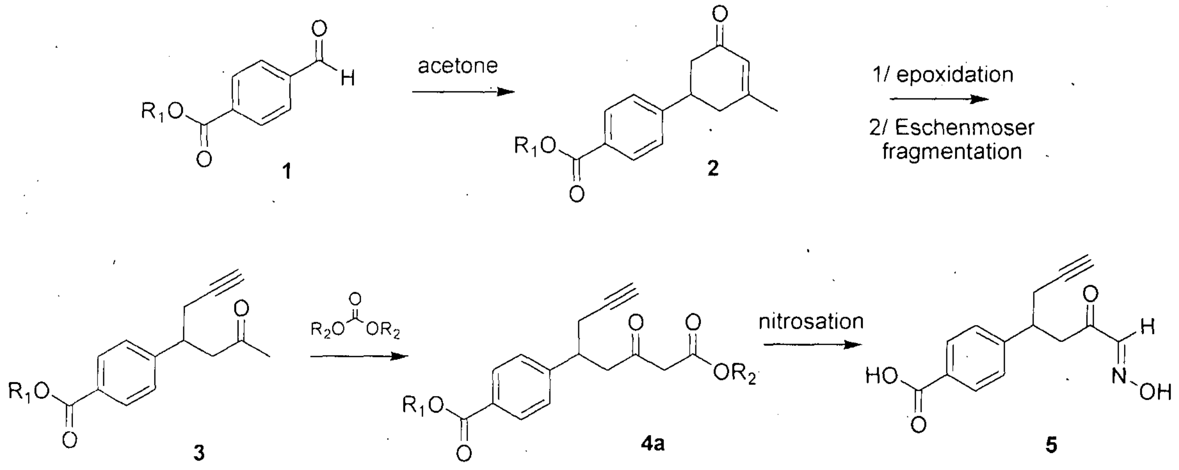
Wherein, R_1, R_2, R_3 and R_4 are alkyl or aryl groups and have same definition wherever used in the description.

Preparation of intermediates alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid

The preparation of novel intermediates alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid can be achieved by three different routes described hereinafter as route A, route B and route C.

Route A-

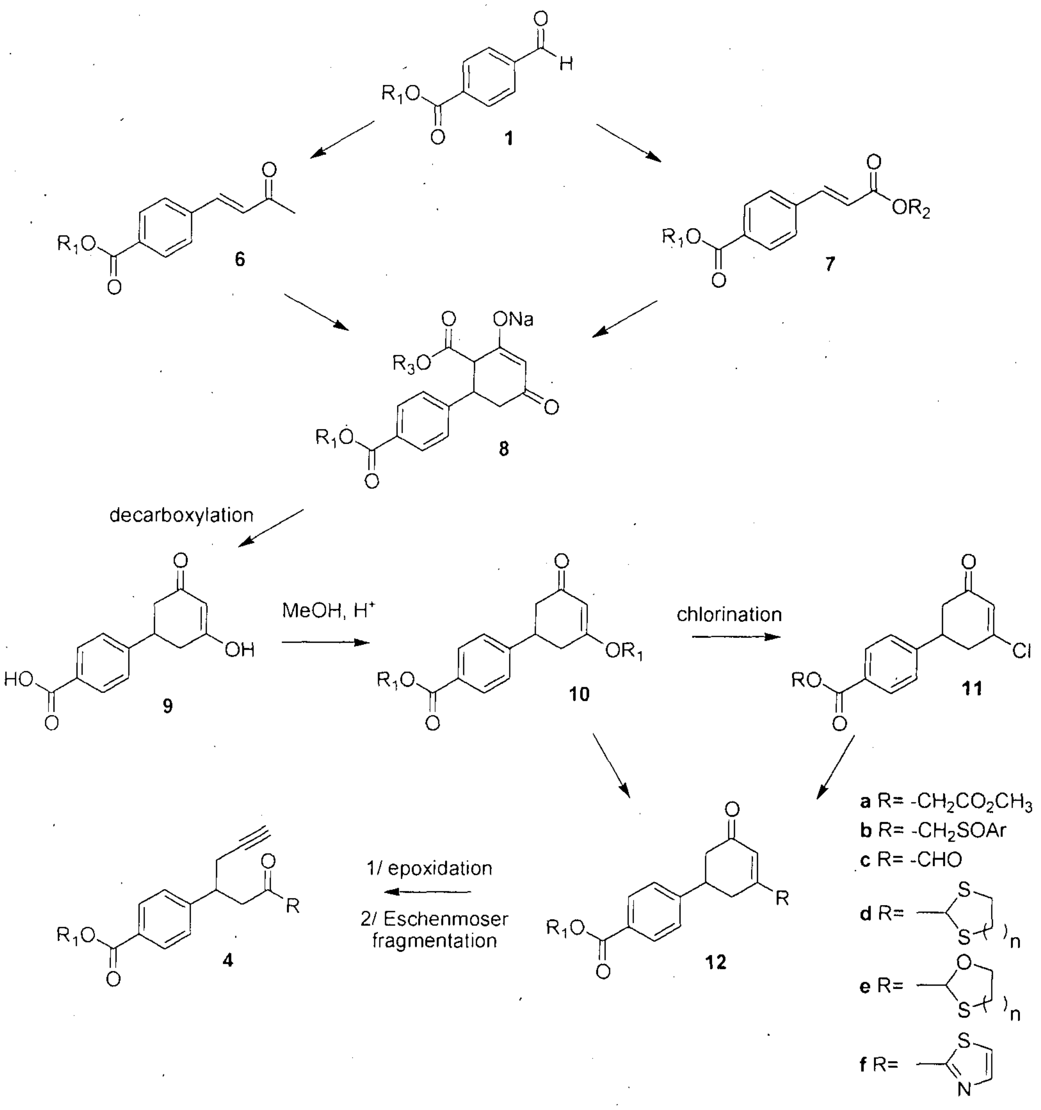
In the first route (Route A), the method comprises: treating 4-formyl benzoate (compound **1**) with a suitable base in an appropriate solvent with acetone or an equivalent of acetone to obtain alkyl 4-(3-methyl-5-oxocyclohex-3-enyl)benzoate (compound **2**); preparing the epoxide compound **3** with suitable oxidizing agents that are used to obtain epoxides from alkenes; reacting the epoxy ketone with a suitable sulfonyl hydrazine *via* formation of a hydrazone to obtain alkyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound **3**) though a process known in the art as the Eschenmoser fragmentation; treating ketone (compound **3**) with a suitable base in an appropriate solvent with alkyl or halogenoformate to obtain the keto-ester (compound **4a**); hydrolyzing the ester functionality of compound **4a** and subsequent nitrosation of the resulting keto acid using a metal or organic nitrite to obtain oxime compound **5**. The method is illustrated below in scheme-1.



