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DESCRIPTION

[0001] The present invention relates to chemical compounds useful in the treatment of cancer.

[0002] In 1957, the antitumour activity of 5-Fluorouracil (5FU) was discovered. More than fifty years since it was first synthesised, 5FU remains widely used in the treatment of solid tumours including breast, gastrointestinal system, head, neck and ovary and in particular of colorectal cancer, as approved by FDA in 1962. The fluoropyrimidine 5-fluorouracil (5FU) and 5-fluoro-2'-deoxyuridine (5-FdUrd) are used in combination with folic acid as standard treatment for a variety of carcinomas, as stomach, colon and breast. Moreover, a combination of 5FU with leucovorin (LV) is considered as standard chemotherapy for colon cancer. The drug 5FU is usually administered by intravenous bolus or by continuous infusion.

[0003] The antitumour activity of 5FU is comparable to that of its analogue 5-FdUrd, which partly acts as prodrug of 5FU. 5-FdUrd was approved by FDA in 1970, and has been used extensively for the clinical treatment of carcinoma of the ovary, breast and gastrointestinal tract. Moreover due to extensive hepatic extraction 5-FdUrd is a useful drug for hepatic arterial chemotherapy of liver metastases thereby it is more efficiently metabolized by the liver than 5FU.

[0004] A problem exists, however, in that activity of both the agents 5FU and 5-FdUrd can be impaired by the development of resistance in tumour cells. Treatment of cancer with 5FU has also been found to cause neurotoxic and cardiotoxic side effects. Toxicity also derives from the lack of selectivity of 5FU towards tumours.

[0005] It is an object of the present invention to provide compounds derived from 5-fluoro-2'-deoxyuridine that show an enhanced activity and/or reduced toxicity in their treatment of cancer, compared to that shown by 5-fluoracil or 5-fluoro-2'-deoxyuridune *per se.*

[0006] It is a further object of the present invention to provide compounds derived from 5-fluoro-2'-deoxyuridine that show a low level of resistance in tumour cells, in particular a resistance in tumour cells that is less than that shown by 5FU or by 5-FdUrd.

[0007] WO 2005/012327A2 relates to phosphoramidate derivatives of nucleotides and their use in the treatment of cancer.

[0008] WO 99/37753A1 relates to a method for identifying potential therapeutic agents by contacting a target cell with a candidate therapeutic agent which is a selective substrate for an endogenous, intracellular enzyme in the cell which is enhanced in its expression as a result of selection by biologic or chemotherapy. It also relates to methods and examples of molecules for selectively killing a pathological cell by contacting the cell with a prodrug that is a selective substrate for an endogenous, intracellular enzyme. The prodrug is subsequently converted to a cellular toxin.

[0009] According to the present invention there is provided a compound selected from the group comprising:

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(benzoxy-L-leucinyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(benzoxy-L-isoleucinyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(benzoxy-L-phenylalaninyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(pentoxy-L-methioninyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(pentoxy-L-leucinyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(benzoxy-L-leucinyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(pentoxy-L-isoleucinyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(pentoxy-L-phenylalaninyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(benzoxy-L-methioninyl)]phosphate

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(pentoxy-α,α-dimethylglycine)] phosphate;

or a pharmaceutically acceptable derivative thereof wherein said pharmaceutically acceptable derivative is any pharmaceutically acceptable salt, hydrate, solvate or crystalline form.

[0010] It has been found that the compounds of the present invention show activity that renders them useful in the prophylaxis or treatment of cancer in homo sapiens. In particular, the present compounds exhibit beneficial properties which indicate their ability to treat cancer in patients whilst showing reduced resistance in tumour cells. Notably, compounds of the present invention can show a cytoactivity comparable to or better than that of 5-fluoracil, but with a resistance that is comparable to or less than that of each of 5-fluoracil and 5-fluoro-2'-deoxyuridine.

[0011] By "resistance" in the present application is meant a low or diminished response to therapy. Resistance can be innate or acquired. An innate resistance is a reduced responsiveness relative to other specimens or patients. An acquired resistance is a reduced effectiveness over the course of time in a given patient, whether or not acquired in conjunction with therapy comprising the administration to the patient of a drug regime to treat cancer, for example, a drug regime comprising 5FU and/or 5-FdUrd. Each of innate resistance and acquired resistance can correspond to the downregulation or low activity of transporter proteins, including nucleoside transporter proteins, or necessary anabolic enzymes or the upregulation of catabolic enzymes.

[0012] Although the applicant does not wish to be bound by any theory, it is postulated, as discussed further below, that causes of resistance in tumour cells to the activity of 5FU and/or 5-FdUrd could be: a) deletion of activating kinase as thymidine kinase (TK), a key enzyme required for the initial phosphorylation step from 5-FdUrd to 5-FdUMP; b) overproduction of thymidylate synthase (TS); and/or c) deficient transport into target cells.

[0013] Surprisingly it has now been found that compounds of the present invention can show significant cytostatic activity in cells with a lowered level of nucleoside transporter proteins and/or with nucleoside kinase-deficient cells and/or in mycoplasma-infected cells.

[0014] The beneficial property of compounds of the present invention to retain marked cytostatic activity in nucleoside kinase-deficient cells may confer *in vivo* a clinical advantage in cellular environments lacking in nucleoside kinases or having decreased levels of nucleoside kinases and thus unable to efficiently activate 5-FdUrd.

[0015] Mycoplasma-infected cells greatly reduce the activity of nucleosides such as 5-FdUrd due, it is believed, to the overproduction of thymidylate synthase (TS). The presently proposed use of the present compounds in mycoplasma-infected cells thus, it is postulated, derives from the beneficial property of the present compounds to act additionally as a TS inhibitor and so permit the present compounds to retain their cytostatic activity in mycoplasma-infected cells. The prodrugs comprising the compounds of the present invention, due to their lipophylic nature may be taken up by the target cells in an at least partially nucleoside transport carrier-independent way, and thus, may circumvent potential resistance mechanisms due to lowered levels of nucleoside or nucleobases transport carriers in the target cell membrane.

[0016] Additionally, the prodrugs comprising the compounds of the present invention are surprisingly insensitive to the action of the catabolic enzyme Thymidine Phosphorylase (TP) that is often upregulated in tumors, and thus, the prodrugs would be more independent of the presence of this catabolic enzyme than 5-FdUrd.

[0017] It has been observed that mycoplasma infection of cells can greatly reduce the activity of nucleosides, including 5-FdUrd. Administration of a TP inhibitor restores the cytostatic activity of 5-FdUrd in mycoplasma-infected cell cultures, providing evidence of the deteriorating role of TP in the eventual cytostatic activity of 5-FdUrd. This may be a limitation in patients that are mycoplasma infected. Unlike 5-FdUrd, the 5-FdUrd prodrugs of the present invention can retain high activity in these mycoplasma-infected cells.

[0018] The present compounds thus have the potential to overcome many of the limitations of 5-FU and 5-FdUrd.

[0019] 5-Fluorouracil (5FU) is one of the first examples of an anticancer drug. The design of 5-FU was based on the available biochemical information: a fluorine atom and a hydrogen atom have a similar size, however a carbon-fluorine bond is much stronger than a carbon-hydrogen bond. Thymidylate synthase acts by replacing the 5-hydrogen of deoxyuridine monophosphate with a methyl group obtained from methylene tetrahydrofolate to make thymidylate. 5FU exerts its cytotoxic effect through three different pathways. The nucleobase 5FU and the deoxyribonucleoside 5-FdUrd enter cells through facilitated nucleoside transport systems. One of the mechanisms of action of these agents is inhibition of the enzyme thymidylate synthase (TS). The nucleobase 5FU is converted to the deoxynucleoside 5-fluoro-2'-deoxyuridine (5-FdUrd) by thymidine phosphorylase. Subsequent phosphorylation of the deoxynucleoside 5-FdURd by thymidine kinase results in formation of the cytotoxic nucleotide 5-fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP). In the presence of the reduced folate, 5,10-methylene-tetrahydrofolate (mTHF), the nucleotide (5-FdUMP) inhibits thymidylate synthase (TS) due to the inability of the enzyme to remove the 5-fluorine atom. Thus, the first and the foremost important mechanism of action of 5FU and FDUR is inhibition of the enzyme thymidylate synthase (TS). Thymidylate synthase (TS) has two substrates for (dUMP and mTHF), both of which bind in the catalytic site to enable the synthesis of dTMP. 5-FdUMP forms a covalent ternary complex with thymidylate synthase (TS), inhibiting this enzyme activity and leading to depletion of deoxythymidine triphosphate, necessary for DNA synthesis. Alternatively, (5-FdUMP) is synthesized after conversion of 5FU to5-FUMP by OPRT, to fluorouridine diphosphate (FUDP), fluorodeoxyuridine diphosphate (5-FdUDP) by ribonucleotide reductase (RR) and eventually to 5'-FdUMP. It has been observed that after drug exposure to 5FU or 5-FdUrd, the cells develop resistance to these chemotherapeutic agents. The overexpression of thymidylate synthase (TS) reduces the therapeutic effect of TS inhibitory drug leading to resistance. It was observed that some individuals are more resistant to TS targeted therapy than others. Secondly, the deoxynucleoside 5-fluoro-2'-deoxyuridine (5-FdUrd) can be converted to its triphosphate 5-FdUTP form which in turn can be incorporated into DNA causing cell damage. Thirdly, 5FU may also inhibit RNA synthesis by its conversion to FUMP by OPRT and subsequently, in two steps, to fluorouridine triphosphate (FUTP) that is incorporated into RNA. This is believed to be another potential action of 5FU.

[0020] The molecule 5FU thus does not result in an optimal TS inhibitory drug because it is inefficiently converted to 5-FdUMP due to the several metabolic steps required for metabolic activation of 5FU. Further resistance can occur if the cell produces excess quantities of dUMP to compete with the drug for the active site.

[0021] 5-FdUrd is a relatively good substrate for thymidine kinase, which converts it directly to 5-FdUMP. *In vitro* studies, in several cancer cell lines have demonstrated that 5-FdURd is about 5000 fold more potent as inhibitor of cell growth than 5FU. Furthermore, the prodrug 5-FdURd shows no significant conversion to ribonucleotide metabolites at cytotoxic concentrations. *In vivo* studies showed that a significant amount of 5-FdUrd is degraded to its relative base 5FU by thymidine phosphorylase, enzyme for which 5-FdUrd shows a good affinity. This rapid phosphorolytic cleavage of 5-FdUrd to 5FU *in vitro* and *in vivo* represents a major obstacle in delivering intact 5-FdUrd to cells for enhanced cytotoxic action. In addition, the degradation of 5-FdUrd in rat intestinal homogenates and in humans, after oral administration, suggests that 5-FdUrd would scarcely be absorbed as intact 5-FdUrd.

[0022] According to a further aspect of the present invention, the compound of the present invention is provided for use in a method of prophylaxis or treatment of cancer in homo sapiens. Suitably, the cancer is selected from the group comprising leukemia, pancreatic, prostate, lung, breast and cervical cancer.

[0023] In particular, the compound of the present invention is for use in a method of prophylaxis or treatment of cancer in a patient who has developed or has the potential to develop resistance in tumour cells with respect to the activity of 5-fluoracil or 5-fluoro-2'-deoxyuridine in the prophylaxis or the treatment of cancer.

[0024] For example, the compound of the present invention can be for use in a method of prophylaxis or treatment of cancer in a patient with cells with a lowered level of nucleoside transporter proteins and/or with nucleoside kinase-deficient cells and/or with mycoplasma-infected cells, particularly where the cancer is leukemia. The compound of the present invention can instead of or as well as be for use in a method of prophylaxis or treatment of cancer in a patient who has cells with a raised level of thymidylate synthase (TS).

[0025] According to a further aspect of the present invention, there is provided a method of propylaxis or treatment of cancer comprising administering to a homo sapiens patient in need of such treatment an effective dose of a compound of the present invention. Suitably the cancer is selected from the group comprising leukemia, pancreatic, prostate, lung, breast and cervical cancer.

[0026] In particular, the present invention comprises a method for treating a patient who has developed or has the potential to develop resistance in tumour cells with respect to the activity of 5-fluoracil or 5-fluoro-2'-deoxyuridine in a method of prophylaxis or treatment of cancer. For example, the method of the present invention can comprise treating a patient with cells with a lowered level nucleoside transporter proteins and/or with nucleoside kinase-deficient cells and/or with mycoplasma-infected cells, particularly where the cancer is leukemia. The method of the present invention for treating a patient can instead of or as well as be for treating a patient that has cells with a raised level of thymidylate synthase (TS).

[0027] "Tumour" or "tumour cell" as used in the present application, unless otherwise indicated, refers to both solid tumours and cancers such as leukemia.

[0028] Compounds of the present invention can be used for treating a patient with cancer either alone *de novo* or in conjunction with other cancer therapy. For example, compounds of the present invention can be used in a cancer treatment regime in conjunction with other anti-cancer drugs, such as 5-FU and/or 5-FdUrd either, with or without leucovorin (LV), and/or other anti-cancer drugs. Alternatively, compounds of the present invention can be used where a patient has failed to respond to other anti-cancer drugs, such as for example 5FU and/or 5-FdUrd either with or without leucovorin (LV), or where the patient has shown resistance to other anti-cancer drugs, such as for example 5-FU and/or 5-FdUrd either with or without leucovorin (LV).

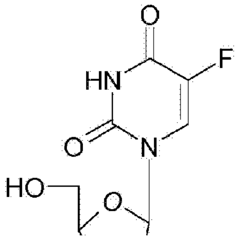
[0029] Compounds of the present invention where Ar is 1-naphthyl, whether substituted or unsubstituted, are particularly suitable for use in the above uses and methods of the present invention, particularly in a patient who has developed, or who has the potential to develop, resistance in tumour cells, such as, for example, a patient with cells with a lowered level of nucleoside transporter cells and/or with kinase-deficient cells and/or with mycoplasma-infected cells and/or a patient who has cells with a raised level of thymidylate synthase (TS).

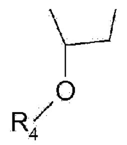
[0030] According to a further aspect of the present invention, there is provided a pharmaceutical composition comprising a compound of the present invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

[0031] According to another aspect of the present invention, there is provided a method of preparing a pharmaceutical composition comprising the step of combining a compound of the present invention with a pharmaceutically acceptable excipient, carrier or diluent.

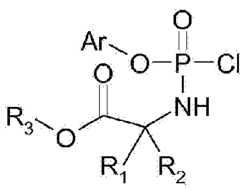
[0032] According to another aspect of the present disclosure, there is provided a process for the preparation of a compound of the present invention comprising reacting a compound of formula (II)

II





with a compound of formula (III)
III



wherein Ar, R_3, R_4, R_1 and R_2 have the meanings required to form the compounds of claim 1.

[0033] By "a pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, ester, salt of such ester, hydrate, solvate, or crystalline form or metabolite or any other compound which upon administration to a recipient is capable of providing (directly or indirectly) a compound of the invention.

[0034] Conventional treatment of cancer using chemotherapeutics is largely based on the use of nucleoside analogues. These molecules are designed to mimic natural pyrimidine and purine nucleosides. After uptake by the cell, they are phosphorylated by cellular enzymes such as (deoxy)cytidine kinase (dCK), thymidine kinase (TK) and/or nucleo(s)(t)ide kinases. These antimetabolites can subsequently interfere with the *de novo* synthesis of DNA/RNA precursors to eventually inhibit DNA/RNA synthesis resulting in cytotoxic/static activity (Hatse *et al.,* 1999; Galmarini *et al.,* 2002).