Scheme-1

Route B-

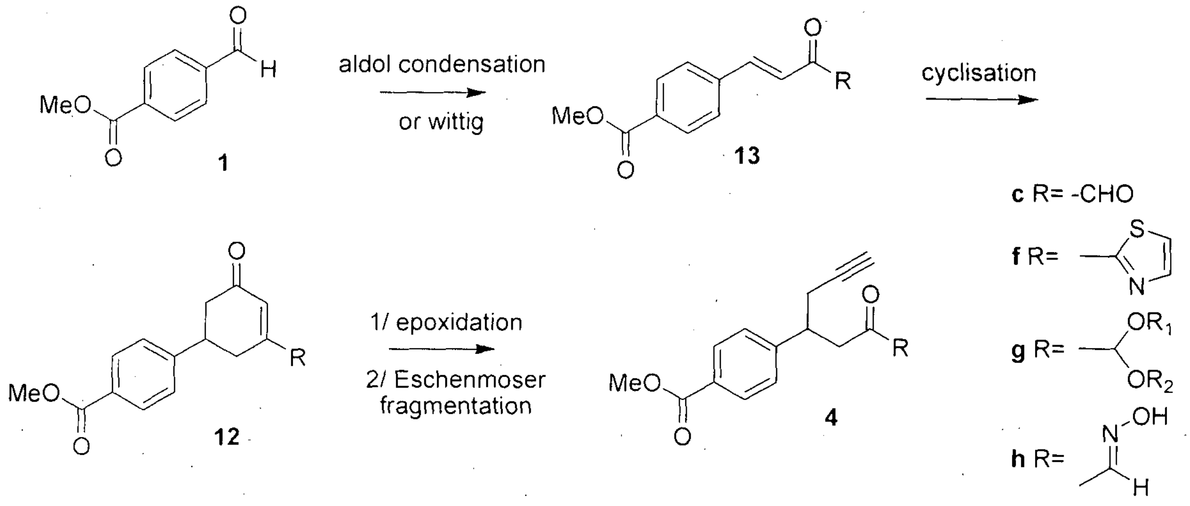
In the second approach (Route B), the method comprises: treating 4-formyl benzoate (compound **1**) with acetone or an equivalent of acetone with a suitable base in an appropriate solvent to afford alkyl 4-(3-oxobut-1-enyl)benzoate (compound **6**); treating compound **6** with a suitable di-ester of malonic acid, preferably dimethyl malonate in a suitable solvent using an appropriate base to obtain compound **8**; treating 4-formyl benzoate (compound **1**) with a suitable di-ester of malonic acid or Wittig reagent with a suitable base in an appropriate solvent to afford give alkyl 4-(2-(alkoxycarbonyl)vinyl)benzoate (compound 7); treating compound 7 with alkyl acetoacetate with a suitable base in an appropriate solvent to afford compound **8**; hydrolysis of the ester functionality in compound **8** and immediate decarboxylation of the resulting carboxylate salt which on acidification with any suitable Bronsted acid gives alkyl 4-(3-hydroxy-5-oxocyclohex-3-enyl)benzoic acid (compound **9**); subjecting the benzoic acid (compound **9**) to esterification and enolate alkylation under acidic conditions to obtain alkyl 4-(3-alkoxy-5-oxocyclohex-3-enyl)benzoate (compound **10**); selective hydrolysis of the enolate with a suitable acid in an appropriate solvent to afford alkyl 4-(3-hydroxy-5-oxocyclohex-3-enyl)benzoate followed by halogenation, preferably chlorination of the 1,3 di-ketone to give alkyl 4-(3-chloro-5-oxocyclohex-3-enyl)benzoate (Compound **11**);); reacting compound **10** or **11** with an appropriate nucleophile (**a**: an alkyl aceto acetate, b : lithium salt of 1-(methylsulfinyl)aryl d: 2-lithio 1,3-dithiane or 2-lithio 1,3-dithiolane, e: 2-lithio 1,3-oxathiane or 2-lithio 1,3-oxathiolane, f: 2-lithio-1,3-thiazole) to obtain compound **12**; epoxidising compound **12** with suitable epoxidising agents to generate an epoxide; reacting this epoxy ketone with a suitable sulfonyl hydrazine *via* formation of a hydrazone to obtain compound **4**. The method is illustrated below in scheme-2.



Scheme 2

__Route C-__

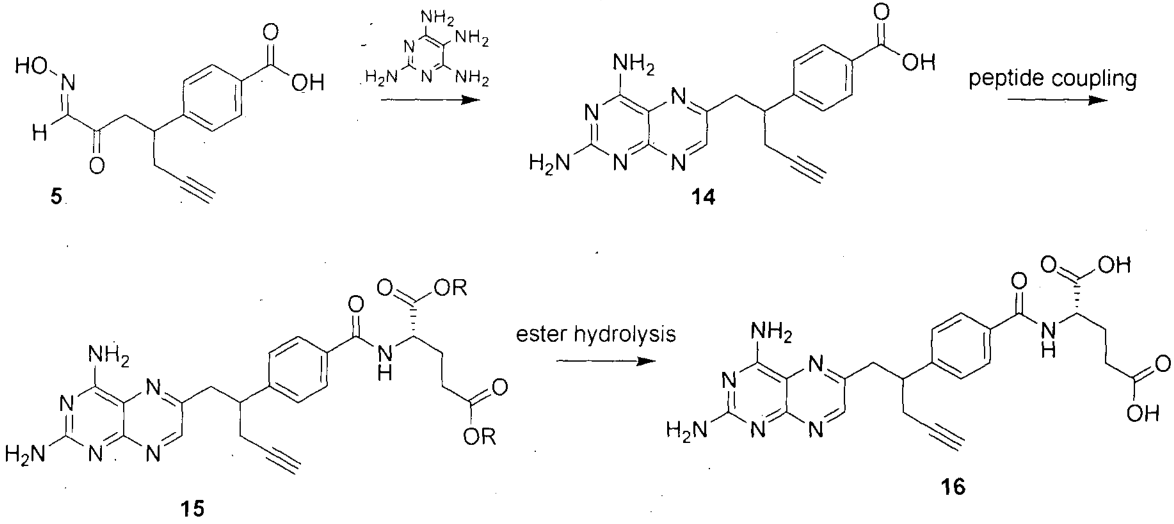
In the third approach (Route C), the method comprises: treating 4-formyl benzoate (compound **1**) with a Wittig reagent or the appropriate methyl ketone and a suitable base in an appropriate solvent to afford an alkene (compound **13**); treating compound **13** with acetone or an equivalent of acetone with a suitable base in an appropriate solvent to afford compound **12**; epoxidising compound **12** with suitable epoxidising agents to generate an epoxide; reacting this epoxy ketone with a suitable sulfonyl hydrazine *via* formation of a hydrazone to obtain compound **4**. The method is illustrated below in scheme-3.



Scheme 3

Preparation of 10-propargyl-10-deazaaminopterin (Pralatrexate)

The method for the preparation of 10-propargyl-10-deazaaminopterin (Pralatrexate) comprises of; treating pyrimidine-2,4,5,6 tetramine or its salt with the oxime compound **5** to obtain 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound **14**); treating the acid compound **14** with an optically active or racemic form of di-alkyl glutamate using a suitable coupling agent to facilitate amide bond formation under ambient conditions to obtain (S)-di-alkyl-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioate (compound **15**); obtaining (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl) benzamido) pentanedioic acid (compound **16**) by ester hydrolysis of compound **15**. The method is illustrated below in scheme 4.



Scheme 4

The obtained compound **16** (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioic acid or 10-propargyl-10-deazaaminopterin or Pralatrexate can be converted into pharmaceutically acceptable salts and can be used for pharmaceutical compositions with pharmaceutically acceptable excipients. In one embodiment, the salt maybe a hydrochloric (HCl) salt. The obtained compound **16** (Pralatrexate) or its salts or pharmaceutical compositions comprising optically active diastereomers can be used in therapeutic applications such as the treatment of cancers and inflammatory ailments. The pharmaceutical compositions can be used for the treatment of prostate cancer, T-cell lymphoma, breast cancer, lung cancer, hematologic malignancies, head and neck cancer, cancer of the gastrointestinal tract, ovarian cancer, osteosarcoma and rheumatoid arthritis.

Such pharmaceutical compositions may used for preparation of oral formulations or injectables for intravenous administration.

DETAILED DESCRIPTION OF THE INVENTION

Detailed embodiments of the present invention are disclosed herein below. However, it is to be understood that the disclosed embodiments are merely exemplary of the invention, which can be embodied in various forms. The scope of the invention is not limited to the disclosed embodiments and terms and phrases used herein are not intended to be limiting but rather to provide an understandable description of the invention. The invention is defined by claims appended hereto.

The invention relates to a process for preparation of Pralatrexate or 10-propargyl-10-deazaaminopterin and its intermediates.

One of the novel intermediates of the process are alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid, represented by figure-1 below, wherein the ester group can be selected from the group consisting of methyl, ethyl, isopropyl, butyl, phenyl and any alkyl or aryl functionality. The aldehyde function could be masked by an N-oxime, an acetal, a thioacetal, 3-alkylthiazolidine or any other group which could generate an aldehyde.

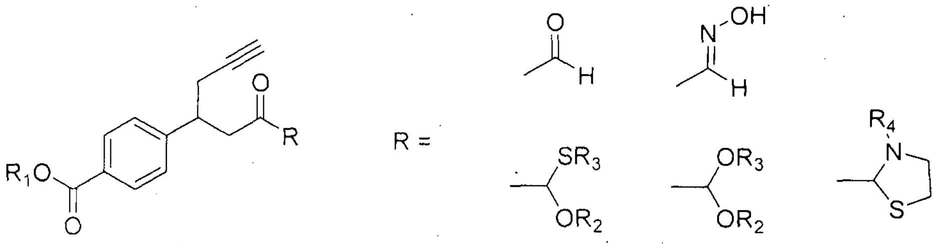


Figure-1

Wherein, R_1, R_2, R_3 and R_4 are alkyl or aryl groups and have same definition wherever used in the description

The compound of the formula shown in Figure-1 is one of the key intermediates of the present process and can be prepared by three alternative processes (route A, route-B and route-C). These alternative processes of preparing the key intermediate alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid are illustrated hereinafter as scheme-1, scheme-2 and scheme-3.

Scheme-4 hereinafter illustrates the method of preparation of 10-propargyl-10-deazaaminopterin (Pralatrexate) from the novel intermediate prepared by the process.

Different novel intermediates prepared by the present process are represented by the compounds of formulae 2-16 illustrated by graphical schemes 1, 2, 3 and 4.

Preparation of intermediates alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid

As described above, among various novel intermediates prepared in the process, alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid are one the key intermediates in the whole process of preparing 10-propargyl-10-deazaaminopterin (Pralatrexate) from 4-formyl benzoate and can be prepared by three different routes described hereinafter as route A, route B and route C.

Route-A

Stage 1: Preparation of alkyl 4-(3-methyl-5-oxocyclohex-3-enyl)benzoate (compound 2)

Compound 2 is obtained by treating an alkyl 4-formyl benzoate with a suitable base in an appropriate solvent with acetone or an equivalent of acetone. The ester group can be selected from the group consisting of methyl, ethyl, isopropyl, butyl, phenyl and any alkyl or aryl functionality and the equivalent of acetone can be selected from any alkyl acetoacetate, 3-oxobutanoic acid, dialkyl 3-oxopentanedioate, 3-oxopentanedioic acid or 2,4 pentadione. In preferred embodiment of the invention, the selected ester group is methyl with a suitable base which can be either an inorganic or organic base in an appropriate solvent to promote the carbon-carbon bond forming step *via* an Aldol type reaction involving the reaction of an enolate derived from the acetone or the equivalent of acetone with the electrophilic aldehyde. The α,β-unsaturated carbonyl compound formed undergo another addition of acetone or the equivalent of acetone followed by an intramolecular Aldol type reaction to give compound **2**. In the preferred embodiment of the invention, this reaction is done in one-pot but the intermediate could also be isolated.

Stage 2: Preparation of alkyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound 3)

The epoxide is prepared by conditions known in the art to generate an epoxide. Several reagents are available to achieve this transformation such as hydrogen peroxide, organic peroxides, peracetic acids, perbenzoic acids and related derivatives, metal hypochlorites, metal salts of peroxy sulfates and other oxidizing agents known in the art as epoxidising agents. In one embodiment, hydrogen peroxide was used while in another embodiment, alkyl peroxide such as *tert*-butyl hydroperoxide was used in alkaline conditions.

Compound 3 was prepared *via* formation of a hydrazone obtained by reaction of the previous epoxy ketone with a suitable sulfonyl hydrazine that can result in the subsequent opening of the 6 membered cyclic ring through a process known in the art as the Eschenmoser fragmentation. The reaction can be done in any organic solvent and a catalytic amount of Bronsted or Lewis acid can help accelerate this reaction. In one preferred embodiment, the solvent was acetic acid and the transformation was done at ambient temperature.

An alternative route to obtain **3** from compound **2** is to form the hydrazone of α, β-unsaturated ketone **2** with a suitable sulfonyl hydrazine which undergoes an alkynone fragmentation when treated with electrophiles under basic conditions

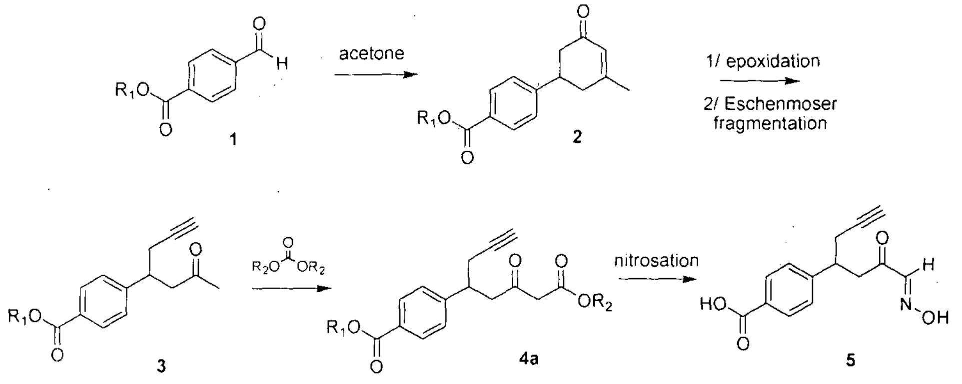
Stage 3: alkyl 4-(1-(alkoxycarbonyl)-2-oxohept-6-yn-4-yl)benzoate (compound 4a)

Compound 4a is obtained by treating the alkyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound 3) with a suitable base in an appropriate solvent with the appropriate source alkoxycarbonyl group which could be selected for example from dialkyl carbonate, alkyl halogenoformate or alkyl cyanoformate.

Stage 4: 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid (compound 5)

Compound 5 was prepared by the hydrolysis of the ester functionality of compound 4a and subsequent nitrosation of the resulting keto acid using a metal or organic nitrite, preferably sodium nitrite in aqueous acidic conditions which also facilitates the decarboxylation of the acid to obtain the oxime compound 5.