[0035] Fluoropyrimidine-based antimetabolites such as fluorouracil (5-FU), capecitabine and 5-fluoro-2'-deoxyuridine (5-FdUrd) are mainly used in the treatment of colon, breast and ovarian carcinoma (de Bruin *et al.,* 2006; Ishikawa *et al.,* 1998; Walko *et al.,* 2005). Intracellularly, these drugs are metabolised to 5-FdUMP, which forms a stable inhibitory complex with thymidylate synthase (TS) and the reduced co-substrate 5,10-methylenetetrathydrofolate, thereby blocking binding of the normal substrate dUMP to the enzyme (Beck *et al.,* 1994; Tanaka *et al.,* 2000; Longley *et al,* 2003). TS is the enzyme responsible for the conversion of dUMP to TMP and is therefore indispensible for cell proliferation, making it an interesting target for drug design. Among the fluoropyrimidines mentioned above, 5-FdUrd requires only one metabolic conversion, a phosphorylation catalysed by TK to generate 5-FdUMP (Longley *et al.,* 2003). This obligatory phosphorylation is often the rate-limiting step in the metabolism of many anti-cancer drugs (including 5-FdUrd), and is therefore still one of the limiting factors for the therapeutic use of nucleoside analogues. Hence, different strategies to improve the antitumour efficacy of nucleoside analogues have been investigated (Galmarini *et al.,* 2002).

[0036] The charged nature of nucleoside monophosphates under physiological conditions results in poor, if any, penetration across the cell membrane (Mehellou *et al.,* 2009). Therefore, the direct administration of phosphorylated molecules to circumvent the first phosphorylation step has little therapeutic advantage. Hence, different strategies for bypassing the rate-limiting phosphorylation using various types of nucleoside 5'-monophosphate prodrugs for more efficient drug-delivery have been explored (Hecker & Erion, 2008). The administration of lipophilic phosphoramidate nucleotide prodrugs (ProTides) has proved successful for several molecules with anti-viral/cancer activity (Harris *et al.,* 2001; Congiatu *et al.,* 2006; McGuigan *et al.,* 2010). By masking the charges of the phosphate motif, good passive membrane diffusion of the prodrugs can be accomplished after which the prodrug is rapidly converted intracellularly into the nucleoside monophosphate by enzymatic cleavage (Mehellou *et al.,* 2009).

[0037] Mycoplasmas are the smallest self-replicating organisms on earth and are characterized by the lack of a cell wall and a strongly reduced genome (600-1,200 kb). Many of these bacteria have a parasitic lifestyle and reside in the human body causing asymptomatic infections (Razin *et al.,* 1998). It was shown that these prokaryotes tend to preferentially colonize tumour tissue: Huang *et* al. (2001) reported that 39.7-56% of human gastric, colon, oesophageal, lung and breast cancers are infected with mycoplasmas compared to 20.9-30% in non-tumourigenic tissue. Pehlivan *et al.* (2005) found >80 % of kidney tissue samples of patients suffering renal cell carcinoma to be infected with mycoplasmas compared to 14 % in control tissue samples. Chan *et al.* (1996) reported a 59% infection rate in ovarian cancer tissues and other studies also report a high mycoplasma infection rate in gastric (Sasaki *et al.,* 1995, Yang *et al.,* 2010) and cervical condyloma tissues (Kidder *et al.,* 1998). Due to their reduced set of genes, mycoplasmas lack the pathway for *de novo* pyrimidine and purine synthesis and therefore express a wide array of salvage nucleo(s)(t)ide-metabolizing enzymes, such as thymidine phosphorylase (TP), deoxycytidine deaminase, etc. (Razin, 1978; Charron & Langelier, 1981; Neale *et al.,* 1983; Tham *et al.,* 1993). Already in 1985 it was observed that mycoplasma-encoded enzymes (e.g. TP), present in contaminated cell cultures, lead to decreased dTTP incorporation in lymphocytes (Sinigaglia & Talmadge, 1985). Recently, it has been demonstrated that these enzymes, in particular the mycoplasma-encoded thymidine phosphorylase, also interfere with the cytostatic activity of several chemotherapeutics, including 5-trifluorothymidine, *in vitro* (Bronckaers *et al.,* 2008; Jetté *et al.,* 2008; Liekens *et al.,* 2009). Therefore it has been hypothesized that the elimination of mycoplasmas by antibiotics or suppression of mycoplasma-encoded enzymes in human tumour tissue may optimize treatment of cancer patients using purine and pyrimidine antimetabolites (Liekens *et al.,* 2009).

[0038] The present invention is derived from the development and assessment of TK-independent phosphoramidate prodrugs of 5-FdUrd and provides compounds that can also be insensitive to the TP-dependent inactivation of its free nucleoside analogue. Compounds of the present invention can thus provide mycoplasma-insensitive nucleoside analogue prodrugs which may optimize treatment of cancer patients using a pyrimidine antimetabolite. From among the presently synthesized phosphoramidate prodrugs of 5-FdUrd, CPF-373 (identified below and mentioned above as a particularly suitable compound of the invention with R_4 as H) was chosen for further in depth studies. This molecule contains a naphthyl and benzylalaninyl group to mask the charged 5'-phosphate on 5-FdUMP.

[0039] Various mechanisms of tumour cell resistance towards fluoropyrimidines such as 5FU, 5-FdUrd and trifluorothymidine (TFT) have been described, including a decreased activity of crucial drug-activating enzymes (e.g. TK and orotate phosphoribosyltransferase), an increased activity of drug-inactivating enzymes (i.e. thymidine phosphorylase) and/or an upregulation of the target enzymes (e.g. TS) (Agarwal *et al.,* 1999; Murakami *et al.,* 2000; Kosaka *et al.,* 2004). Also, high TP levels found in several types of cancer tissue were reported to be predictive of a poorer prognosis upon treatment with fluoropyrimidines (Kamoshida *et al.,* 2005; Ciaparrone *et al.,* 2006; Koopman *et al.,* 2009), although other studies have not confirmed these findings (Ciccolini *et al.,* 2004; Koopman *et al.,* 2009). The present invention derives from the development of a prodrug for 5-FdUrd, to circumvent possible resistance mechanisms and susceptibility to degradation by catabolic enzymes, present in the tumour micro-environment.

[0040] Compounds embodying the present invention, for example CPF-373, are phosphoramidate prodrugs of 5-FdUrd and are described herein and can fulfil these aims. After uptake into the tumour cells, CPF-373, for example, generates 5-FdUMP intracellularly upon enzymatic cleavage. Stability studies and enzymatic/serum studies by ^{31}P NMR technology revealed that the prodrug CPF-373, for example, is fully stable in acid and alkaline conditions, but subject to hydrolysis in the presence of serum or carboxypeptidase Y, resulting in the formation of the nucleoside 5'-phosphoramidate derivative. Whereas TK is a key enzyme in the activation of 5-FdUrd, CPF-373, for example, was found to be much less dependent on TK to exert its cytostatic action in both murine (L1210) and human (CEM) cell cultures. Due to the lipophilic nature of ProTides, these molecules can deliver nucleoside-monophosphates directly into the intact tumour cell after conversion to their nucleoside phosphoramidate derivative by enzymes such as carboxyesterases or carboxypeptidases (i.e. carboxypeptidase Y), eliminating the need for an initial phosphorylation by specific nucleoside kinases such as TK. In this regard, CPF-373, for example, may be an adequate tool for the treatment of tumour cells with a modified TK activity (be it acquired or inherent). Also, since TK expression is S-phase-dependent, it is expected that CPF-373, for example, can also efficiently deliver 5-FdUMP in tumour cells that are not in the S-phase of their replication cycle. TS activity studies revealed that, CPF-373, for example, was able to inhibit TS in both wild-type and TK-deficient tumour cell lines, pointing again to an efficient intracellular delivery of the 5'-monophosphate of 5-FdUrd, and its virtual independence of cellular TK for metabolic activation.

[0041] Compounds of the present invention, are unlikely to be inactivated by catabolic enzymes involved in nucleoside metabolism. Indeed, whereas 5-FdUrd is highly susceptible to enzymatic hydrolysis by TP resulting in the formation of 5-FU and 2-deoxyribose-1-phosphate, its prodrug, for example CPF-373, is not a substrate for prokaryotic (i.e. *E.coli*) or mammalian (i.e. human erythrocyte) TP. Also, uridine phosphorylase does not recognize, for example CPF-373, as a substrate, whereas 5-FdUrd is (poorly, but measurably) hydrolyzed by this enzyme. Several studies revealed that many tumour cells have elevated levels of TP, which also acts as an angiogenic factor (Koopman *et al.,* 2009; Bronckaers *et al.,* 2009). Moreover, there are several reports on the preferential colonization of tumour tissue by mycoplasmas (Sasaki *et al.,* 1995; Chan *et al.,* 1996; Huang *et al.,* 2001; Pehlivan *et al.,* 2005) which interfere with the cytostatic activity of several conventional chemotherapeutics *in vitro* through its encoded TP (Bronckaers *et al.,* 2008; Jetté *et al.,* 2008; Liekens *et al.,* 2009). The present observations that 5-FdUrd, but not, for example, CPF-373, markedly loses cytostatic activity when the tumour cells are infected by (TP-expressing) mycoplasmas, is in full agreement with these observations. Therefore, the administration of a TP-insensitive anti-cancer prodrug such as CPF-373, demonstrated to be chemically stable at extreme pH conditions, may further improve cancer chemotherapy. In conclusion, ProTides, such as CPF-373, provide an interesting new approach towards the development of more resilient anti-cancer drugs. For instance CPF-373 may have at least several advantages over its parent drug 5-FdUrd: it exerts its cytostatic activity independent of TK and it is resistant to metabolic breakdown by TP, an enzyme that is often upregulated in tumours or may be externally expressed by mycoplasma infection of the tumour tissue.

[0042] The compound having formula I or the pharmaceutical composition according to the present invention can be administered to a homo sapiens patient by any suitable means.

[0043] The medicaments employed in the present invention can be administered by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration.

[0044] For oral administration, the compounds of the invention will generally be provided in the form of tablets or capsules, as a powder or granules, or as an aqueous solution or suspension.

[0045] Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while cornstarch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

[0046] Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

[0047] Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

[0048] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[0049] For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

[0050] The compounds of the invention may also be presented as liposome formulations.

[0051] In general a suitable dose will be in the range of 0.1 to 300 mg per kilogram body weight of the recipient per day. A preferred lower dose is 0.5 mg per kilogrm body weight of recpient per day, a more preferred lower dose is 6 mg per kilogram body weight of recipient per day, an even more preferred lower dose is 10 mg per kilogram body weight per recipient per day. A suitable dose is preferably in the range of 6 to 150 mg per kilogram body weight per day, and most preferably in the range of 15 to 100 mg per kilogram body weight per day. The desired dose is preferably presented as two, three, four, five or six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 10 to 1500 mg, preferably 20 to 1000 mg, and most preferably 50 to 700 mg of active ingredient per unit dosage form.

[0052] Examples of the present invention will now be described, by way of example only, with reference to the accompanying drawings comprising Figures 1 to 11, wherein:

Fig. 1 shows structural formula of 5-FdUrd and its phosphoramidate prodrug CPF-373;

Fig. 2 shows the effect of thymidine phosphorylase and uridine phosphorylase on dThd, Urd, 5-FdUrd and CPF-373, where data are the mean of at least 2 independent experiments (± S.D.);

Fig. 3 shows the inhibition of TS by 5-FdUrd and CPF-373 as measured by tritium release from [5-^{3}H]dUrd (panels A and B) and [5-^{3}H]dCyd (panels C and D) in L1210/0 cell cultures and by tritium release from [5-^{3}H]dCyd (panels E and F) in L1210/TK- cell cultures, where data are the mean of 2 independent experiments (± S.E.M.);

Fig. 4 shows a proposed putative mechanism of activation of 5-FdUrd ProTides;

Fig. 5 shows carboxypeptidase-mediated cleavage of prodrug CPF-373 monitored by ^{31}P NMR;

Fig. 6 shows ^{31}P NMR spectrum of compound CPF-373 in serum;

Fig. 7 shows ^{31}P NMR spectrum of compound CPF-373 in buffer pH = 1;

Fig. 8 shows ^{31}P NMR spectrum of compound CPF-373 in buffer pH = 8;

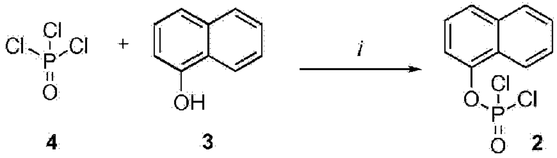
Fig. 9 shows spectra of nucleoside and relative base by ^{19}F NMR: a) 5-FdUrd submitted to the phosphorylase assay (**A**); b) 5-FdUrd and the base 5FU under condition of the assay in absence of the enzyme (TP) (**B**);

Fig. 10 shows spectra of nucleoside and base in potassium phosphate buffer (205 nM) by ^{19}F NMR: a) 5-FdUrd submitted to the phosphorylase assay in absence of enzyme (**A**); b) Result after the addition of enzyme (TP) (**B**); and

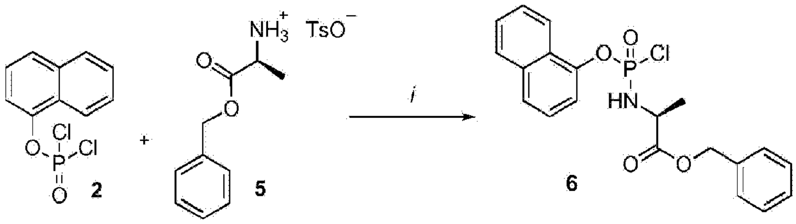
Fig. 11 shows spectra of prodrug compound CPF373 in phosphorylase assay: a) prodrug CPF373 under conditions of the assay in absence of the enzyme (TP) (**A**); b) prodrug CPF373 submitted to the action of thymidine phosphorylase (TP) (**B**).

Compound synthesis

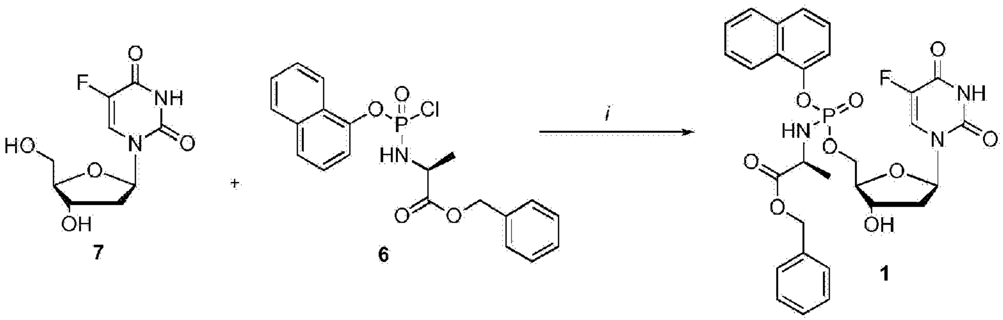
[0053] With reference to Fig. 1 and Schemes 1 to 3 below, compounds of the present invention - as exemplified by the compound CPF-373 (**1**) which does not form part of the current invention but has been provided as a reference example - have been synthesized using phosphorochloridate chemistry. Phosphorochloridate chemistry has previously been reported by McGuigan *et al.* (1993, 1996, 1997). For example, arylphosphorodichlorophosphate (**2**) has been prepared coupling 1-naphthol (**3**) with phosphorus oxychloride (**4**) in the presence of Et_3N (Scheme 1) and this was allowed to react with L-alanine benzyl ester tosylate (**5**) in the presence of Et_3N to generate the phosphorochloridate derivative (**6**) (Scheme 2). The nucleoside 5-FdUrd (**7**) was converted to the 5' ProTide by coupling with the phosphorochloridate derivative (**6**) in THF, in the presence of *N*-methyl imidazole (NMI) to give the target compound CPF-373 (**1**) (Scheme 3). The sample was obtained as a mixture of two diastereoisomers as confirmed by the presence of two peaks in the ^{31}P NMR.



Scheme 1. *Reagents and Conditions*: (*i*) 1-naphthol (**3**), phosphorus oxychloride (**4**), dry Et_2O, dry Et_3N, -78 °C., 30min. then R.T., 3h.



Scheme 2. *Reagents and Conditions*: (*i*) dry Et_3N, CH_2Cl_2, -78 °C., 1h then R.T., 3h.



Scheme 3. *Reagents and Conditions*: (*i*) NMI, dry THF, 10 min., then phosphorochloridate (**6**), R.T., overnight.

[0054] Anhydrous solvents were obtained from Aldrich and used without further purification. All reactions were carried out under an argon atmosphere. Reactions were monitored with analytical TLC on Silica Gel 60-F254 precoated aluminium plates and visualised under UV (254 nm) and/or with ^{31}P NMR spectra. Column chromatography was performed on silica gel (35-70 μM). Proton (^{1}H), carbon (^{13}C), phosphorus (^{31}P) and fluorine (^{19}F) NMR spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C. Spectra were auto-calibrated to the deuterated solvent peak and all ^{13}C NMR and ^{31}P NMR were proton-decoupled. Analytical HPLC was conducted by Varian Prostar (LC Workstation-Varian prostar 335 LC detector) using Varian Polaris C18-A (10 μM) as an analytic column.

[0055] Low and High resolution mass spectra were performed as a service by Birmingham University, using electrospray (ES). CHN microanalysis was performed as a service by MEDAC Ltd., Surrey.

Standard Procedure A: Synthesis of Dichlorophosphate (2).

[0056] Phosphorus oxychloride (1.0 equiv) was added to a solution of 1-naphthol (1.0 equiv) in diethyl ether under argon atmosphere, then anhydrous triethylamine (1.0 equiv) was added dropwise at -78 °C and the resulting reaction mixture was stirred for 1 h. Subsequently the reaction mixture was allowed to slowly warm up to room temperature for 3 h. Formation of the desired compound was monitored by ^{31}P NMR. The resulting mixture was filtered and then evaporated *in vacuo* under nitrogen to afford the crude colourless oil as product, which was used without further purification in the next step.

[0057] **Synthesis of 1-Naphthyl dichlorophosphate (2):** Prepared according to Standard Procedure **A,** from 1-naphthol (3.00 g, 20.81 mmol), phosphorus oxychloride (1.94 mL, 20.81 mmol), triethylamine (2.9 mL, 20.81 mmol) and anhydrous diethyl ether (70 mL). After 1 h at -78 °C the reaction was left to rise to room temperature and stirred for 3 h. The crude product was obtained as an oil. The resulting mixture was filtered and then evaporated *in vacuo,* after purification by column chromatography eluting with hexane-EtOAc, (1:1) to afford a colorless oil (4.59 g, 84%) [R_f = 0.93 (hexane-EtOAc, 1:1)], ^{31}P NMR (202 MHz, $CDCl_3$): δ_P 5.07; ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.52-7.71 (m, 4H, Ar*H*), 7.86-7.89 (m, 1H, Ar*H*), 7.95-7.98 (m, 1H, Ar*H*), 8.16-8.19 (m, 1H, Ar*H*).

Standard Procedure B: Synthesis of Phosphorochloridate (6).

[0058] A solution of aryl phosphorodichloridate (1.0 equiv.) and appropriate amino acid ester salt (1.0 equiv.) in dichloromethane under argon atmosphere was added dropwise to anhydrous triethylamine (2.0 equiv.) at -78 °C. After 1 h the reaction mixture was allowed to slowly warm to room temperature for 3 h and the formation of the desired compound was monitored by ^{31}P NMR. The reaction mixture was concentrated under reduced pressure, the residue was redissolved in diethyl ether, filtered and evaporated *in vacuo* under nitrogen to afford a crude colourless oil, which in some cases was used without further purification in the next step. The aryl phosphorochloridate synthesized was purified by column chromatography eluting with hexane-EtOAc, (7:3) to afford the title compound as a colorless oil.

[0059] **Synthesis of 1-Naphthyl(benzyl-L-alaninyl) phosphorochloridate (6):** The phosphorochloridate was prepared using 1-naphthyl dichlorophosphate (2.50 g, 9.57 mmol), L-alanine benzyl ester tosylate salt (3.36 g, 9.57 mmol), dry triethylamine (2.66 mL, 19.14 mmol) and dry dichloromethane (35.7 mL) according to the general procedure B. Purification by column chromatography eluting with hexane-EtOAc, (7:3) afforded the title compound as a colourless oil (1.82 g, 47%) [R_f = 0.90 (hexane-EtOAc, 7:3)], ^{31}P NMR (202 MHz, $CDCl_3$, mixture of diastereoisomers): δ_P 7.92, 8.14 (Int.: 1.00:1.00); ^1H NMR (500 MHz, $CDCl_3$, mixture of diastereoisomers with a ratio of 1:1): δ_H 1.42-1.45 (m, 3H, CHCH_3), 4.20-4.23 (m, 1H, C*H*CH_3), 4.78-4.81 (m, 1H, N*H*), 5.09 (s, 2H, OCH_2Ph), 7.09-7.73 (m, 11H, Ar*H*), 7.97-8.12 (m, 1H, Ar*H*).

Standard Procedure C: Synthesis of the Nucleoside Phosphoramidate (1).

[0060] A solution of the appropriate nucleoside (1.0 equiv.) in dry THF (10 mL) was added to NMI (5.0 equiv.) at room temperature under argon atmosphere. After 10 min the reaction mixture was added dropwise to a solution of phosphorochloridate (3.0 equiv) in anhydrous THF. The reaction was stirred at room temperature overnight and evaporated *in vacuo*. The oil obtained was dissolved in CH_2Cl_2, washed twice with H_2O, then with HCl 0.5 M or in alternative the crude product was washed with diethyl ether. Then the crude product was purified by column chromatography on silica, eluting with CH_2Cl_2-MeOH as a gradient to afford the phosphoramidate.

[0061] Synthesis of 5-Fluoro-2'deoxyuridine-5'-*O*-[α-naphthyl (benzyl-L-alaninyl)] phosphate (1) (provided as a reference example): The phosphoramidate was prepared using 5-Fluoro-2'deoxyuridine (0.25 g, 1.01 mmol), NMI (0.40 mL, 5.07 mmol) and naphthyl(benzyl-L-alaninyl) phosphorochloridate (0.82 g, 3.04 mmol) according to the general procedure **C.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (47.0 mg, 8%) [R_f = 0.19 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 636.1520. $C_{29}H_{29}N_3O_9FNaP$ requires [MNa^+], 636.1523); ^{31}P NMR (202 MHz, MeOD, mixture of diastereoisomers): δ_P 4.24, 4.59; ^{19}F NMR (470 MHz, MeOD): δ_F -167.36, -167.18; ^1H NMR (500 MHz, MeOD): δ_H 1.34-1.38 (m, 3H, $CHCH_3$), 1.67-1.79 (m, 1H, *H*-2'), 2.08-2.17 (m, 1H, *H*-2'), 4.03-4.15 (m, 2H, $CHCH_3$, *H*-4'), 4.24-4.36 (m, 3H, CH_2OP, *H*-3'), 5.08 (d, 1H, *J* = 12.0 Hz, OC*H*HPh), 5.13 (d, 1H, *J* = 12.0 Hz, OCH*H*Ph), 6.09-6.16 (m, 1H, *H*-1'), 7.27-7.45 (m, 6H, Ar*H*), 7.47-7.55 (m, 3H, Ar*H*), 7.67-7.72 (m, 2H, Ar*H*, *H*-6), 7.86-7.90 (m, 1H, Ar*H*), 8.12-8.18 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): δ_C 20.3 (d, $^3J_{C-P}$ = 7.6 Hz, CH_3), 20.5 (d, $^3J_{C-P}$ = 6.5 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.8 (CH), 51.9 (CH), 67.6 (d, $^2J_{C-P}$ = 5.3 Hz, CH_2), 67.8 (d, $^2J_{C-P}$ = 5.2 Hz, CH_2), 68.0 (CH_2), 68.1 (CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 8.1 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 8.1 Hz, CH), 86.9 (CH), 87.0 (CH), 116.2 (d, $^3J_{C-P}$ = 3.3 Hz, CH), 116.5 (d, $^3J_{C-P}$ = 3.5 Hz, CH), 122.6 (CH), 125.3 (CH), 125.4 (CH), 125.6 (CH), 125.7 (CH), 126.2 (CH), 126.5 (CH), 126.6 (CH), 127.6 (CH), 127.7 (CH), 127.8 (C), 127.9 (C), 128.0 (CH), 128.1 (CH), 128.9 (CH), 129.0 (CH), 129.4 (CH), 129.5 (CH), 129.6 (CH), 129.7 (CH), 136.2 (C), 137.1 (C), 137.2 (C), 141.6 (d, $^1J_{C-F}$ = 233.8 Hz, C), 141.7 (d, $^1J_{C-F}$ = 233.9 Hz, C), 147.8 (d, $^2J_{C-P}$ = 7.7 Hz, C), 147.9 (d, $^2J_{C-P}$ = 7.4 Hz, C), 150.5 (d, $^4J_{C-F}$ = 4.0 Hz, C), 159.3 (d, $^2J_{C-F}$ = 26.1 Hz, C), 174.6 (d, $^3J_{C-P}$ = 5.0 Hz, C), 174.9 (d, $^3J_{C-P}$ = 4.3 Hz, C), m/z (ES) 636 (MH^+, 100%), Reverse HPLC eluting with (H_2O/MeOH from 100/0 to 0/100) in 45 min., showed two peaks of the diastereoisomers with t_R 34.23 min. and t_R 34.59 min. Anal. Calcd for $C_{29}H_{29}FN_3O_9P$: C, 56.77; H, 4.76; N, 6.85. Found: C, 56.57; H, 5.06; N, 6.72.

Radioactive pyrimidine deoxynucleosides

[0062] [5-^{3}H]dCyd (radiospecificity: 22 Ci/mmol) and [5-^{3}H]dUrd (radiospecificity: 15.9 Ci/mmol) were obtained from Moravek Biochemicals Inc. (Brea, CA).

Standard procedure D: synthesis of phosphoramidates (NMI method)

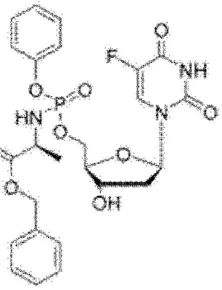
[0063] To a stirring solution of 5-F-dUrd (1.0 eq.) in anhydrous THF, an appropriate phosphorochloridate (3.0 eq.) dissolved in anhydrous THF was added dropwise under an Ar atmosphere. To that reaction mixture at -78°C was added dropwise over 5 minutes NMI (5.0 eq.). After 15 minutes, the reaction mixture was let to rise to room temperature and stirred overnight. The solvent was removed under *vacuum* and the residue was re-dissolved in DCM and washed with 0.5 M HCl three times. The organic layer was dried over $MgSO_4$, filtered, reduced to dryness and purified by column chromatography with gradient of eluent (DCM/MeOH 99:1 to 97:3 to 95:5).

Standard procedure E: synthesis of phosphoramidates (*t*BuMgCl method)

[0064] To a stirring solution of 5-FdUrd (1.0 eq.) dissolved in anhydrous THF, *t*BuMgCl (1.1 mol eq. 1M solution in THF) was added dropwise under an Ar atmosphere, followed by addition (after 30 min.) of the appropriate phosphorochloridate (2.0 mol eq.) dissolved in anhydrous THF. The resulting reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography using gradient of eluent (DCM/MeOH 99:1 to 97:3 to 95:5)

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(benzoxy-L-alaninyl)]phosphate (CPF381) (provided as a reference example)

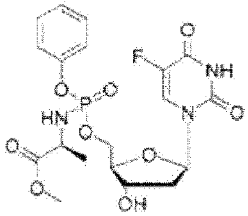
[0065]



[0066] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.40 g, 1.62 mmol), *tert*-butylmagnesium chloride in tetrahydrofuran (^{t}BuMgCl) (1.0 M, 2.43 mL, 2.43 mmol) and phenyl(benzoxy-L-alaninyl) phosphorochloridate (1.08 g, 3.20 mmol) according to general procedure **E.** Purification by gradient column chromatography on silica, eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (71.0 mg, 8%) [R_f = 0.35 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 586.1360. $C_{25}H_{27}N_3O_9NaPF$ requires [MNa^+], 586.1367); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.74, 4.14; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.57,-167.46; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.35 (d, 3H, *J* = 7.4 Hz, CHC*H*$_3$, one diast.), 1.37 (d, 3H, *J* = 6.9 Hz, CHC*H*$_3$, one diast.), 1.96-2.32 (m, 2H, *H*-2'), 3.95-4.08 (m, 2H, C*H*CH_3, *H*-4'), 4.23-4.34 (m, 3H, CH_2OP, *H*-3'), 5.13 (br d, 1H, *J* = 12.3 Hz, OC*H*HPh), 5.16 (br d, 1H, *J* = 12.3 Hz, OCH*H*Ph, one diast.), 5.17 (br d, 1H, *J* = 12.2 Hz, OCH*H*Ph, one diast.), 6.16-6.22 (m, 1H, *H*-1'), 7.17-7.25 (m, 3H, Ar*H*), 7.26-7.40 (m, 7H, Ar*H*), 7.81-7.85 (m, 1H, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.2 (d, $^3J_{C-P}$ = 7.5 Hz, CH_3), 20.4 (d, $^3J_{C-P}$ = 6.2 Hz, CH_3), 40.6 (CH_2), 40.9 (CH_2), 51.6 (CH), 51.8 (CH), 67.5 (d, $^2J_{C-P}$ = 5.3 Hz, CH_2), 67.6 (d, $^2J_{C-P}$ = 5.5 Hz, CH_2), 68.0 (CH_2), 71.8 (CH), 71.9 (CH), 86.6 (d, $^3J_{C-P}$ = 8.0 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 8.3 Hz, CH), 86.9 (CH), 87.0 (CH), 121.4 (d, $^3J_{C-P}$ = 5.1 Hz, CH), 121.5 (d, $^3J_{C-P}$ = 5.6 Hz, CH), 125.5 (d, $^5J_{C-P}$ = 3.2 Hz, CH), 125.8 (d, $^5J_{C-P}$ = 3.2 Hz, CH), 126.3 (CH), 129.0 (CHx2), 129.3 (CHx2), 129.6 (CHx2), 130.8 (CHx2), 140.9 (C), 141.6 (d, $^1J_{C-F}$ = 233.6 Hz, C), 141.7 (d, $^1J_{C-F}$ = 233.6 Hz, C), 150.7 (d, $^4J_{C-F}$ = 5.7 Hz, C), 152.1 (d, $^2J_{C-F}$ = 6.5 Hz, C), 159.2 (d, $^2J_{C-F}$ = 26.3 Hz, C), 174.6 (d, $^3J_{C-P}$ = 4.9 Hz, C), 174.7 (d, $^3J_{C-P}$ = 4.9 Hz, C), m/z (ES) 586 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, └ = 275 nm, showed one peak of the mixture of diastereoisomers with t_R 25.08 min. (97%).