Stages 1 to 4 of Route-A are illustrated in Scheme-1 below-



Scheme-1

Route-B

Stage 1: Preparation of methyl 4-(3-oxobut-1-enyl)benzoate (compound 6)

Compound 6 is obtained by treating 4-formyl benzoate with a suitable base in an appropriate solvent, wherein the ester group can be selected from the group consisting of methyl, ethyl, isopropyl, butyl, phenyl and any alkyl or aryl functionality. In preferred embodiment of the invention, the selected ester group is methyl with a suitable base which can be either an inorganic or organic base in an appropriate solvent to promote the carbon-carbon bond forming step via an Aldol type reaction involving the reaction of an enolate derived from the ketone with the electrophilic aldehyde. The subsequent dehydration of the resulting hydroxyl intermediate can be achieved by treatment with a suitable Bronsted acid at ambient or elevated temperatures to yield the compound of formula 6.

These reactions can be done in ambient and elevated temperatures and preferred base for this process is a metal hydroxide or alkoxide in an aqueous media or a polar solvent or by using the reactant acetone as the solvent.

Stage 2: Preparation of compound 8

Compound 8 can be obtained by treatment of compound **6** with a suitable di-ester of malonic acid, preferably dimethyl malonate in a suitable solvent using an appropriate base that allows for the deprotonation of the malonate, resulting in a conjugate addition to the unsaturated ketone 8 and subsequent cyclization *via* a Dieckmann type condensation to give the compound formula **3** in the form of a salt. The ideal conditions for this transformation are elevated temperatures ranging between 50^0 C to 90^0 C in an alcoholic solvent such as methanol.

Stage 3: Preparation of alkyl 4-(3-hydroxy-5-oxocyclohex-3-enyl)benzoic acid (compound 9)

Compound 9 is prepared by hydrolysis of the ester functionality in compound 8 and immediate decarboxylation of the resulting carboxylate salt which on acidification with any suitable Bronsted acid gives the di-keto substituted benzoic acid. This can be achieved by treating the ester **8** with any metal hydroxide, preferably sodium hydroxide in an aqueous solution and subjecting the resulting carboxylate salt to acidic treatment preferably with sulfuric acid. Both these transformations are done at elevated temperatures.

Stage 4: Preparation of alkyl 4-(3-alkoxy-5-oxocyclohex-3-enyl)benzoate (compound 10)

Compound 10 is prepared by subjecting the benzoic acid 9 to conditions known in the art as esterification and enolate alkylation under acidic conditions. This involves treating the acid with a suitable Bronsted acid, preferably sulfuric acid with an alcohol that will give the required ester functionality. This transformation and the formation of an alkyl enolate which involves a conjugate addition of the alcohol and dehydration of the 1, 3 di-keto functionality is achieved by heating the acidic alcoholic mixture at elevated temperatures which allow for the solvent to reflux for a period of 8 to 20 hours or until completion of the reaction.

Stage 5: Preparation of alkyl 4-(3-chloro-5-oxocyclohex-3-enyl)benzoate (Compound 11)

Hydrolysis of the enolate 10 can be achieved using any weak Bronsted acid such as *para*-toluene sulfonic acid in aqueous conditions. A suitable co-solvent such as acetone can be used to assist dissolution of the organic substrate. The reaction can be performed in ambient or elevated temperatures and is monitored by thin layer chromatography (TLC) for ensuring completion.

Compound 11 is prepared by the treatment of the previous 1,3 di keto by a suitable chlorinating agent to transform the enol into a vinyl chloride. Other halogenating agents can also be used to obtain the related vinyl halogenated species which can be used for the subsequent step and serve the same function as the vinyl chloride 11. The vinyl chloride was obtained by treatment of compound 1,3 diketo with phosphorus tri-chloride and the reaction was performed in a suitable organic solvent, preferably toluene at elevated temperatures ranging between 60^0 C to 120^0 C whilst monitoring the conversion to the product using TLC. A standard work-up of the reaction mixture results in the product 11 which can be used in the next step.

Stage 6: preparation of compound 12

Compound 12 is prepared by the reaction of compound 10 or 11 with an appropriate nucleophile (a: an alkyl aceto acetate, b : lithium salt of 1-(methylsulfinyl)aryl d: 2-lithio 1,3-dithiane or 2-lithio 1,3-dithiolane, e: 2-lithio 1,3-oxathiane or 2-lithio 1,3-oxathiolane, f: 2-lithio-1,3-thiazole). In the preferred embodiment, sodium salt of methyl acetoacetate reacts with compound 11 in refluxing toluene. The transformation involves the conjugate addition and subsequent elimination of the halogen or chlorine group and deacetylation to give compound 12.