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(methoxy-L-alaninyl)]phosphate (CPF382) (provided as a reference example)

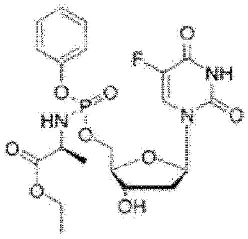
[0067]



[0068] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), *N*-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and phenyl(methoxy-L-alaninyl) phosphorochloridate (0.84 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (16.0 mg, 4%) [R_f = 0.30 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 510.1045. $C_{19}H_{23}N_3O_9NaPF$ requires [MNa^+], 510.1054); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.79, 4.09; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.78, -167.72; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.34 (d, 3H, *J* = 7.1 Hz, CHC*H*$_3$, one diast.), 1.36 (d, 3H, *J* = 7.1 Hz, CHC*H*$_3$, one diast.), 2.02-2.16 (m, 1H, *H*-2'), 2.25-2.34 (m, 1H, *H*-2'), 3.69 (s, 3H, OC*H*$_3$, one diast.), 3.70 (s, 3H, OC*H*$_3$, one diast.), 3.93-4.02 (m, 1H, C*H*CH_3), 4.08-4.13 (m, 1H, *H*-4'), 4.27-4.45 (m, 3H, CH_2OP, *H*-3'), 6.20-6.29 (m, 1H, *H*-1'), 7.18-7.28 (m, 3H, Ar*H*), 7.35-7.40 (m, 2H, Ar*H*), 7.85 (d, 1H, $^3J_{H-F}$ = 6.4 Hz, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.2 (d, $^3J_{C-P}$ = 7.5 Hz, CH_3), 20.5 (d, $^3J_{C-P}$ = 6.7 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.5 (CH_3), 51.6 (CH_3), 52.7 (CH), 52.8 (CH), 67.5 (d, $^2J_{C-P}$ = 5.5 Hz, CH_2), 67.6 (d, $^2J_{C-P}$ = 5.1 Hz, CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 8.2 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 8.2 Hz, CH), 86.9 (CH), 87.0 (CH), 121.2 (d, $^{3}J_{C\text{-}P}$ = 4.5 Hz, CH), 121.4 (d, $^{3}J_{C\text{-}P}$ = 4.7 Hz, CH), 125.6 (d, $^{5}J_{C\text{-}P}$ = 2.9 Hz, CH), 125.9 (d, $^{5}J_{C\text{-}P}$ = 2.9 Hz, CH), 126.2 (CH), 130.8 (CH), 130.9 (CH), 141.6 (d, $^{1}J_{C\text{-}F}$ = 233.8 Hz, C), 141.7 (d, $^{1}J_{C\text{-}F}$ = 233.9 Hz, C), 150.6 (d, $^{4}J_{C\text{-}F}$ = 3.6 Hz, C), 152.1 (d, $^{2}J_{C\text{-}P}$ = 6.8 Hz, C), 152.2 (d, $^{2}J_{C\text{-}P}$ = 6.8 Hz, C), 159.4 (d, $^{2}J_{C\text{-}F}$ = 26.0 Hz, C), 175.2 (d, $^{3}J_{C\text{-}P}$ = 4.8 Hz, C), 175.5 (d, $^{3}J_{C\text{-}P}$ = 3.7 Hz, C), m/z (ES) 510 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 23.11 min. and t_R 24.11 min. (74% : 24%).

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(ethoxy-L-alaninyl)]phosphate (CPF383) (provided as a reference example)

[0069]

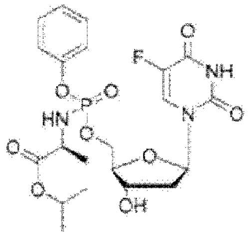


[0070] The phosphoramidate was prepared using 5-fluoro-2'deoxyuridine (0.10 g, 0.40 mmol), *N*-methylimidazole (NMI) (0.16 mL, 2.03 mmol) and phenyl(ethoxy-L-alaninyl) phosphorochloridate (0.35 g, 1.21 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (10.0 mg, 5%) [R_f = 0.11 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 524.1202. $C_{20}H_{25}N_3O_9NaPF$ requires [MNa^+], 524.1210); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.83, 4.11; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.67, -167.61; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.25 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.26 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.34 (d, 3H, *J* = 7.2 Hz, $CHCH_3$, one diast.), 1.36 (d, 3H, *J* = 7.2 Hz, $CHCH_3$, one diast.), 2.02-2.15 (m, 1H, *H*-2'), 2.24-2.34 (m, 1H, *H*-2'), 3.90-4.00 (m, 1H, $CHCH_3$,), 4.08-4.19 (m, 3H, CH_2CH_3, *H*-4'), 4.27-4.45 (m, 3H, CH_2OP, *H*-3'), 6.20-6.28 (m, 1H, *H*-1'), 7.18-7.28 (m, 3H, Ar*H*), 7.34-7.39 (m, 2H, Ar*H*), 7.85 (d, 1H, $^{3}J_{H\text{-}F}$ = 6.4 Hz, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 14.4 (CH_3), 15.4 (CH_3), 20.3 (d, $^{3}J_{C\text{-}P}$ = 7.6 Hz, CH_3), 20.5 (d, $^{3}J_{C\text{-}P}$ = 6.5 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.6 (CH), 51.7 (CH), 62.4 (CH_2), 62.5 (CH_2), 67.5 (d, $^{2}J_{C\text{-}P}$ = 5.4 Hz, CH_2), 67.6 (d, $^{2}J_{C\text{-}P}$ = 5.4 Hz, CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^{3}J_{C\text{-}P}$ = 8.1 Hz, CH), 86.8 (d, $^{3}J_{C\text{-}P}$ = 8.3 Hz, CH), 86.9 (CH), 87.0 (CH), 121.3 (d, $^{3}J_{C\text{-}P}$ = 4.8 Hz, CH), 121.4 (d, $^{3}J_{C\text{-}P}$ = 4.6 Hz, CH), 125.6 (d, $^{5}J_{C\text{-}P}$ = 4.6 Hz, CH), 125.8 (d, $^{5}J_{C\text{-}P}$ = 4.8 Hz, CH), 126.3 (CH), 130.8 (CH), 130.9 (CH), 141.6 (d, $^{1}J_{C\text{-}F}$ = 233.7 Hz, C), 141.8 (d, $^{1}J_{C\text{-}F}$ = 233.8 Hz, C), 150.8 (br C), 152.0 (d, $^{2}J_{C\text{-}P}$ = 7.1 Hz, C), 152.1 (d, $^{2}J_{C\text{-}P}$ = 7.1 Hz, C), 159.6 (d, $^{2}J_{C\text{-}F}$ = 26.0 Hz, C), 174.8 (d, $^{3}J_{C\text{-}P}$ = 5.4 Hz, C), 175.1 (d, $^{3}J_{C\text{-}P}$ = 4.4 Hz, C), m/z (ES) 524 (MNa^+, 100%); Reverse-phase HPLC eluting with

H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, └ = 275 nm, showed two peaks of the diastereoisomers with t_R 25.63 min. and t_R 26.40 min. (71%: 27%).

5-Fluoro-2'deoxyuridine-5'-*O*-[phenyl(isopropoxy-L-alaninyl)]phosphate (CPF384) (provided as a reference example)

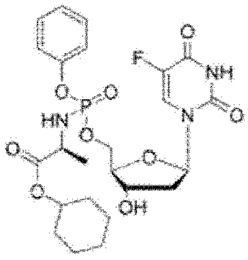
[0071]



[0072] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), N-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and phenyl(isopropoxy-L-alaninyl) phosphorochloridate (0.93 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (31.0 mg, 6%) [R_f = 0.21 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 538.1370. $C_{21}H_{27}N_3O_9NaPF$ requires [MNa^+], 538.1367); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.87, 4.13; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.64, -167.56; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.22-1.26 (m, 6H, $CH(CH_3)_2$), 1.33 (d, 3H, J = 7.1 Hz, $CHCH_3$, one diast.), 1.35 (d, 3H, J = 7.1 Hz, $CHCH_3$, one diast.), 2.00-2.15 (m, 1H, *H*-2'), 2.23-2.34 (m, 1H, *H*-2'), 3.88-3.96 (m, 1H, $CHCH_3$), 4.08-4.14 (m, 1H, *H*-4'), 4.27-4.45 (m, 3H, CH_2OP, *H*-3'), 4.98 (hept, 1H, J = 6.1 Hz, $CH(CH_3)_2$), 6.20-6.29 (m, 1H, *H*-1'), 7.17-7.29 (m, 3H, Ar-*H*), 7.34-7.40 (m, 2H, Ar-*H*), 7.84 (d, 1H, $^3J_{H\text{-}F}$ = 6.4 Hz, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.3 (d, $^3J_{C\text{-}P}$ = 7.6 Hz, CH_3), 20.5 (d, $^3J_{C\text{-}P}$ = 6.4 Hz, CH_3), 21.9 (CH_3x2), 22.0 (CH_3x2), 40.8 (CH_2), 40.9 (CH_2), 51.7 (CH), 51.8 (CH), 67.5 (d, $^2J_{C\text{-}P}$ = 5.4 Hz, CH_2), 67.6 (d, $^2J_{C\text{-}P}$ = 5.2 Hz, CH_2), 70.2 (CH), 70.3 (CH), 72.0 (CH), 72.1 (CH), 86.6 (d, $^3J_{C\text{-}P}$ = 8.2 Hz, CH), 86.8 (d, $^3J_{C\text{-}P}$ = 8.2 Hz, CH), 86.9 (CH), 87.0 (CH), 121.2 (d, $^3J_{C\text{-}P}$ = 4.7 Hz, CH), 121.4 (d, $^3J_{C\text{-}P}$ = 4.9 Hz, CH), 125.6 (d, $^5J_{C\text{-}P}$ = 7.1 Hz, CH), 125.9 (d, $^5J_{C\text{-}P}$ = 7.1 Hz, CH), 126.3 (CH), 130.8 (CH), 130.9 (CH), 141.8 (d, $^1J_{C\text{-}F}$ = 234.5 Hz, C), 141.9 (d, $^1J_{C\text{-}F}$ = 234.4 Hz, C), 150.7 (d, $^4J_{C\text{-}F}$ = 3.7 Hz, C), 152.0 (d, $^3J_{C\text{-}P}$ = 6.2 Hz, C), 152.1 (d, $^3J_{C\text{-}P}$ = 6.2 Hz, C), 159.3 (d, $^2J_{C\text{-}F}$ = 26.3 Hz, C), 159.4 (d, $^2J_{C\text{-}F}$ = 26.0 Hz, C), 174.3 (d, $^3J_{C\text{-}P}$ = 5.6 Hz, C), 174.6 (d, $^3J_{C\text{-}P}$ = 4.6 Hz, C), m/z (ES) 538 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, └ = 275 nm, showed two peaks of the diastereoisomers with t_R 28.93 min. and t_R 29.45 min. (44% : 52%).

5-Fluoro-2'deoxyuridine-5'-*O*-[phenyl(cyclohexoxy-L-alaninyl)]phosphate (CPF508) (provided as a reference example)

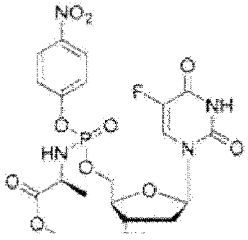
[0073]



[0074] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.30 g, 1.21 mmol), N-methylimidazole (NMI) (0.48 mL, 6.09 mmol) and phenyl(cyclohexoxy-L-alaninyl) phosphorochloridate (1.026 g, 3.65 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (6.7 mg, 3%) [R_f = 0.45 (CH_2Cl_2-MeOH, 95:5)]; (Found: MNa^+, 565.48. $C_{24}H_{31}N_3O_9NaPF$ requires [MNa^+], 565.49); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.86, 4.15; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.68, -167.62; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.26-1.40 (m, 3H, CHCH_3), 1.41-1.50 (m, 4H, CH(CH_2)$_5$), 1.52-1.61 (m, 1H, CH(CH_2)$_5$), 1.70-1.88 (m, 5H, CH(CH_2)$_5$), 2.00-2.14 (m, 1H, *H*-2'), 2.23-2.34 (m, 1H, *H*-2'), 3.90-3.98 (m, 1H, C*H*CH_3), 4.07-4.14 (m, 1H, *H*-4'), 4.29-4.39 (m, 2H, CH_2OP), 4.40-4.45 (m, 1H, *H*-3'), 4.72-4.78 (m, 1H, C*H*(CH_2)$_5$), 6.20-6.28 (m, 1H, *H*-1'), 7.18-7.29 (m, 3H, Ar*H*), 7.34-7.39 (m, 2H, Ar*H*), 7.85 (d, 1H, $^3J_{H\text{-}F}$ = 6.6 Hz, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.3 (d, $^3J_{C\text{-}P}$ = 7.3 Hz, CH_3), 20.6 (d, $^3J_{C\text{-}P}$ = 6.5 Hz, CH_3), 24.6 (CH_2), 26.4 (CH_2), 32.3 (CH_2), 32.4 (CH_2), 40.9 (CH_2), 51.7 (CH), 51.9 (CH), 67.5 (d, $^2J_{C\text{-}P}$ = 5.3 Hz, CH_2), 67.7 (d, $^2J_{C\text{-}P}$ = 5.3 Hz, CH_2), 72.0 (CH), 72.1 (CH), 74.9 (CH), 86.6 (d, $^3J_{C\text{-}P}$ = 8.5 Hz, CH), 86.8 (d, $^3J_{C\text{-}P}$ = 8.5 Hz, CH), 86.9 (CH), 87.0 (CH), 121.3 (CH), 121.4 (CH), 121.5 (CH), 121.6 (CH), 125.6 (CH), 125.7 (CH), 125.8 (CH), 125.9 (CH), 126.3 (CH), 130.1 (CH), 141.5 (d, $^1J_{C\text{-}F}$ = 234.0 Hz, C), 150.7 (d, $^4J_{C\text{-}P}$ = 4.0 Hz, C), 152.0 (d, $^2J_{C\text{-}P}$ = 7.2 Hz, C), 152.1 (d, $^2J_{C\text{-}P}$ = 7.2 Hz, C), 159.4 (d, $^2J_{C\text{-}F}$ = 26.3 Hz, C), 174.3 (d, $^3J_{C\text{-}P}$ = 4.6 Hz, C), 174.5 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, C); m/z (ES) 565 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, L = 275 nm, showed two peaks of the diastereoisomers with t_R 30.00 min. and t_R 30.45 min. (33% : 65%).

5-Fluoro-2'deoxyuridine-5'-*O*-[*p*-nitro-phenyl(ethoxy-L-alaninyl)]phosphate (CPF430) (provided as a reference example)

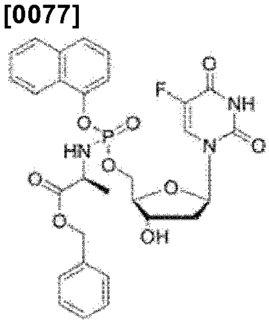
[0075]



[0076] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), *N*-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and *p*-nitro-phenyl(ethoxy-L-alaninyl)phosphorochloridate (1.02 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (77.0 mg, 14%) [R_f = 0.24 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 569.1066. $C_{20}H_{24}N_4O_{11}NaPF$ requires [MNa^+], 569.1061); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 3.63, 3.67; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.89, -167.82; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.24 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.25 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.36-1.40 (m, 3H, $CHCH_3$), 2.16-2.25 (m, 1H, *H*-2'), 2.30-2.38 (m, 1H, *H*-2'), 3.95-4.00 (m, 1H, $CHCH_3$), 4.09-4.19 (m, 3H, CH_2CH_3, *H*-4'), 4.32-4.48 (m, 3H, CH_2OP, *H*-3'), 6.21-6.29 (m, 1H, *H*-1'), 7.46 (d, 1H, *J* = 8.7 Hz, Ar*H*), 7.49 (d, 1H, *J* = 8.7 Hz, Ar*H*), 7.85 (d, 1H, $^3J_{H\text{-}F}$ = 6.6 Hz, *H*-6), 7.87 (d, 1H, $^3J_{H\text{-}F}$ = 6.6 Hz, *H*-6), 8.29 (d, 2H, *J* = 8.7 Hz, Ar*H*); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 14.5 (CH_3), 14.6 (CH_3), 20.3 (d, $^3J_{C\text{-}P}$ = 7.5 Hz, CH_3), 20.4 (d, $^3J_{C\text{-}P}$ = 6.4 Hz, CH_3), 40.8 (CH_2), 51.6 (CH), 51.7 (CH), 62.5 (CH_2), 67.8 (d, $^2J_{C\text{-}P}$ = 5.5 Hz, CH_2), 68.0 (d, $^2J_{C\text{-}P}$ = 5.2 Hz, CH_2), 71.8 (CHx2), 86.4 (CH), 86.5 (CH), 87.0 (d, $^3J_{C\text{-}P}$ = 7.5 Hz, CH), 122.1 (d, $^3J_{C\text{-}P}$ = 5.2 Hz, CH), 122.5 (d, $^3J_{C\text{-}P}$ = 5.0 Hz, CH), 125.7 (CH), 126.0 (CH), 126.6 (CH), 141.3 (d, $^1J_{C\text{-}F}$ = 233.6 Hz, C), 141.5 (d, $^1J_{C\text{-}F}$ = 233.7 Hz, C), 146.2 (C), 150.6 (d, $^4J_{C\text{-}P}$ = 4.6 Hz, C), 156.9 (d, $^2J_{C\text{-}P}$ = 2.6 Hz, C), 157.0 (d, $^2J_{C\text{-}P}$ = 2.6 Hz, C), 159.3 (d, $^2J_{C\text{-}F}$ = 26.3 Hz, C), 174.6 (d, $^3J_{C\text{-}P}$ = 4.6 Hz, C), 174.9 (d, $^3J_{C\text{-}P}$ = 3.7 Hz, C), m/z (ES) 569 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 min., 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 31.63 min. and t_R 31.89 min. (11%: 85%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(benzoxy-L-alaninyl)]phosphate (CPF373) (provided as a reference example)

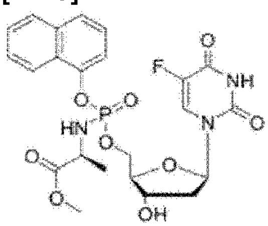
[0077]



[0078] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), N-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and 1-naphthyl(benzoxy-L-alaninyl) phosphorochloridate (0.82 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (47.0 mg, 8%) [R_f = 0.19 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 636.1520. $C_{29}H_{29}N_3O_9NaPF$ requires [MNa^+], 636.1523); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 4.24, 4.59; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.36, -167.18; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.34-1.38 (m, 3H, CHC*H*$_3$), 1.67-1.79 (m, 1H, *H*-2'), 2.08-2.17 (m, 1H, *H*-2'), 4.03-4.15 (m, 2H, C*H*CH$_3$, *H*-4'), 4.24-4.36 (m, 3H, C*H*$_2$OP, *H*-3'), 5.08 (d, 1H, *J* = 12.0 Hz, OC*H*HPh), 5.13 (d, 1H, *J* = 12.0 Hz, OCH*H*Ph), 6.09-6.16 (m, 1H, *H*-1'), 7.27-7.45 (m, 6H, Ar*H*), 7.47-7.55 (m, 3H, Ar*H*), 7.67-7.72 (m, 2H, Ar*H*, *H*-6), 7.86-7.90 (m, 1H, Ar*H*), 8.12-8.18 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.3 (d, $^3J_{C-P}$ = 7.6 Hz, CH_3), 20.5 (d, $^3J_{C-P}$ = 6.5 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.8 (CH), 51.9 (CH), 67.6 (d, $^2J_{C-P}$ = 5.3 Hz, CH_2), 67.8 (d, $^2J_{C-P}$ = 5.2 Hz, CH_2), 68.0 (CH_2), 68.1 (CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 8.1 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 8.1 Hz, CH), 86.9 (CH), 87.0 (CH), 116.2 (d, $^3J_{C-P}$ = 3.3 Hz, CH), 116.5 (d, $^3J_{C-P}$ = 3.5 Hz, CH), 122.6 (CH), 125.3 (CH), 125.4 (CH), 125.6 (CH), 125.7 (CH), 126.2 (CH), 126.5 (CH), 126.6 (CH), 127.6 (CH), 127.7 (CH), 127.8 (C), 127.9 (C), 128.0 (CH), 128.1 (CH), 128.9 (CH), 129.0 (CH), 129.4 (CH), 129.5 (CH), 129.6 (CH), 129.7 (CH), 136.2 (C), 137.1 (C), 137.2 (C), 141.6 (d, $^1J_{C-F}$ = 233.8 Hz, C), 141.7 (d, $^1J_{C-F}$ = 233.9 Hz, C), 147.8 (d, $^2J_{C-P}$ = 7.7 Hz, C), 147.9 (d, $^2J_{C-P}$ = 7.4 Hz, C), 150.5 (d, $^4J_{C-F}$ = 4.0 Hz, C), 159.3 (d, $^2J_{C-F}$ = 26.1 Hz, C), 174.6 (d, $^3J_{C-P}$ = 5.0 Hz, C), 174.9 (d, $^3J_{C-P}$ = 4.3 Hz, C), m/z (ES) 636 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, L = 275 nm, showed two peaks of the diastereoisomers with t_R 34.23 min. and t_R 34.59 min. (23% : 76%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(methoxy-L-alaninyl)]phosphate (CPF385) (provided as a reference example)

[0079]

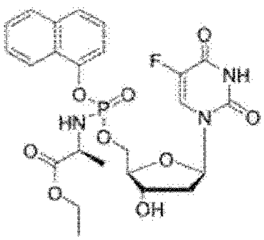


[0080] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), N-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and 1-naphthyl(methoxy-L-alaninyl) phosphorochloridate (0.99 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (7.0 mg, 1%) [R_f = 0.23 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 560.1198. $C_{23}H_{25}N_3O_9NaPF$ requires [MNa^+], 560.1210); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 4.31, 4.56; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.51, -167.37; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.34 (d, 3H, *J* = 6.7 Hz, CHC*H*$_3$, one diast.), 1.36 (d, 3H, *J* = 6.7 Hz, CHC*H*$_3$, one diast.), 1.76-1.87 (m, 1H, *H*-2'), 2.12-2.22

(m, 1H, *H*-2'), 3.64 (s, 3H, OCH_3, one diast.), 3.65 (s, 3H, OCH_3, one diast.), 4.03-4.13 (m, 2H, C*H*CH_3, *H*-4'), 4.30-4.38 (m, 2H, CH_2OP), 4.41 (dd, 1H, *J* = 2.5 Hz, *J* = 5.8 Hz, *H*-3'), 6.12-6.19 (m, 1H, *H*-1'), 7.41-7.46 (m, 1H, Ar*H*), 7.50-7.58 (m, 3H, Ar*H*), 7.70-7.76 (m, 2H, *H*-6, Ar*H*), 7.87-7.91 (m, 1H, Ar*H*), 8.15-8.20 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): $™_C$ 20.3 (d, $^3J_{C-P}$ = 7.1 Hz, CH_3), 20.4 (d, $^3J_{C-P}$ = 6.5 Hz, CH_3), 40.7 (CH_2), 40.8 (CH_2), 51.6 (CH_3), 51.7 (CH_3), 52.7 (CH), 52.8 (CH), 67.8 (d, $^2J_{C-P}$ = 5.7 Hz, CH_2), 67.5 (d, $^2J_{C-P}$ = 5.7 Hz, CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 7.9 Hz, CH), 86.9 (d, $^3J_{C-P}$ = 8.5 Hz, CH), 86.9 (CH), 87.0 (CH), 116.2 (d, $^3J_{C-P}$ = 3.1 Hz, CH), 116.5 (d, $^3J_{C-P}$ = 3.5 Hz, CH), 122.5 (CH), 122.6 (CH), 125.4 (CH), 125.5 (CH), 125.6 (CH), 125.7 (CH), 126.1 (CH), 126.2 (CH), 126.5 (CH), 126.6 (CH), 127.6 (CH), 127.7 (Cx2), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.0 (CH), 136.3 (C), 141.6 (d, $^1J_{C-F}$ = 233.4 Hz, C), 141.7 (d, $^1J_{C-F}$ = 234.1 Hz, C), 147.8 (d, $^2J_{C-P}$ = 7.9 Hz, C), 148.0 (d, $^2J_{C-P}$ = 7.2 Hz, C), 150.6 (C), 159.4 (d, $^2J_{C-F}$ = 27.0 Hz, C), 175.2 (d, $^3J_{C-P}$ = 3.9 Hz, C), 175.5 (d, $^3J_{C-P}$ = 3.9 Hz, C), m/z (ES) 560 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, L = 275 nm, showed two peaks of the diastereoisomers with t_R 28.45 min. and t_R 28.85 min. (73% : 25%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(ethoxy-L-alaninyl)]phosphate (CPF386) (provided as a reference example)

[0081]

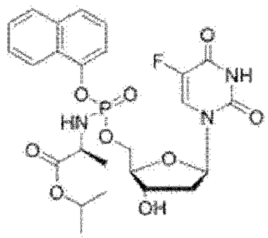


[0082] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), N-methylimidazole (NMI) (0.40 mL, 5.07 mmol) and 1-naphthyl(ethoxy-L-alaninyl) phosphorochloridate (1.04 g, 3.04 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (47.0 mg, 4%) [R_f = 0.25 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 574.1360. $C_{24}H_{27}N_3O_9NaPF$ requires [MNa^+], 574.1367); ^{31}P NMR (202 MHz, MeOD): $™_P$ 4.34, 4.55; ^{19}F NMR (470 MHz, MeOD): $™_F$ -167.31, -167.16; ^{1}H NMR (500 MHz, MeOD): $™_H$ 1.20 (t, 3H, *J* = 7.0 Hz, CH_2CH_3, one diast.), 1.21 (t, 3H, *J* = 7.0 Hz, CH_2CH_3, one diast.), 1.33-1.37 (m, 3H, $CHCH_3$), 1.73-1.86 (m, 1H, *H*-2'), 2.12-2.21 (m, 1H, *H*-2'), 4.01-4.07 (m, 1H, C*H*CH_3), 4.08-4.13 (m, 3H, CH_2CH_3, *H*-4'), 4.31-4.43 (m, 3H, CH_2OP, *H*-3'), 6.11-6.19 (m, 1H, *H*-1'), 7.39-7.46 (m, 1H, Ar*H*), 7.50-7.57 (m, 3H, Ar*H*), 7.68-7.75 (m, 2H, Ar*H*, H-6), 7.86-7.91 (m, 1H, Ar*H*), 8.15-8.20 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): $™_C$ 14.4 (CH_3), 20.3 (d, $^3J_{C-P}$ = 7.4 Hz, CH_3), 20.5 (d, $^3J_{C-P}$ = 6.2 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.8 (CH), 51.9 (CH), 62.4 (CH_2), 62.5 (CH_2), 67.8 (d, $^2J_{C-P}$ = 4.6 Hz, CH_2), 67.9

(d, $^2J_{C-P}$ = 4.6 Hz, CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 8.4 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 8.4 Hz, CH), 86.9 (CH), 87.0 (CH), 116.1 (d, $^3J_{C-P}$ = 3.5 Hz, CH), 116.5 (d, $^3J_{C-P}$ = 3.5 Hz, CH), 122.6 (CH), 125.4 (CH), 125.5 (CH), 125.7 (CH), 125.8 (CH), 126.1 (CH), 126.2 (CH), 126.5 (CH), 126.6 (CH), 127.5 (CH), 127.6 (C), 127.7 (C), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.0 (CH), 136.3 (C), 141.6 (d, $^1J_{C-F}$ = 233.3 Hz, C), 141.7 (d, $^1J_{C-F}$ = 233.4 Hz, C), 147.8 (d, $^2J_{C-P}$ = 6.9 Hz, C), 148.0 (d, $^2J_{C-P}$ = 6.9 Hz, C), 150.6 (C), 159.3 (d, $^2J_{C-F}$ = 26.3 Hz, C), 174.8 (d, $^3J_{C-P}$ = 4.8 Hz, C), 175.1 (d, $^3J_{C-P}$ = 4.0 Hz, C); m/z (ES) 574 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, L = 275 nm, showed two peaks of the diastereoisomers with t_R 30.77 min. and t_R 31.20 min. (51%: 48%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(isopropoxy-L-alaninyl)] phosphate (CPF387) (provided as a reference example)

[0083]

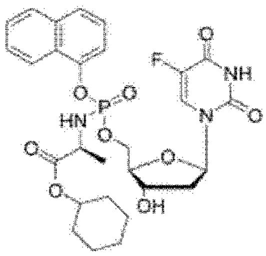


[0084] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.10 g, 0.40 mmol), tert-butylmagnesium chloride in tetrahydrofuran (^tBuMgCl) (1.0 M, 0.61 mL, 0.61 mmol) and 1-naphthyl(isopropoxy-L-alaninyl) phosphorochloridate (0.31 g, 0.89 mmol) according to general procedure **E.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (71.0 mg, 17%) [R_f = 0.21 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 588.1521. $C_{25}H_{29}N_3O_9NaPF$ requires [MNa^+], 588.1523); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 4.38, 4.58; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.43, -167.26; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.19-1.23 (m, 6H, $CH(CH_3)_2$), 1.34-1.38 (m, 3H, $CHCH_3$), 1.68-1.84 (m, 1H, *H*-2'), 2.09-2.20 (m, 1H, *H*-2'), 3.96-4.05 (m, 1H, $CHCH_3$), 4.07-4.12 (m, 1H, *H*-4'), 4.29-4.38 (m, 2H, CH_2OP), 4.39-4.42 (m, 1H, *H*-3'), 4.93-5.01 (m, 1H, $CH(CH_3)_2$), 5.10-6.18 (m, 1H, *H*-1'), 7.40-7.46 (m, 1H, Ar*H*), 7.50-7.57 (m, 3H, Ar*H*), 7.70-7.75 (m, 2H, *H*-6, Ar*H*), 7.87-7.92 (m, 1H, Ar*H*), 8.16-8.20 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.3 (d, $^3J_{C-P}$ = 7.1 Hz, CH_3), 20.5 (d, $^3J_{C-P}$ = 6.6 Hz, CH_3), 21.8 (CH_3), 21.9 (CH_3), 22.0 (CH_3), 22.1 (CH_3), 40.8 (CH_2), 40.9 (CH_2), 51.9 (CH), 52.0 (CH), 67.8 (d, $^2J_{C-P}$ = 4.5 Hz, CH_2), 67.9 (d, $^2J_{C-P}$ = 4.8 Hz, CH_2), 70.2 (CH), 70.3 (CH), 72.0 (CH), 72.1 (CH), 86.6 (CH), 86.7 (CH), 86.9 (d, $^3J_{C-P}$ = 8.6 Hz, CH), 87.0 (d, $^3J_{C-P}$ = 8.6 Hz, CH), 116.2 (d, $^3J_{C-P}$ = 2.5 Hz, CH), 116.5 (d, $^3J_{C-P}$ = 2.7 Hz, CH), 122.6 (CH), 125.5 (CH), 125.7 (CH), 126.1 (CH), 126.2 (CH), 126.5 (CH), 127.5 (CH), 127.6 (C), 127.7 (C), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.0 (CH), 136.3 (C), 141.6 (d, $^1J_{C-F}$ = 233.2 Hz, C), 141.7 (d, $^1J_{C-F}$ = 233.4 Hz, C), 147.7 (d, $^2J_{C-P}$ = 7.6 Hz, C), 147.9

(d, $^2J_{C-P}$ = 7.7 Hz, C), 150.5 (C), 159.4 (d, $^2J_{C-F}$ = 26.2 Hz, C), 174.4 (d, $^3J_{C-P}$ = 5.0 Hz, C), 174.7 (d, $^3J_{C-P}$ = 5.1 Hz, C); m/z (ES) 588 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 32.20 min. and t_R 32.80 min. (27% : 69%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(cyclohexoxy-L-alaninyl)] phosphate (CPF509) (provided as a reference example)

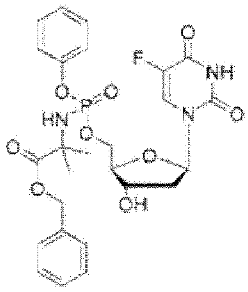
[0085]



[0086] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.30 g, 1.21 mmol), *N*-methylimidazole (NMI) (0.48 mL, 6.09 mmol) and phenyl(cyclohexoxy-L-alaninyl) phosphorochloridate (1.45 g, 3.65 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (6.7 mg, 3%) [R_f = 0.47 (CH_2Cl_2-MeOH, 95:5)]; (Found: MNH_4^+, 623.2261. $C_{28}H_{37}N_4O_9NaPF$ requires [MNH_4^+], 623.2282); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 4.35, 4.52; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.31, -167.17; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.30-1.43 (m, 3H, $CHCH_3$), 1.44-1.56 (m, 4H, $CH(CH_2)_5$), 1.57-1.66 (m, 1H, $CH(CH_2)_5$), 1.67-1.83 (m, 5H, $CH(CH_2)_5$), 1.84-1.93 (m, 1H, *H*-2'), 2.09-2.20 (m, 1H, *H*-2'), 3.98-4.06 (m, 1H, $CHCH_3$), 4.07-4.15 (m, 1H, *H*-4'), 4.29-4.38 (m, 2H, CH_2OP), 4.39-4.44 (m, 1H, *H*-3'), 4.67-4.76 (m, 1H, $CH(CH_2)_5$), 6.09-6.19 (m, 1H, *H*-1'), 7.38-7.57 (m, 5H, Ar*H*), 7.68-7.75 (m, 1H, Ar*H*), 7.79-7.92 (m, 1H, Ar*H*), 8.17 (d, 1H, $^3J_{H-F}$ = 6.6 Hz, *H*-6); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 20.4 (d, $^3J_{C-P}$ = 8.0 Hz, CH_3), 20.6 (d, $^3J_{C-P}$ = 6.5 Hz, CH_3), 24.5 (CH_2), 26.3 (CH_2), 32.3 (CH_2), 40.8 (CH_2), 51.8 (CH), 51.9 (CH), 67.8 (CH_2), 72.0 (CH), 72.2 (CH), 75.0 (CH), 86.7 (d, $^3J_{C-P}$ = 8.2 Hz, CH), 87.0 (CH), 116.1 (d, $^3J_{C-P}$ = 2.5 Hz, CH), 116.4 (d, $^3J_{C-P}$ = 3.0 Hz, CH), 122.6 (CH), 124.8 (CH), 125.9 (CH), 126.1 (CH), 126.2 (CH), 126.4 (CH), 126.5 (CH), 126.6 (CH), 127.6 (CH), 127.7 (Cx2), 127.8 (CH), 127.9 (CH), 128.9 (CH), 129.0 (CH), 136.3 (C), 141.6 (C), 148.0 (d, $^2J_{C-P}$ = 7.2 Hz, C), 150.6 (C), 159.4 (d, $^2J_{C-F}$ = 27.0 Hz, C), 175.2 (d, $^3J_{C-P}$ = 3.9 Hz, C), 175.5 (d, $^3J_{C-P}$ = 3.9 Hz, C); m/z (ES) 623 (MNH_4^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 30.50 min. and t_R 31.48 min. (27% : 69%).