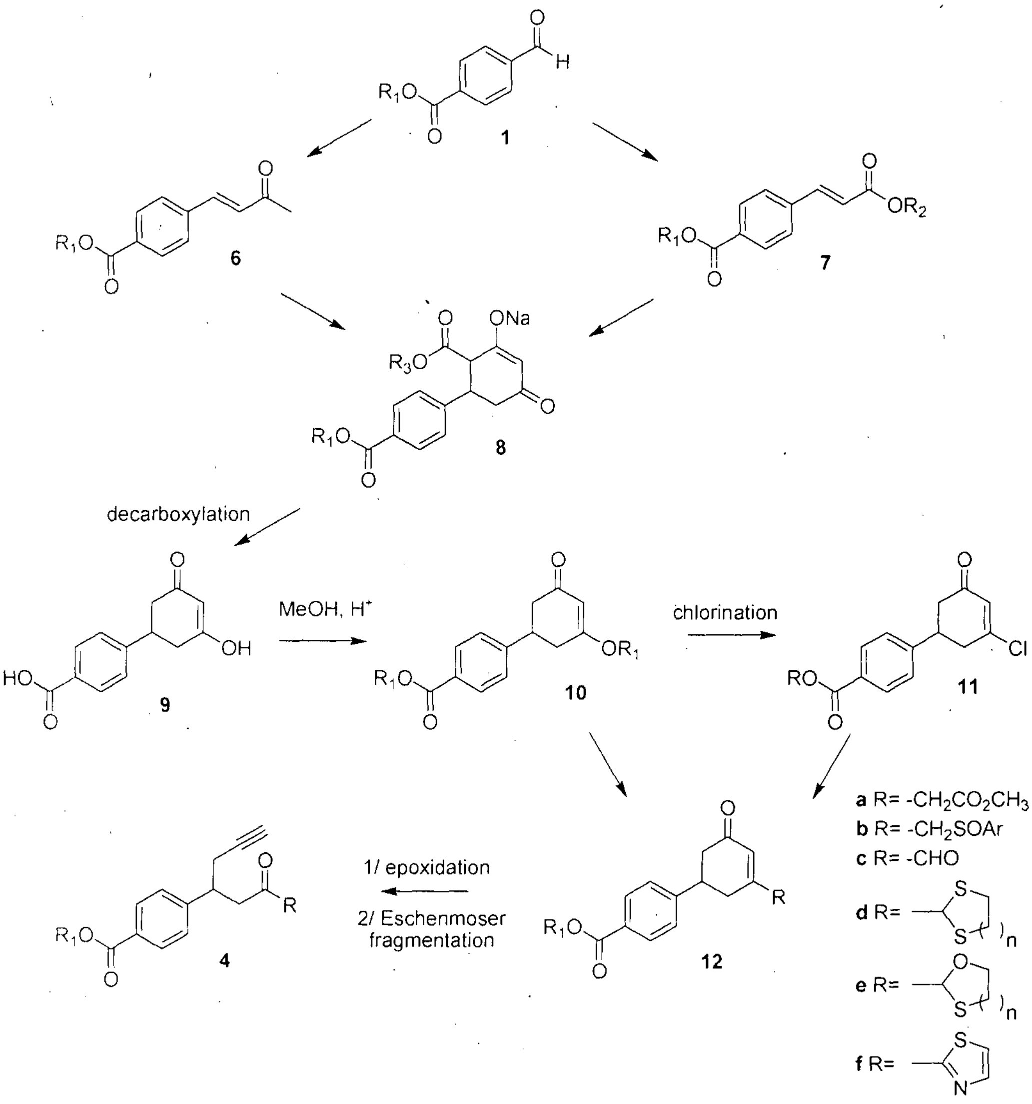
Stage 7: preparation of compound 4

The epoxide is prepared by conditions known in the art to generate an epoxide. Several reagents are available to achieve this transformation such as hydrogen peroxide, organic peroxides, peracetic acids, perbenzoic acids and related derivatives, metal hypochlorites, metal salts of peroxy sulfates and other oxidizing agents known in the art as epoxidising agents. In one embodiment, hydrogen peroxide was used while in another embodiment, alkyl peroxide such as *tert*-butyl hydroperoxide was used in alkaline conditions.

Compound 4 was prepared *via* formation of a hydrazone obtained by reaction of the previous epoxy ketone with a suitable sulfonyl hydrazine that can result in the subsequent opening of the 6 membered cyclic ring through a process known in the art as the Eschenmoser fragmentation. The reaction can be done in any organic solvent and a catalytic amount of Bronsted or Lewis acid can help accelerate this reaction. In one preferred embodiment, the solvent was acetic acid and the transformation was done at ambient temperature. At this stage an additional step maybe required to regenerate the masked aldehyde, for example, an acid or base treatment or a Pummerer rearrangement.

An alternative route to obtain 4 from compound 12 is to form the hydrazone of α, β-unsaturated ketone 12 with a suitable sulfonyl hydrazine which undergoes an alkynone fragmentation when treated with electrophiles under basic conditions.

Stages 1 to 7 of Route-B are illustrated in Scheme-2 below-



Scheme-2

Route-C

Stage 1: preparation of compound 13

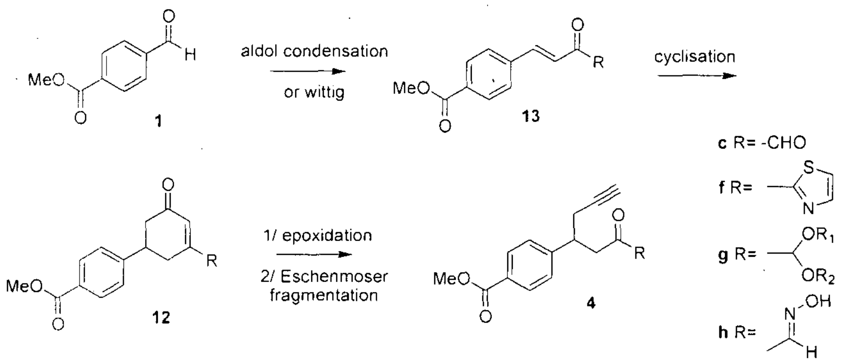
Compound 2 is obtained by treating an alkyl 4-formyl benzoate with a suitable base in an appropriate solvent with the appropriate methylketone (for example: pyruvaldehyde dialkyl acetal or pyruvic aldehyde oxime). The ester group can be selected from the group consisting of methyl, ethyl, isopropyl, butyl, phenyl and any alkyl or aryl functionality. In a preferred embodiment of the invention, the selected ester group is methyl with a suitable base which can be either an inorganic or organic base in an appropriate solvent to promote the carbon-carbon bond forming step *via* an Aldol type reaction involving the reaction of an enolate derived from the ketone with the electrophilic aldehyde. The subsequent dehydration of the resulting hydroxyl intermediate can be achieved by treatment with a suitable Bronsted acid at ambient or elevated temperatures to yield the compound 13.

Stage 2: preparation of compound 12

The α, β-unsaturated carbonyl compound 13 undergoes an addition of acetone or an equivalent of acetone (alkyl acetoacetate, 3-oxobutanoic acid, dialkyl 3-oxopentanedioate, 3-oxopentanedioic acid or 2,4 pentadione) followed by an intramolecular Aldol type reaction to give compound 12. In a preferred embodiment of the invention, this reaction is done in one-pot but the intermediate could also be isolated.

Compound 12 is converted into compound 4 by the process disclosed in stage-7 of Route-B above. The intermediate compound 4 is then converted into oxime compound 5 for next step of preparation of 10-propargyl-10-deazaaminopterin (Pralatrexate).

Various stages of Route-C are illustrated in Scheme-3 on next page.



Scheme-3

Preparation of 10-propargyl-10-deazaaminopterin (Pralatrexate)

10-propargyl-10-deazaaminopterin or Pralatrexate is prepared by following process from the intermediate compound 5 prepared by any of the process route-A or B or C described herein above. The process of preparing 10-propargyl-10-deazaaminopterin from the intermediate compound 5 is also illustrated herein below in scheme-4.

Stage 1: Preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14)

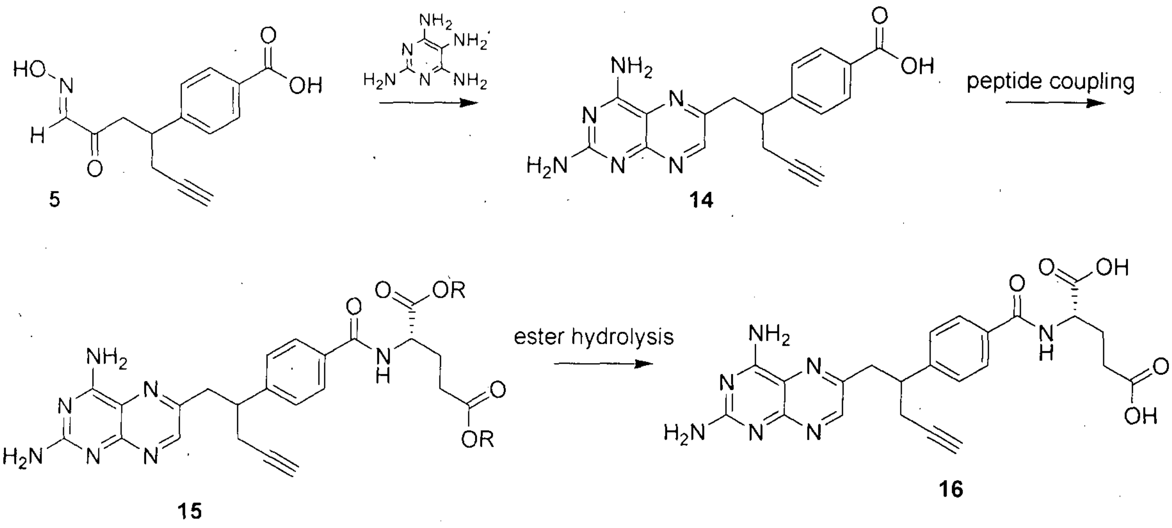
Compound 14 is prepared by treating pyrimidine-2,4,5,6 tetramine or its salt with the oxime compound, 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid (compound 5) in a suitable solvent that facilitates the dissolution of the contents, preferably an alcoholic solvent such as the inexpensive methanol. The reaction can be done in ambient conditions but faster conversion is obtained by performing the process at elevated temperature ranging between 60^0 C to 90^0 C resulting in compound 14.