5-Fluoro-2'deoxyuridine-5'-*O*-[phenyl(benzoxy-α,α-dimethylglycine)] phosphate (CPF393) (provided as a reference example)

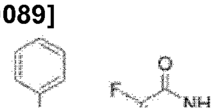
[0087]

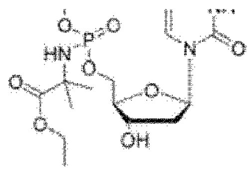


[0088] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.40 g, 1.62 mmol), *tert*-butylmagnesium chloride in tetrahydrofuran (^{t}BuMgCl) (1.0 M, 2.43 mL, 2.43 mmol) and phenyl(benzoxy-α,α-dimethylglycine) phosphorochloridate (1.17 g, 3.20 mmol) according to general procedure **E.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (69.0 mg, 7%) [R_f = 0.27 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 600.1527. $C_{26}H_{29}N_3O_9NaPF$ requires [MNa^+], 600.1523); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 2.42, 2.47; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.80, -167.62; ^{1}H NMR (500 MHz, MeOD): ™$_H$ 1.51-1.60 (m, 6H, C(C*H*$_3$)$_2$), 1.89-1.97 (m, 1H, *H*-2', one diast.), 2.07-2.15 (m, 1H, *H*-2', one diast.), 2.21 (ddd, 1H, *J* = 3.4 Hz, 5.9 Hz, 13.5 Hz, *H-2',* one diast.), 2.29 (ddd, 1H, *J* = 3.2 Hz, 6.1 Hz, 13.5 Hz, *H-2',* one diast.), 4.00-4.07 (m, 1H, *H*-4'), 4.22-4.31 (m, 2H, C*H*$_2$OP), 4.32-4.36 (m, 1H, *H*-3', one diast.), 4.37-4.41 (m, 1H, *H*-3', one diast.), 5.08-5.18 (m, 2H, OC*H*$_2$Ph), 6.19-6.25 (m, 1H, *H*-1'), 7.20-7.26 (m, 3H, Ar*H*), 7.27-7.39 (m, 7H, Ar*H*), 7.74 (d, $^3J_{H\text{-}F}$ = 6.4 Hz, *H*-6, one diast.), 7.80 (d, $^3J_{H\text{-}F}$ = 6.4 Hz, *H*-6, one diast.); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 27.5 (CH_3), 27.7 (d, $^3J_{C\text{-}P}$ = 7.1 Hz, CH_3), 27.8 (d, $^3J_{C\text{-}P}$ = 7.1 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 58.2 (C), 58.3 (C), 67.6 (d, $^2J_{C\text{-}P}$ = 5.5 Hz, CH_2), 67.7 (d, $^2J_{C\text{-}P}$ = 5.5 Hz, CH_2), 68.3 (CH_2), 71.9 (CH), 72.0 (CH), 86.6 (d, $^3J_{C\text{-}P}$ = 8.1 Hz, CH), 86.8 (d, $^3J_{C\text{-}P}$ = 7.3 Hz, CH), 86.9 (CH), 121.4 (d, $^3J_{C\text{-}P}$ = 4.8 Hz, CH), 121.6 (d, $^3J_{C\text{-}P}$ = 4.5 Hz, CH), 125.6 (CH), 125.8 (CH), 125.9 (CH), 126.1 (CH), 126.2 (CH), 129.3 (CH), 129.4 (CH), 129.6 (CH), 130.7 (CH), 130.8 (CH), 137.2 (C), 137.3 (C), 141.8 (d, $^1J_{C\text{-}F}$ = 233.7 Hz, C), 150.6 (C), 152.1 (d, $^4J_{C\text{-}F}$ = 7.0 Hz, C), 152.1 (d, $^4J_{C\text{-}F}$ = 7.6 Hz, C), 159.3 (d, $^2J_{C\text{-}F}$ = 26.1 Hz, C), 159.4 (d, $^2J_{C\text{-}F}$ = 26.1 Hz, C), 176.5 (d, $^3J_{C\text{-}P}$ = 4.0 Hz, C), 176.6 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, C), m/z (ES) 600.1 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 35 minutes, 1 ml/min, └ = 275 nm, showed one peak of the mixture of diastereoisomers with t_R 17.71 (96%).

5-Fluoro-2'deoxyuridine-5'-*O*-[phenyl(ethoxy-α,α-dimethylglycine)] phosphate (CPF394) (provided as a reference example)

[0089]

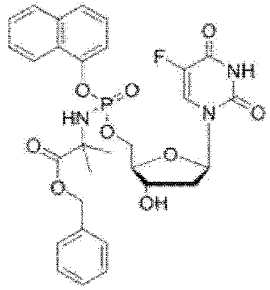




[0090] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.20 g, 0.80 mmol), *N*-methylimidazole (NMI) (0.31 mL, 4.0 mmol) and phenyl(ethoxy-α,α-dimethylglycine) phosphorochloridate (0.73 g, 2.40 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (25.0 mg, 6%) [R_f = 0.24 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 538.1367. $C_{21}H_{27}N_3O_9NaPF$ requires [MNa^+], 538.1367); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 2.49, 2.52; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.62, -167.58; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.24 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.26 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.44-1.54 (m, 6H, $C(CH_3)_2$), 1.95-2.04 (m, 1H, *H-2',* one diast.), 2.13-2.21 (m, 1H, *H*-2', one diast.), 2.24 (ddd, 1H, *J* = 3.1 Hz, *J* = 6.3 Hz, *J* = 13.5 Hz, *H*-2', one diast.), 2.31 (ddd, 1H, *J* = 3.2 Hz, *J* = 6.1 Hz, *J* = 13.7 Hz, *H*-2', one diast.), 4.08-4.19 (m, 3H, CH_2CH_3, *H*-4'), 4.33-4.49 (m, 3H, CH_2OP, *H*-3'), 6.20-6.30 (m, 1H, *H*-1'), 7.23-7.28 (m, 3H, Ar*H*), 7.33-7.40 (m, 2H, Ar*H*), 7.80 (d, $^3J_{H-F}$ = 6.4 Hz, *H*-6, one diast.), 7.88 (d, $^3J_{H-F}$ = 6.4 Hz, *H*-6, one diast.); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 14.4 (CH_3), 14.5 (CH_3), 27.5 (d, $^3J_{C-P}$ = 7.3 Hz, CH_3), 27.7 (d, $^3J_{C-P}$ = 7.6 Hz, CH_3), 27.8 (d, $^3J_{C-P}$ = 7.6 Hz, CH_3), 40.8 (CH_2), 40.9 (CH_2), 58.1 (C), 62.6 (CH_2), 62.7 (CH_2), 67.6 (d, $^2J_{C-P}$ = 6.7 Hz, CH_2), 67.7 (d, $^2J_{C-P}$ = 5.8 Hz, CH_2), 71.9 (CH), 72.0 (CH), 86.6 (d, $^3J_{C-P}$ = 8.1 Hz, CH), 86.8 (d, $^3J_{C-P}$ = 7.6 Hz, CH), 86.9 (CH), 121.4 (d, $^3J_{C-P}$ = 4.4 Hz, CH), 121.6 (d, $^3J_{C-P}$ = 4.4 Hz, CH), 125.6 (CH), 125.8 (CH), 125.9 (CH), 126.1 (CH), 126.2 (CH), 130.7 (CH), 130.8 (CH), 130.9 (CH), 141.8 (d, $^1J_{C-F}$ = 233.5 Hz, C), 150.6 (C), 150.7 (C), 152.2 (d, $^4J_{C-F}$ = 7.3 Hz, C), 152.3 (d, $^4J_{C-F}$ = 6.9 Hz, C), 159.2 (d, $^2J_{C-F}$ = 20.3 Hz, C), 159.4 (d, $^2J_{C-F}$ = 20.4 Hz, C), 176.6 (d, $^3J_{C-P}$ = 4.2 Hz, C), 176.8 (d, $^3J_{C-P}$ = 4.6 Hz, C), m/z (ES) 538.1 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 18.76 min. and t_R 20.44 min. (68% : 30%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(benzoxy-α,α-dimethylglycine)] phosphate (CPF395) (provided as a reference example)

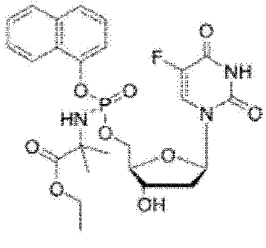
[0091]



[0092] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.40 g, 1.62 mmol), N-methylimidazole (NMI) (0.64 mL, 8.0 mmol) and 1-naphthyl(benzoxy-α,α-dimethylglycine) phosphorochloridate (2.00 g, 4.80 mmol) according to general procedure **D.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (16.4 mg, 6%) [R_f = 0.15 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 650.1678. $C_{30}H_{31}N_3O_9NaPF$ requires [MNa^+], 650.1680); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 2.87, 3.03; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.95, -167.13; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.37-1.42 (m, 6H, C(C*H*$_3$)$_2$), 1.61-1.69 (m, 1H, *H*-2', one diast.), 1.79-1.87 (m, 1H, *H*-2', one diast.), 2.06 (ddd, 1H, *J* = 3.0 Hz, *J* = 6.1 Hz, *J* = 13.6 Hz, *H*-2', one diast.), 2.15 (ddd, 1H, *J* = 3.2 Hz, *J* = 5.9 Hz, *J* = 13.7 Hz, *H*-2', one diast.), 3.98-4.04 (m, 1H, *H*-4'), 4.19-4.35 (m, 3H, C*H*$_2$OP, *H*-3'), 5.09-5.13 (m, 1H, OC*H*HPh), 5.18-5.19 (m, 1H, OCH*H*Ph), 6.05-6.15 (m, 1H, *H*-1'), 7.28-7.40 (m, 7H, Ar*H*), 7.48-7.55 (m, 3H, Ar*H*), 7.62 (d, $^3J_{H\text{-}F}$ = 6.4 Hz, *H*-6, one diast.), 7.70 (d, $^3J_{H\text{-}F}$ = 6.4 Hz, *H*-6, one diast.), 7.86-7.90 (m, 1H, Ar*H*), 8.17-8.22 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 27.5 (d, $^3J_{C\text{-}P}$ = 4.4 Hz, CH_3), 27.9 (d, $^3J_{C\text{-}P}$ = 7.3 Hz, CH_3), 28.0 (d, $^3J_{C\text{-}P}$ = 7.3 Hz, CH_3), 40.7 (CH_2), 40.8 (CH_2), 65.2 (C), 67.8 (d, $^2J_{C\text{-}P}$ = 6.5 Hz, CH_2), 68.3 (CH_2), 72.0 (CH), 72.1 (CH), 86.6 (d, $^3J_{C\text{-}P}$ = 8.2 Hz, CH), 86.8 (d, $^3J_{C\text{-}P}$ = 7.8 Hz, CH), 86.9 (CH), 116.3 (d, $^3J_{C\text{-}P}$ = 3.2 Hz, CH), 116.7 (d, $^3J_{C\text{-}P}$ = 2.9 Hz, CH), 122.8 (CH), 122.9 (CH), 125.4 (CH), 125.5 (CH), 125.6 (CH), 126.0 (CH), 126.1 (CH), 126.4 (CH), 126.5 (CH), 127.4 (CH), 127.5 (CH), 127.7 (CH), 127.8 (CH), 127.9 (C), 128.0 (CH), 128.9 (CH), 129.3 (CH), 129.4 (CH), 129.6 (CH), 136.2 (C), 137.3 (C), 141.8 (d, $^1J_{C\text{-}F}$ = 234.4 Hz, C), 147.9 (d, $^3J_{C\text{-}P}$ = 7.7 Hz, C), 148.0 (d, $^3J_{C\text{-}P}$ = 8.2 Hz, C), 150.7 (d, $^4J_{C\text{-}F}$ = 3.7 Hz, C), 159.5 (d, $^2J_{C\text{-}F}$ = 25.8 Hz, C), 159.6 (d, $^2J_{C\text{-}F}$ = 25.8 Hz, C), 176.5 (C), 176.6 (C), m/z (ES) 650.0 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, ^{L} = 275 nm, showed two peaks of the diastereoisomers with t_R 20.80 min. and t_R 21.00 min. (72% : 24%).

5-Fluoro-2'deoxyuridine-5'-*O*-[1-naphthyl(ethoxy-α,α-dimethylglycine)] phosphate (CPF396) (provided as a reference example)

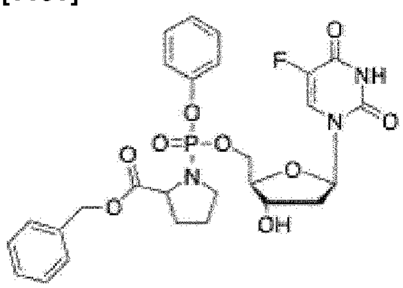
[0093]



[0094] The phosphoramidate was prepared using 5-fluoro-2'-deoxyuridine (0.40 g, 1.62 mmol), *tert*-butylmagnesium chloride in tetrahydrofuran (^tBuMgCl) (1.0 M, 2.43 mL, 2.43 mmol) and 1-naphthyl(ethoxy-α,α-dimethylglycine) phosphorochloridate (1.14 g, 3.20 mmol) according to general procedure **E.** Purification by gradient column chromatography eluting with CH_2Cl_2 until CH_2Cl_2-MeOH (95:5) afforded the title compound as a colourless solid (54.0 mg, 2%) [R_f = 0.10 (CH_2Cl_2-MeOH, 95:5)], (Found: MNa^+, 588.1528. $C_{25}H_{29}N_3O_9NaPF$ requires [MNa^+], 588.1523); ^{31}P NMR (202 MHz, MeOD): ™$_P$ 2.91, 3.03; ^{19}F NMR (470 MHz, MeOD): ™$_F$ -167.38, -167.21; ^1H NMR (500 MHz, MeOD): ™$_H$ 1.24 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.25 (t, 3H, *J* = 7.1 Hz, CH_2CH_3, one diast.), 1.50-1.55 (m, 6H, $C(CH_3)_2$), 1.68-1.76 (m, 1H, *H*-2', one diast.), 1.87-1.94 (m, 1H, *H*-2', one diast.), 2.09 (ddd, 1H, *J* = 2.9 Hz, *J* = 6.3 Hz, *J* = 13.4 Hz, *H-2',* one diast.), 2.19 (ddd, 1H, *J* = 3.0 Hz, *J* = 6.3 Hz, *J* = 13.8 Hz, *H-2',* one diast.), 4.07-4.10 (m, 1H, *H*-4'), 4.16 (q, 2H, *J* = 7.1 Hz, CH_2CH_3), 4.36-4.41 (m, 3H, CH_2OP, *H*-3'), 6.10-6.18 (m, 1H, *H*-1'), 7.40-7.46 (m, 1H, Ar*H*), 7.50-7.59 (m, 3H, Ar*H*), 7.66-7.72 (m, 2H, Ar*H*, *H*-6), 7.85-7.91 (m, 1H, Ar*H*), 8.18-8.24 (m, 1H, Ar*H*); ^{13}C NMR (125 MHz, MeOD): ™$_C$ 14.4 (CH_3), 27.5 (br s, CH_3), 27.9 (d, $^3J_{C-P}$ = 6.1 Hz, CH_3), 28.0 (d, $^3J_{C-P}$ = 6.1 Hz, CH_3), 40.7 (CH_2), 40.8 (CH_2), 58.2 (C), 58.3 (C), 62.6 (CH_2), 67.8 (d, $^2J_{C-P}$ = 4.9 Hz, CH_2), 67.9 (d, $^2J_{C-P}$ = 4.5 Hz, CH_2), 72.0 (CH), 72.1 (CH), 86.7 (d, $^3J_{C-P}$ = 7.7 Hz, CH), 86.9 (d, $^3J_{C-P}$ = 7.3 Hz, CH), 87.0 (CH), 116.3 (d, $^3J_{C-P}$ = 3.2 Hz, CH), 116.6 (d, $^3J_{C-P}$ = 2.9 Hz, CH), 122.8 (CH), 122.9 (CH), 125.4 (CH), 125.6 (CH), 125.7 (CH), 126.0 (CH), 126.1 (CH), 126.5 (CH), 127.4 (CH), 127.5 (CH), 127.7 (CH), 127.8 (CH), 127.9 (C), 128.0 (C), 128.9 (CH), 136.2 (C), 141.8 (d, $^1J_{C-F}$ = 233.5 Hz, C), 148.0 (d, $^2J_{C-P}$ = 7.3 Hz, C), 148.1 (d, $^2J_{C-P}$ = 7.6 Hz, C), 150.5 (C), 150.6 (C), 159.3 (d, $^2J_{C-F}$ = 26.2 Hz, C), 159.4 (d, $^2J_{C-F}$ = 26.6 Hz, C), 176.8 (C), 176.9 (C); m/z (ES) 588.1 (MNa^+, 100%); Reverse-phase HPLC eluting with H_2O/MeOH from 100/0 to 0/100 in 45 minutes, 1 ml/min, └ = 275 nm, showed one peak of the mixture of diastereoisomers with t_R 16.05 min. (96%).

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl(benzoxy-L-prolinyl)]phosphate (CPF583) (provided as a reference example)

[0095]



[0096] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and phenyl(benzoxy-L-prolinyl)-phosphochloridate (0.77 g, 2.03 mmol) in THF (10 mL). Column purification followed by two preparative TLC purifications gave the product as a white solid (0.010 g, 2%).

^{31}P-NMR (MeOD, 202 MHz) δ 1.82

^{19}F-NMR (MeOD, 470 MHz) δ - 167.91

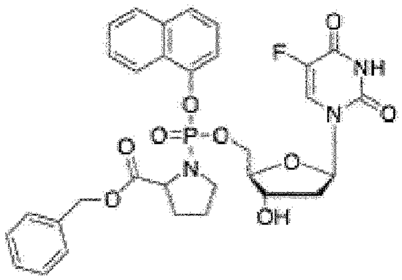
^{1}H-NMR (MeOD, 500 MHz) δ 7.84 (d, *J* = 7.18 Hz, 1H, *H*-base), 7.39 - 7.33 (m, 7H, *H*-Ar), 7.22 - 7.19 (m, 3H, *H*-Ar), 6.26 - 6.23 (m, 1H, *H*-1'), 5.22 - 5.13 (m, CH_2Ph ester), 4.40 - 4.35 (m, 3H, NC*H*, 2 x *H*-5'), 4.33 - 4.28 (m, 1H, *H*-3'), 4.06 - 4.04 (m, 1H, *H*-4'), 3.36 - 3.32 (m, 2H, NCH_2), 2.26 - 2.19 (m, 1H, *H*-2'), 2.18 - 2.13 (m, 1H, CH_2-L-Pro), 2.00 - 1.81 (m, 4H, 3 x H, CH_2-L-Pro, 1 x H, *H*-2')

^{13}C-NMR (MeOD, 125 MHz) δ 174.81 (*C*=O, ester), 159.40 (*C*=O, base), 152.0 (d, $^{2}J_{C-P}$ = 6.32 Hz, O*C*-Ar), 150.71 (*C*=O, base), 141.88 ($^{1}J_{C-F}$ = 232 Hz, *C*F, base), 137.23 (*C*Ar), 131.33, 129.70, 129.48, 129.45, 129.30, 126.45 (*C*H-Ar), 125.80, 125.53 (2 x d, $^{2}J_{C-F}$ = 29.0 Hz, *C*H-base), 121.00, 120.96 (*C*H-Ar), 87.80 (*C*-1'), 86.80 (*C*-4'), 72.02 (*C*-3'), 68.16 (*C*H_2Ph), 67.64 (d, $^{2}J_{C-P}$ = 4.65 Hz, *C*-5'), 62.40 (d, $^{2}J_{C-P}$ = 5.60 Hz, N*C*H), 48.03 (d, $^{2}J_{C-P}$ = 4.80 Hz, N*C*H_2), 41.07 (*C*-2'), 32.18, 32.11 (*C*H_2-L-Pro), 26.29, 26.21 (*C*H_2-L-Pro).

MS (ES+) m/e: 612 (MNa^{+}, 100%), 590 (MH+, 1%) Accurate mass: $C_{27}H_{29}FN_3O_9P$ required 589.51

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(benzoxy-L-prolinyl)]phosphate (CPF577) (provided as a reference example)

[0097]



[0098] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl(benzoxy-L-prolinyl)-phosphochloridate (0.84 g, 2.03 mmol) in THF (10 mL). Column purification followed by two preparative TLC purifications gave the product as a white solid (0.006 g, 1%).

^{31}P-NMR (MeOD, 202 MHz) δ 2.27

^{19}F-NMR (MeOD, 121 MHz) δ - 167.46

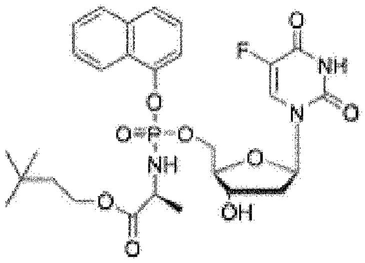
^{1}H-NMR (MeOD, 500 MHz) δ 8.14 - 8.12 (m, 1H, *H*-Ar), 7.90 - 7.89 (m, 1H, *H*-Ar), 7.74 - 7.71 (m, 2H, 1 x *H*-Ar, 1 x *H*-base), 7.56 - 7.42 (m, 4H, *H*-Ar), 7.36 - 7.33 (m, 5H, *H*-Ar), 6.13 (t, *J* = 6.38 Hz, *H*-1'), 5.22 - 5.13 (m, 2H, CH_2Ph), 4.49 - 4.46 (m, 1H, NC*H*), 4.42 - 4.33 (m, 2H, *H*-5'), 4.25 - 4.23 (m, 1H, *H*-3'), 4.06 - 4.04 (m, 1H, *H*-4'), 3.36 - 3.34 (m, 2H, NCH_2), 2.23 - 2.15 (m, 1H, CH_2-L-Pro), 2.10 - 2.02 (m, 2H, 1 x H, CH_2-L-Pro, 1 x H, *H*-2'), 1.97 - 1.77 (m, 2H, CH_2-L-Pro), 1.63 - 1.57 (m, 1H, *H*-2')

^{13}C-NMR (MeOD, 125 MHz) δ 174.82 (*C*=O, ester), 159.52 (*C*=O, base), 150.54 (*C*=O, base), 147.84, 147.78 (d, $^{2}J_{C-P}$ = 6.03 Hz, O*C*-Ar), 141.75, 139.97 (2 x d, $^{1}J_{C-F}$ = 232 Hz, *C*F, base), 137.20, 136.34 (*C*-Ar), 129.76, 129.65, 129.44, 129.36, 129.27, 129.06, 128.95, 128.04, 128.75, 126.56 (*C*H-Ar), 125.41 (d, $^{2}J_{C-F}$ = 30.0 Hz, *C*H-base), 122.13 (*C*H-Ar), 115.76 (d, $^{3}J_{C-P}$ = 3.3 Hz, *C*H-Ar), 87.06

(*C*-1'), 86.79 (*C*-4'), 72.23 (*C*-3'), 68.15 (d, $^{2}J_{C-P}$ = 5.46 Hz, C-5'), 68.08 (CH_2Ph), 62.53 (d, $^{2}J_{C-P}$ = 5.60 Hz, NCH), 48.26 (d, $^{2}J_{C-P}$ = 5.34 Hz, NCH_2), 40.97 (C-2'), 32.16, 32.09 (CH_2-L-Pro), 26.22, 26.15 (CH_2-L-Pro).

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl(3,3-dimethyl-1-butoxy-L-alaninyl)]phosphate (CPF585) (provided as a reference example)

[0099]



[0100] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl-(3,3-dimethyl-1-butoxy-L-alaninyl)-phosphochloridate (1.21 g, 3.04 mmol) in THF (10 mL). Column purification followed by two preparative TLC purifications gave the product as a white solid (0.010 g, 2%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.48, 4.33

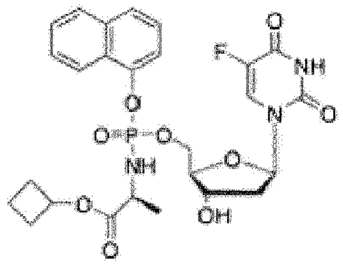
^{19}F-NMR (MeOD, 470 MHz) δ - 167.30, - 167.47

^{1}H-NMR (MeOD, 500 MHz) δ 8.20 - 8.17 (m, 1H, *H*-Ar), 7.91 - 7.89 (m, 1H, *H*-Ar), 7.77 - 7.72 (m, 2H, *H*-Ar), 7.58 - 7.51 (m, 3H, *H*-base, 2 x *H*-Ar), 7.46 - 7.41 (2 x t, 1H, *J* = 7.8 Hz, *H*-Ar), 6.19 - 6.13 (m, 1H, *H*-1'), 4.42 - 4.40 (m, 1H, 1 x *H*-5'), 4.38 - 4.32 (m, 2H, *H*-3', 1 x *H*-5'), 4.14 - 4.00 (m, 4H, *H*-4', C*H*CH_3, OC$H_2$$CH_2$($CH_3$)$_3$), 2.21 - 2.13 (m, 1H, 1 x *H*-2'), 1.91 - 1.76 (m, 1H, 1 x *H*-2'), 1.52 - 1.48 (m, 2H, OCH_2CH_2(CH_3)$_3$), 1.37 - 1.35 (m, 3H, CHCH_3), 0.92, 0.91 (2 x s, 9H, O$CH_2$$CH_2$(C$H_3$)$_3$) ^{13}C-NMR (MeOD, 125 MHz) δ 175.16, 174.84 (2 x d, $^{3}J_{C-P}$ = 4.75 Hz, *C*=O, ester), 159.56, 159.35 (*C*=O, ester), 150.61 (*C*=O, ester), 148.00, 147.86 (2 x d, $^{2}J_{C-P}$ = 6.25 Hz, O*C*-Ar), 141.78, 141.73 (2 x d, $^{1}J_{C-F}$ = 232 Hz, *C*F, base), 136.28 (*C*-Ar), 128.98, 128.95, 127.92, 127.90, 127.58, 126.57, 126.20, 126.14 (*C*H-Ar), 125.63, 125.55 (2 x d, $^{2}J_{C-F}$ = 34 Hz, *C*H, base), 122.65, 122.63 (*C*H-Ar), 116.48, 116.15 (2 x d, $^{3}J_{C-P}$ = 3.0 Hz, *C*H-Ar), 87.01, 86.94 (*C*-1'), 86.73, 86.68 (d, $^{3}J_{C-P}$ = 7.75 Hz, *C*-4'), 72.18, 72.07 (*C*-3'), 67.87, 67.85 (2 x d, $^{2}J_{C-P}$ = 5.0 Hz, *C*-5'), 64.08, 64.05 (O$CH_2$$CH_2$($CH_3$)$_3$), 51.86 (d, $^{3}J_{C-P}$ = 5.5 Hz, *C*HCH_3), 42.74 (O$CH_2$$CH_2$($CH_3$)$_3$), 40.91, 40.83 (*C*-2'), 29.96 (O$CH_2$$CH_2$(*C*$H_3$)$_3$), 20.50, 20.34 (2 x d, $^{3}J_{C-P}$ = 6.5 Hz, CH*C*H_3).

MS (ES+) m/e: 630 (MNa^+, 100%), 608 (MH+, 10%) Accurate mass: $C_{28}H_{35}FN_3O_9P$ required 607.56

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(cyclobutoxy-L-alaninyl)] phosphate (CPF578) (provided as a reference example)

[0101]



[0102] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.23 g, 0.93 mmol), NMI (0.38 g, 4.67 mmol, 0.37 mL) and 1-naphthyl-(cyclobutoxy-L-alaninyl)-phosphochloridate (0.85 g, 2.33 mmol) in THF (10 mL). Column purification followed by preparative TLC purification gave the product as a white solid (0.010 g, 2%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.54, 4.36

^{19}F-NMR (MeOD, 470 MHz) δ - 167.12, - 167.29

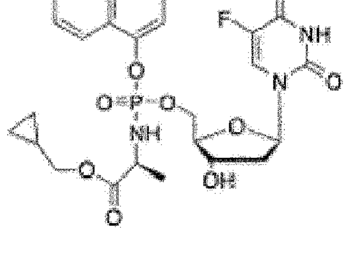
^{1}H-NMR (MeOD, 500 MHz) δ 8.18 - 8.17 (m, 1H, *H*-Ar), 7.81 - 7.87 (m, 1H, *H*-Ar), 7.74 - 7.71 (m, 2H, 1 x *H*-Ar, 1 x *H*-base), 7.60 - 7.53 (m, 3H, *H*-Ar), 7.46 - 7.43 (2 x t, *J* = 8.0 Hz, 1H, *H*-Ar), 6.18 - 6.12 (m, 1H, *H*-1'), 5.00 - 4.95 (m, 1H, OC*H* ester), 4.41 - 4.36 (m, 3H, 2 x *H*-5', *H*-3'), 4.11 - 4.00 (m, 2H, *H*-4', C*H*CH_3), 2.36 - 2.27 (m, 2H, CH_2), 2.18 - 1.98 (m, 3H, CH_2 ester, 1 x *H*-2'), 1.82 - 1.56 (m, 3H, CH_2 ester, 1 x *H*-2'), 1.36 - 1.34 (m, 3H, CHCH_3)

^{13}C-NMR (MeOD, 125 MHz) δ 175.97, 173.34 (*C*=O, ester), 159.88 (*C*=O, base), 151.64 (*C*=O, base), 146.58 (O*C*-Ar), 141.15 (d, $^{1}J_{C-F}$ = 220 Hz, *C*F, base), 136.28 (*C*Ar), 128.93, 127.89, 127.54, 126.52, 126.18, 126.14 (*C*H-Ar), 125.53, 125.44 (2 x d, $^{2}J_{C-F}$ = 32.5 Hz, *C*H-base), 122.63 (*C*H-Ar), 116.46, 116.44 (2 x d, $^{3}J_{C-P}$ = 2.5 Hz, *C*H-Ar), 86.98 (d, $^{3}J_{C-P}$ = 6.25 Hz, *C*-4'), 86.71 (*C*-1'), 72.14, 72.04 (*C*-3'), 71.07 (O*C*H ester), 67.83 (d, $^{2}J_{C-P}$ = 7.38 Hz, *C*-5'), 51.66 (d, $^{2}J_{C-P}$ = 8.75 Hz, *C*HCH_3), 40.89, 40.83 (*C*-2'), 31.03 (OCHCH_2), 20.43 (CHCH_3), 14.23 (CH_2 ester).