Stage 2: Preparation of (S)-di-alkyl 2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioate (compound 15)

Compound 15 is prepared by treating the acid compound 14 with an optically active or racemic form of di-alkyl or diaryl glutamate using a suitable coupling agent to facilitate amide bond formation under ambient conditions. Such coupling agents are selected from substituted triazoles or carbodiimides that allow for amide formation without racemization to obtain compound 15.

Stage 3: Preparation of (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido)pentanedioic acid (compound 16)

Compound 16 is prepared by hydrolysis of alkyl ester that constitutes the framework of compound 15. Several methods are known in the art that allow for hydrolysis of the ester without epimerization of the amino acid. In the general embodiment, the alkyl group is a *tert*-butyl and trifluoro acetic acid was used in a suitable organic solvent such as dichloromethane. The hydrolysis of the ester 15 was done to obtain di-acid compound 16 under ambient conditions.



Scheme-4

The obtained compound 16 (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioic acid or 10-propargyl-10-deazaaminopterin or Pralatrexate can be converted into pharmaceutically acceptable salts and can be used for pharmaceutical compositions with pharmaceutically acceptable excipients. In one embodiment, the salt may be a hydrochloric (HCl) salt. The obtained compound 16 (Pralatrexate) or its salts or pharmaceutical compositions comprising optically active diastereomers can be used in therapeutic applications such as the treatment of cancers and inflammatory ailments. The pharmaceutical compositions can be used for the treatment of prostate cancer, T-cell lymphoma, breast cancer, lung cancer, hematologic malignancies, head and neck cancer, cancer of the gastrointestinal tract, ovarian cancer, osteosarcoma and rheumatoid arthritis.

Such pharmaceutical compositions may used for preparation of oral formulations or injectables for intravenous administration.

The above process and different stages of the process are further explained with the help of following examples.

Examples

Example-1: Preparation of methyl 4-(3-methyl-5-oxocyclohex-3-enyl)benzoate (compound 2)

4-formyl methyl benzoate (5g, 30.4 mmol) was charged in a 250ml 3N RBF equipped with guard tube, reflux condenser and addition funnel. To this was added pyrrolidine (0.650g, 9.1 mmol), propanoic acid (0.67g, 9.1 mmol) and acetone 25 ml was added and heated at 50 °C for 14 h. Progress of the reaction was monitored by TLC (20% EtOAc/hexane). After completion of the reaction acetone was distilled under reduced pressure. The obtained residue was purified by column chromatography.

Example-2: Preparation of methyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound 3)

Methanol 150ml, hydrogen peroxide (41ml, 36 mmol) were mixed together in a 250 ml 3N RBF to this was added potassium carbonate (8.4g, 61 mmol) dissolved in 15 ml of water at 0°C. Compound **2** (15g, 61 mmol) dissolved in 30 ml of methanol was added to the reaction mixture at same temperature, and stirred for 3h at 0°C. Progress of the reaction was monitored by TLC (20% EtOAc/hexane). After completion of the reaction, it was diluted with water (100 ml) and extracted with CH_2Cl_2 (2 x 100 ml). Organic layer was washed with water (2x100ml), brine solution (100ml), dried over anhydrous sodium sulphate and concentrated under reduced pressure. The obtained crude residue was stirred with hexane and filtered to give 13.2 g of a white solid.

The previous epoxide (2 g, 7.8 mmol) was charged in 10 ml of acetic acid 10 ml in a 50 ml 3N RBF equipped with guard tube. To this was added tosyl hydrazide (1.45g, 7.8 mmol) and stirred for 1 h at ambient temperature. Progress of the reaction was monitored by TLC (20% EtOAc/hexane). After completion of the reaction, reaction mixture was poured into ice water (50 ml) and extracted with DCM (2x25ml).The organic layer was washed with ice water and dried over sodium sulphate, filtered and concentrated under reduced pressure thus obtained crude was purified by column chromatography.

Example-3: Preparation of methyl 4-(1-(methoxycarbonyl)-2-oxohept-6-yn-4-yl)benzoate (compound 4a)

Sodium hydride (196 mg, 8.4 mmol) was charged in 50ml 2N RBF and washed with anhydrous hexane, THF 5ml was added to it under inert atmosphere. Di-methyl carbonate 2ml was added to it and stirred for 10 min. Compound **3** (1 g, 4 mmol) was diluted with 1ml of di-methyl carbonate and added to reaction mixture at ambient temperature. The overall reaction mixture was heated at 80°C for 4 h. Progress of the reaction was monitored by TLC (20% EtOAc/hexane), after completion of the reaction, reaction mixture was poured in to the ice water and extracted with ethyl acetate (2 X 15ml). Organic layer washed with water and dried over sodium sulphate and concentrated. The obtained residue was purified by column chromatography.

Example-4: Preparation of 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid (compound 5)

Compound 4a (1.4 g, 0.0046 moles, 1 eq) was charged into 100ml 3N RB flask, cooled to 0°C. KOH (0.9 g, 16.07 mmol, 10 ml water) solution was added drop wise, stirred overnight at ambient temperature. Progress of the reaction was monitored by TLC (50% ethyl acetate/hexane). On completion of the reaction, $NaNO_2$ (0.368g, 5.3 mmol, 1.16 eq) was added to reaction mixture at ambient temperature. 50% H_2SO_4 (1.194 g, 12.1 mmol, 2.65 eq) was added drop wise for 15 min at 0°C, stirred for 6 h at ambient temperature. Progress of the reaction was monitored by TLC (50% ethyl acetate/hexane). On completion of the reaction, reaction contents were diluted with water and extracted with ethyl acetate (2x50 ml). Organic layer was dried over sodium sulfate, concentrated to give the crude compound which was purified by column chromatography (10% ethyl acetate/hexane) to yield the required product as a solid.

Example-5: Preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14)

Pyrimidine-2,4,5,6-tetraamine hydrochloride (0.7 g, 3.2 mmol, 1 eq), methanol (25.5ml, 30 times) were charged into 50ml 3N RB flask. Compound **5** (0.88 g, 3.6 mmol, 1 eq) was added to reaction mixture, heated to refluxed for overnight. Progress of the reaction was monitored by TLC (10% methanol/chloroform). On completion of the reaction, reaction contents were cooled to 0°C. The reaction mixture was basified to pH-9-10 was adjusted with aq.NH_3, stirred for 15 min at 0°C to give the solid which was filtered and dried to give the required compound.

Example-6: Preparation of (S)-di-tert-butyl 2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioate (compound 15)

Compound 14 (400 mg, 1.14 mmol, 1 eq) was charged into 100ml 3N RB flask. DMF (10 ml, 25 times) was added, followed by *t*-butyl glutamic ester (327 mg, 1.26 mmol, 1.1 eq). BOP reagent (0.605 g, 1.36 mmol, 1.2 eq) was added to reaction mixture, followed by TEA (461 mg, 4.56 mmol, 4 eq). Overall reaction was stirred for overnight at ambient temperature under nitrogen atmosphere. Progress of the reaction was monitored by TLC (20% methanol/chloroform). On completion of the reaction, reaction contents were quenched with water (20 ml), compound was extracted with DCM (50mlx2) and dried over sodium sulfate, concentrated to give crude which was purified by column chromatography to yield the required product as a solid.

Example-7: Preparation of (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido)pentanedioic acid (compound 16)

Compound 15 (0.47 g, 0.79 mmol, 1 eq) in DCM (20 ml) was charged into 100ml 3N RB flask, cooled to 0°C. TFA (1.88 ml, 4 times) was added drop wise to reaction mixture for 10 min, stirred for overnight at ambient temperature. Progress of the reaction was monitored by TLC (10% methanol/chloroform). On completion of the reaction, DCM was concentrated on rotavapor (at low temperature 35°C) to give the crude which was dissolved in cool water (15 ml). pH was adjusted to 4 with Aq. ammonia solution, stirred for 30 min at ambient temperature to give the solid which was filtered, washed with acetone (20 ml) to yield the required product as off white solid.

Example 8: Preparation of methyl 4-(3-oxobut-1-enyl)benzoate (compound 6)

Starting material, 4-formyl benzoate (25 g, 0.15 moles, 1 eq) and acetone (250 ml, 10 times) were charged into 1L 3N RB flask. Water (50 ml, 2 times) was added and the reaction mixture was cooled to 15°C. 10% NaOH Solution (10 ml) was added dropwise for 30 min at 15°C and the reaction mixture was stirred for 3 hrs at RT. Progress of the reaction was monitored by TLC (30% ethyl acetate/hexane). On completion of the reaction, acetone was removed under vacuum and the residue was poured into ice water. The solid obtained was filtered and washed with water. Aqueous layers were extracted with DCM (2x100ml), dried over sodium sulphate and solvent was removed to give compound which was mixed with above obtained solid. Total compound was charged into 250ml 3N RB flask. 50 ml of methanol, 4 ml of Con. HCl was added to reaction mixture, stirred at 70°C for 4 hrs. Progress of the reaction was monitored by TLC. On completion of the reaction, reaction mixture was cooled to ambient temperature, filtered and dried to give pale yellow solid compound.

Example-9: Preparation of compound 8

Sodium methoxide (1.98 g, 0.036 moles, 1.5 eq), methanol (50 ml, 10 times) were charged into 250ml 3N RB flask. Dimethyl malonate (4.7 g, 0.036 moles, 1.5 eq) was added to reaction mixture, stirred for 15 min. Compound **6** (5 g, 0.024 moles, 1 eq) was added to reaction mixture, refluxed for 3 hrs at 90°C. Progress of the reaction was monitored by TLC (30% ethyl acetate/hexane). On completion of the reaction, methanol was distilled to give the solid which was washed with ethyl acetate to yield the required product as off white solid.

Example-10: Preparation of methyl 4-(3-hydroxy-5-oxocyclohex-3-enyl)benzoate (compound 9)

Compound 8 (6 g, 18.00 mmol, 1 eq) was charged into 250ml 3N RB flask. NaOH (3 g in 20 ml of water, 75 mmol, 4 eq) solution was added, refluxed for 2 hrs at 100°C. Overall reaction was cooled to ambient temperature. H_2SO_4 (10 g in 20ml of water, 100 mmol, 5.5 eq) solution was added drop wise to reaction mixture at 0°C. After completion of the addition, reaction contents were refluxed for 2 hrs at 100°C. The reaction mixture was allowed to cool to ambient temperature to give the solid which was filtered and washed with water, dried to yield the required product as of white solid.

Example-11: Preparation of methyl 4-(3-methoxy-5-oxocyclohex-3-enyl)benzoate (compound 10)

Compound 9 (2.7 g, 11.6 mmol, 1 eq), methanol (27 ml, 10 times) were charged into 250ml 3N RB flask. H_2SO_4 (0.2 ml) was added to reaction mixture, refluxed for overnight. Progress of the reaction was monitored by TLC (Ethyl acetate). On completion of the reaction, methanol was distilled completely to give the residue which was basified with $NaHCO_3$ solution, extracted with ethyl acetate (2 x 50 ml). Combined organic layers were dried over sodium sulfate and concentrated to yield the required product as off white solid (2.1g, 70% yield). Aqueous layer was acidified with dil. HCl and recovered to methoxy acid.

Example-12: Preparation of methyl 4-(3-chloro-5-oxocyclohex-3-enyl)benzoate (compound 11)

Compound 10 (15 g, 57.9 mmol, 1 eq), acetone (150 ml, 10 times) were charged into 500ml 3N RB flask. Water (15 ml, 1 time) was added to reaction mixture, followed by PTSA (1.99 g, 11.5 mmol, 0.2 eq). Overall reaction was refluxed for 5 hrs. Progress of the reaction was monitored by TLC (5% methanol/chloroform). On completion of the reaction, DCM (150 ml) was added to reaction mixture and washed with water (2x150 ml) and the layer were separated, organic layer was dried over sodium sulfate and concentrated to give the crude which was washed with MTBE (30 ml) to yield the required product as off white solid.

The previous solid (37 g, 0.151 moles, 1 eq), toluene (185 ml, 5 times) were charged into 500ml 3N RB flask. PCl_3 (12.4 g, 0.090 moles, 0.6 eq) was added to reaction mixture, refluxed for 1½ hr at 120°C. Progress of the reaction was monitored by TLC (5% methanol/chloroform). On completion of the reaction, reaction contents were poured into ice water and the layer were separated, aqueous layer was extracted with ethyl acetate (150 ml). Organic layer was washed with $NaHCO_3$ (2x150 ml), dried over sodium sulfate and concentrated to give the crude which was washed with IPA (2 times) to yield the required product as off white solid.

Example-13: Preparation of compound 12a

Compound 11 (27 g, 0.102 moles, 1 eq), toluene (135 ml, 5 times) were charged into 500ml 3N RB flask. Sodium salt of methyl aceto acetate (21.1 g, 0.153 moles, 1.5 eq) was added to reaction mixture, refluxed for 4 hrs at 120°C. Progress of the reaction was monitored by TLC (40% ethyl acetate/hexane). On completion of the reaction, reaction contents were poured into ice water and the layer were separated, aqueous layer was extracted with ethyl acetate (100 ml). Organic layer was dried over sodium sulfate and concentrated to give the crude which was purified by column chromatography to yield the required product as liquid.

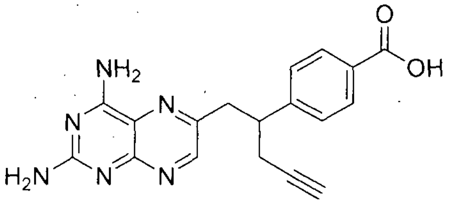
Example-14: Preparation of compound methyl 4-(1-(methoxycarbonyl)-2-oxohept-6-yn-4-yl)benzoate 4a

Compound 12a (2.1 g, 6.9 mmol, 1 eq),.methanol (21 ml, 10 times), water (2.1 ml, 1 time) were charged into 100ml 3N RB flask, cooled to 0°C. 30% H_2O_2 (4.1 ml, 41.7 mmol, 6 eq), K_2CO_3 (0.497 g, 0.0034 moles, 0.5 eq) were added to reaction mixture at 0°C, stirred for 5 hrs at ambient temperature. Progress of the reaction was monitored by TLC (40% ethyl acetate/hexane, Rf-0.4). On completion of the reaction, reaction contents were quenched with water (50 ml), extracted with DCM (2x50 ml). Organic layer was dried over sodium sulfate and concentrated to yield the required product as thick liquid.

Previous epoxide (5 g, 15.7 mmol, 1 eq), DCM (50 ml, 10 times) were charged into the 250ml 3N RB flask. p-nitro phenyl sulfonyl hydrazine (2.98 g, 15.7 mmol, 1 eq) was added to reaction mixture. TFA (0.2 ml) was added to reaction mixture, stirred for 2h at ambient temperature. Progress of the reaction was monitored by TLC (40% EtOAc/hexane). On completion of the reaction, reaction contents were quenched with water and the layers were separated, organic layer was dried over sodium sulfate and concentrated to give crude compound which was purified by column chromatography.

We claim:

1. A process for preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid compound represented by compound 14-



14

wherein the process comprises treating pyrimidine-2,4,5,6 tetramine or its salt with an oxime compound or with a compound depicted in figure-I, to obtain 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid compound (14).

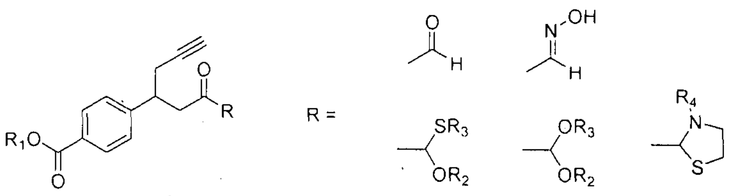


Figure-I

2. A process as claimed in claim 1, wherein said compound of figure-I is alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid.

3. A process as claimed in claim 1, wherein said oxime compound is 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid and is obtained from alkyl 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoate or 4-(1-formyl-1-oxohex-5-yn-3-yl)benzoic acid, depicted in Figure-I.

4. A process as claimed in claim 3, wherein said oxime compound, 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid, is obtained optionally by a process of route-A, route-B or route-C.

5. A process as claimed in claim 4, wherein said oxime compound, 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid, is optionally obtained from a process of route-A comprising:

a). treating alkyl 4-formyl benzoate with acetone or an equivalent of acetone to obtain alkyl 4-(3-methyl-5-oxocyclohex-3-enyl)benzoate compound (2);

b). treating compound 2 with an epoxidizing agent to obtain alkyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound 3) or optionally, obtaining compound (3) from compound (2) by Eschenmoser fragmentation reaction;

c). treating the alkyl 4-(6-oxohept-1-yn-4-yl)benzoate (compound **3**) with a source alkoxycarbonyl group to obtain alkyl 4-(1-(alkoxycarbonyl)-2-oxohept-6-yn-4-yl)benzoate compound (4a);

d). hydrolyzing ester functionality of compound 4a and performing subsequent nitrosation of the resulting keto acid using a metal or organic nitrite in aqueous acidic conditions to obtain the oxime compound 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid compound (5).

6. A process as claimed in claim 5, wherein said equivalent of acetone is selected from alkyl acetoacetate, 3-oxobutanoic acid, dialkyl 3-oxopentanedioate, 3-oxopentanedioic acid and 2,4 pentadione.

7. A process as claimed in claim 5, wherein said epoxidizing agent is selected from the group consisting of hydrogen peroxide, organic peroxides, peracetic acids, perbenzoic acids and related derivatives, metal hypochlorites, metal salts of peroxy sulfates and other oxidizing agents. .

8. A process as claimed in claim 5, wherein said alkoxycarbonyl group is selected from dialkyl carbonate, alkyl halogenoformate and alkyl cyanoformate.

9. A process as claimed in claim 4, wherein said oxime compound, 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid, is optionally obtained from a process of route-B comprising:

a). treating 4-formyl benzoate with a base to obtain methyl 4-(3-oxobut-1-enyl)benzoate compound (6);

b). treating compound (6) with a di-ester of malonic acid to obtain compound (8);

c). hydrolyzing ester functionality in compound (8) and immediate decarboxylation of resulting carboxylate salt followed by acidification to obtain alkyl 4-(3-hydroxy-5-oxocyclohex-3-enyl)benzoic acid compound (9);

d). subjecting compound (9) to esterification and enolate alkylation under acidic conditions to obtain alkyl 4-(3-alkoxy-5-oxocyclohex-3-enyl)benzoate compound (10);

e). hydrolyzing enolate compound (10) with a weak Bronsted acid in aqueous conditions to obtain alkyl 4-(3-chloro-5-oxocyclohex-3-enyl)benzoate compound (11);

f). reacting compound (10) or (11) with a nucleophile to obtain compound (12);

g). epoxidising compound (12) with an epoxidizing agent to obtain compound (4), or optionally, obtaining compound (4) from compound (12) by Eschemoser fragmentation reaction;

h). converting compound (4) into corresponding oxime compound 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid.

10. A process as claimed in claim 9, wherein said nucleophile is a metal salt of alkyl acetoacetate.

11. A process as claimed in claim 4, wherein said oxime compound, 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid, is optionally obtained from a process of route-C comprising :

a). treating alkyl 4-formyl benzoate with a base in a solvent with methylketone to obtain compound (13);

b). adding acetone or an equivalent of acetone to α, β-unsaturated carbonyl compound (13) to obtain compound (12);

c). epoxidising compound 12 with an epoxidizing agent to obtain compound 4, or optionally, obtaining compound (4) from compound (12) by Eschemoser fragmentation reaction;

d). converting compound (4) into corresponding oxime compound 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid.

12. A process as claimed in claim 11, wherein said methylketone is pyruvaldehyde dialkyl acetal or pyruvic aldehyde oxime.

13. A process for preparing (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioic acid comprising subjecting 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid obtained from the process of claim 1, to peptide formation and ester hydrolysis to obtain (S)-2-(4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzamido) pentanedioic acid.

14. A compound 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoate compound (14) obtained from the process as claimed in claim 1.

15. A compound alkyl 4-(6-oxohept-1-yn-4-yl)benzoate compound (3) obtained from the process as claimed in claim 5.

16. A compound alkyl 4-(1-(alkoxycarbonyl)-2-oxohept-6-yn-4-yl)benzoate compound (4a) obtained from the process as claimed in claim 5.

17. A compound 4-(1-formyl oxime-1-oxohex-5-yn-3-yl)benzoic acid compound (5) obtained from the process as claimed in claim 5.

18. A pharmaceutical composition comprising a pharmaceutically acceptable salt of (S)-2-(4-(1-(2.4-diaminopteridin-6-yl)pent-4-yn-2-yl) benzamido) pentanedioic acid or its optically active diastereomers obtained from the process as claimed in claim 13, for treatment of

prostate cancer, T-cell lymphoma, breast cancer, lung cancer, hematologic malignancies, head and neck cancer, cancer of the gastrointestinal tract, ovarian cancer, osteosarcoma and rheumatoid arthritis.

19. A process for preparation of 4-(1-(2,4-diaminopteridin-6-yl)pent-4-yn-2-yl)benzoic acid (compound 14) and key intermediates of 10-propargyl-10-deazaaminopterin synthesis such as herein described in description with reference to the examples.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2013/000284

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D475/08
ADD.

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)
C07D

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)

EPO-Internal, WPI Data, BEILSTEIN Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 354 751 A (DEGRAW JOSEPH I [US] ET AL) 11 October 1994 (1994-10-11) cited in the application	14,19
A	column 39; example 7; compounds IV-4b Rection Scheme 4; column 19 - column 20 -----	1-13
X	US 2011/190305 A1 (PRONK GIJSBERTUS J [US]) 4 August 2011 (2011-08-04) cited in the application	14,18,19
A	paragraph [0068]; example 1; compound 5 claim 5 ----- -/--	1-13

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Stroeter, Thomas

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2013/000284

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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