MS (ES+) m/e: 600 (MNa$^+$, 100%), 578 (MH+, 10%) Accurate mass: $C_{26}H_{29}FN_3O_9P$ required 577.50

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(cyclopropylmethanoxy-L-alaninyl)]phosphate (CPF579) (provided as a reference example)

[0103]



[0104] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl-(cyclopropylmethanoxy-L-alaninyl)-phosphochloridate (0.93 g, 2.54 mmol) in THF (10 mL). Column purification gave the product as a white solid (0.056 g, 10 %).

^{31}P-NMR (MeOD, 202 MHz) δ 4.58, 4.30

^{19}F-NMR (MeOD, 470 MHz) δ -167.18, -167.22

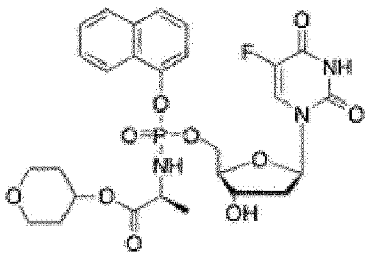
^{1}H-NMR (MeOD, 500 MHz) δ 8.18 (d, *J* = 7.0 Hz, 1H, *H*-Ar), 7.89 - 7.87 (m, 1H, *H*-Ar), 7.73 - 7.70 (m, 2H, *H*-Ar), 7.58 - 7.53 (m, 3H, *H*-Ar), 7.45 - 7.40 (2 x t, *J* = 8.0 Hz, 1H, *H*-Ar), 6.17 - 6.11 (m, 1H, *H*-1'), 4.43 - 4.41 (m, 1H, *H*-5'), 4.38 - 4.32 (m, 2H, *H*-5', *H*-3'), 4.11 - 4.04 (m, 2H, *H*-4', C*H*CH_3), 3.95 - 3.85 (m, 2H, OCH_2 ester), 2.19 - 2.11 (m, 1H, *H*-2'), 1.84 - 1.72 (m, 1H, *H*-2'), 1.38, 1.36 (2 x d, *J* = 5.0 Hz, 3H, CHCH_3), 1.15 - 1.07 (m, 1H, OCH_2C*H* ester), 0.59 - 0.50 (m, 2H, CH_2 ester), 0.30 - 0.24 (m, 2H, CH_2 ester)

^{13}C-NMR (MeOD, 125 MHz) δ 175.25, 174.94 (2 x d, $^{3}J_{C-P}$ = 4.75 Hz, *C*=O, ester), 159.54, 159.35 (*C*=O, base), 150.60, 150.56 (*C*=O, base), 148.05, 147.86 (2 x d, $^{2}J_{C-P}$ = 7.5 Hz, O*C*-Ar), 141.79, 141.73 (2 x d, $^{1}J_{C-F}$ = 232 Hz, *C*F, base), 136.29 (*C*-Ar), 128.94 (d, $^{3}J_{C-P}$ = 4.4 Hz, *C*H-Ar), 127.89 (d, $^{4}J_{C-P}$ = 3.7 Hz, *C*H-Ar), 127.56, 126.55, 126.52, 126.19, 126.16 (*C*H-Ar), 125.64, 125.53 ($^{2}J_{C-F}$ = 34 Hz, *C*H-base), 122.65 (*C*H-Ar), 116.54, 116.24 (2 x d, $^{4}J_{C-P}$ = 2.6 Hz, *C*H-Ar), 87.04, 86.99 (*C*-1'), 86.90, 86.73 (2 x d, $^{3}J_{C-P}$ = 7.1 Hz, *C*-4'), 72.18, 72.07 (*C*-3'), 71.21, 71.18 (OCH_2, ester), 67.87, 67.84 (apparent t, $^{2}J_{C-P}$ = 5.0 Hz, *C*-5'), 51.88 (d, $^{2}J_{C-P}$ = 10.0 Hz, *C*HCH_3), 40.91, 40.83 (*C*-2'), 20.60, 20.46 (2 x d, $^{3}J_{C-P}$ = 6.5 Hz, CHCH_3), 10.69 (OCH_2*C*H ester), 3.70, 3.65 (2 x CH_2, ester).

MS (ES+) m/e: 600 (MNa^+, 100%), 578 (MH+, 15%) Accurate mass: $C_{26}H_{29}FN_3O_9P$ required 577.50.
$HPLC_b$ (H_2O/Acetonitrile from 100/0 to 0/100 in 35 min) Rt 12.91 min.

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(tetrahydropyroxy-L-alaninyl)] phosphate (CPF580) (provided as a reference example)

[0105]



[0106] Prepared according to the standard procedure **E** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), *t*BuMgCl (1.1 mL, 1.1 mmol) and 1-naphthyl-(tetrahydropyroxy-L-alaninyl)-phosphochloridate (0.80 g, 2.03 mmol) in THF (10 mL). Column purification followed by two preparative TLC purifications gave the product as a white solid (0.010 g, 1.6%).

^{31}P-NMR (MeOD, 202 MHz) δ 3.77, 3.22

^{19}F-NMR (MeOD, 470 MHz) δ - 168.27, -168.35

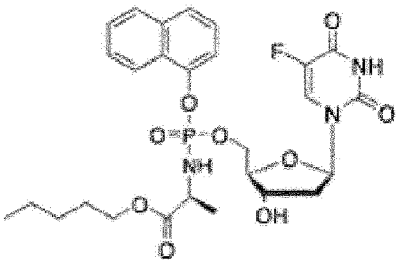
^{1}H-NMR (MeOD, 500 MHz) δ 8.60 (d, *J* = 7.0 Hz, 2H, *H*-Ar), 8.22 - 8.19 (m, 1H, *H*-Ar), 7.92 - 7.91 (d, *J* = 5.50 Hz, 1H, *H*-Ar), 7.60 - 7.45 (m, 4H, *H*-Ar, *H*-base), 6.29 - 6.25 (m, 1H, *H*-1'), 5.25 - 5.17 (m, 1H, *H*-3'), 4.96 - 4.87 (m, 1H, C*H*-ester), 4.28 - 4.26 (m, 1H, *H*-4'), 4.11 - 4.03 (m, 1H, C*H*CH_3), 3.88 - 3.66 (m, 4H, 2 x O$CH_{2a'}$ ester, 2 x *H*-5'), 3.55 - 3.50 (m, 2H, 2 x O$CH_{2a''}$ ester), 2.63 - 2.30 (m, 2H, *H*-2'), 1.91 - 1.85 (m, 2H, 2 x $CH_{2b'}$ ester), 1.65 - 1.54 (m, 2H, $CH_{2b''}$ ester), 1.39 - 1.35 (m, 3H, CHCH_3).

^{13}C-NMR (MeOD, 125 MHz) δ 174.34 (*C*=O, ester), 159.24 (*C*=O, base), 150.76 (*C*=O, base), 148.03 (O*C*-Ar), 141.97 (d, $^{1}J_{C-F}$ = 238 Hz, *C*F, base), 136.37 (*C*-Ar), 128.97, 128.56, 127.61, 127.57, 126.58, 126.23, 126.16, 126.12, 125.84 (*C*H-Ar), 122.70 (d, $^{2}J_{C-F}$ = 24.0 Hz, *C*H-base), 116.62, 116.37 (*C*H-Ar), 87.54 (d, $^{3}J_{C-P}$ = 5.40 Hz, *C*-4'), 86.60, 86.57 (*C*-1'), 79.82, 79.47 (*C*-3'), 71.45 (*C*H-ester), 66.12, 66.08 (2 x OCH_{2a} ester), 66.02 (*C*-5'), 51.83 (*C*HCH_3), 39.97, 39.94 (*C*-2'), 32.65, 32.57 (2 x CH_{2b} ester), 20.45, 20.30 (CHCH_3).

MS (ES+) m/e: 630 (MNa^+, 100%), 608 (MH+, 10%) Accurate mass: $C_{27}H_{31}FN_3O_{10}P$ required 607.52.

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(pentoxy-L-alaninyl)]phosphate (CPF581) (provided as a reference example)

[0107]



[0108] Prepared according to the standard procedure **E** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), *t*BuMgCl (1.1 mL, 1.1 mmol) and 1-naphthyl-(pentoxy-L-alaninyl)-phosphochloridate (0.78 g, 2.03 mmol) in THF (10 mL). Column purification gave the product as a white solid (0.047 g, 8%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.48, 4.32

^{19}F-NMR (MeOD, 470 MHz) δ -167.18, -167.29

^{1}H-NMR (MeOD, 500 MHz) δ 8.25 - 8.17 (m, 1H, *H*-Ar), 8.05 - 7.95 (m, 2H, *H*-Ar), 7.85 - 7.60 (m, 2H, *H*-Ar, *H*-base), 7.65 - 7.48 (m, 3H, *H*-Ar), 6.30 - 6.18 (m, 1H, *H*-1'), 4.60 - 4.37 (m, 3H, 2 x *H*-5', *H*-3'), 4.28 - 4.00 (m, 4H, *H*-4', C*H*CH3, O$CH_2CH_2CH_2CH_2CH_3$), 2.32 - 2.12 (m, 1H, *H*-2'), 1.95 - 1.75 (m, 1H, *H*-2'), 1.70 - 1.55 (m, 2H, O$CH_2CH_2CH_2CH_2CH_3$), 1.50 - 1.28 (m, 7H, 4 x H O$CH_2CH_2CH_2CH_2CH_3$, CHCH_3), 0.83, 0.82 (2 x d, *J* = 7.9 Hz, 3H, O$CH_2CH_2CH_2CH_2CH_3$)

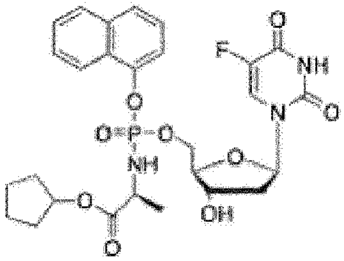
^{13}C-NMR (MeOD, 125 MHz) δ 175.22, 174.91 (*C*=O, ester), 159.5 (*C*=O, base), 150.54 (*C*=O, base), 147.90, 147.88 (O*C*-Ar), 141.75 (d, $^{1}J_{C-F}$ = 225 Hz, *C*F, base), 136.37 (*C*Ar), 128.95, 127.90, 127.56, 126.55, 126.19 (*C*H-Ar), 125.64, 125.53 (2 x d, $^{2}J_{C-F}$ = 34.0 Hz, *C*H-base), 122.65 (*C*H-Ar), 116.51, 116.21 (*C*H-Ar), 87.03, 86.96 (*C*-1'), 86.85, 86.74 (*C*-4'), 72.16, 72.05 (*C*-3'), 67.87 (d, $^2J_{C-P}$ = 5.0 Hz, *C*-5'), 66.54 (O*C*H_2), 51.87, 51.81 (d, $^2J_{C-P}$ = 7.5 Hz, *C*HCH_3), 40.87, 40.80 (*C*-2'), 29.35, 29.10 (*C*H_2 ester), 23.33 (*C*H_2 ester), 20.60, 20.43 (2 x d, $^3J_{C-P}$ = 6.5 Hz, CH*C*H_3), 14.28 (*C*H_3, ester).

MS (ES+) m/e: 616 (MNa^+, 100%), 594 (MH+, 10%) Accurate mass: $C_{27}H_{33}FN_3O_9P$ required 593.54.

$HPLC_b$ (H_2O/Acetonitrile from 100/0 to 0/100 in 35 min) Rt 15.56 min.

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(cyclopentoxy-L-alaninyl)] phosphate (CPF582) (provided as a reference example)

[0109]



[0110] Prepared according to the standard procedure **E** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), *t*BuMgCl (1.1 mL, 1.1 mmol) and 1-naphthyl-(cyclopentoxy-L-alaninyl)-phosphochloridate (0.77 g, 2.03 mmol) in THF (10 mL). Column purification gave the product as a white solid (0.030 g, 5%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.53, 4.37

^{19}F-NMR (MeOD, 470 MHz) δ - 167.07, -167.19

^{1}H-NMR (MeOD, 500 MHz) δ 8.18 - 8.16 (m, 1H, *H*-Ar), 7.89 - 7.85 (m, 1H, *H*-Ar), 7.70 (apparent t, *J* = 6.50 Hz, 2H, *H*-Ar), 7.57 - 7.50 (m, 3H, 2 x *H*-Ar, *H*-base), 7.45 - 7.40 (m, 1H, *H*-Ar), 6.16 - 6.11 (m, 1H, *H*-1'), 5.15 - 5.09 (m, 1H, OC*H* ester), 4.41 - 4.30 (m, 3H, 2 x *H*-5', *H*-3'), 4.11 - 4.08 (m, 1H, *H*-4'), 4.04 - 3. 98 (m, 1H, C*H*CH_3), 2.19 - 2.10 (m, 1H, *H*-2'), 1.86 - 1.73 (m, 3H, OCHCH_2 ester), 1.73 - 1.56 (m, 6H, *H*-2', CH_2 ester), 1.35, 1.34 (2 x d, *J* = 6.57 Hz, CHCH_3)

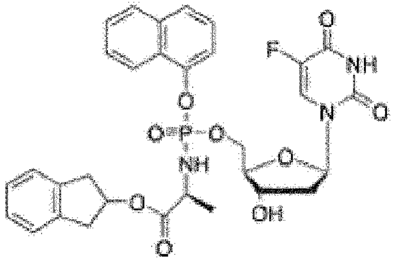
^{13}C-NMR (MeOD, 125 MHz) δ 174.68, 174.64 (*C*=O, ester), 159.27 (*C*=O, base), 150.51 (*C*=O, base), 147.86 (d, $^2J_{C-P}$ = 7.5 Hz, O*C*-Ar), 141.78, 141.72 (2 x d, $^1J_{C-F}$ = 232 Hz, *C*F-base), 136.30 (*C*-Ar), 128.95, 128.54, 127.94, 127.80, 127.60, 127.56, 127.17, 126.80, 126.54, 126.19, 126.16 (*C*H-Ar), 125.66, 125.53 (2 x d, $^2J_{C-F}$ = 34 Hz, *C*H-base), 122.65, 122.61 (*C*H-Ar), 116.53, 116.22 (2 x d, $^4J_{C-P}$ = 3.75 Hz, *C*H-Ar), 86.99, 86.96 (*C*-1'), 86.70 (d, $^3J_{C-P}$ = 7.50 Hz, *C*-4'), 79.64, 79.61 (O*C*H ester), 72.21, 72.07 (*C*-3'), 67.89, 67.85 (2 x d, $^2J_{C-P}$ = 5.0 Hz, *C*-5'), 51.92 (d, $^2J_{C-P}$ = 5.0 Hz, *C*HCH_3), 40.92, 40.86 (*C*-2'), 33.65, 33.61, 33.52, 33.47 (2 x CH_2 ester), 24.68, 24.66 (*C*H_2 ester), 20.45, 20.30 (2 x d, $^3J_{C-P}$ = 6.25 Hz, CH*C*H_3).

MS (ES+) m/e: 614 (MNa^+, 100%), 592 (MH+, 30%) Accurate mass: $C_{27}H_{31}FN_3O_9P$ required 591.52

$HPLC_b$ (H_2O/Acetonitrile from 100/0 to 0/100 in 35 min) Rt 14.03 min.

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(2-indanoxy-L-alaninyl)] phosphate (CPF597) (provided as a reference example)

[0111]



[0112] Prepared according to the standard procedure **E** from 5-Fluoro-2'-deoxyuridine (0.30 g, 1.22 mmol), *t*BuMgCl (1.34 mL, 1.34 mmol) and 1-naphthyl-(2-indanoxy-L-alaninyl)-phosphochloridate (1.06 g, 2.43 mmol) in THF (20 mL). Column purification gave the product as a white solid (0.045 g, 6%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.62, 4.30

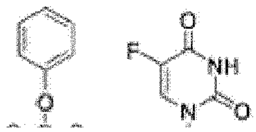
^{19}F-NMR (MeOD, 470 MHz) δ - 167.14, - 167.34

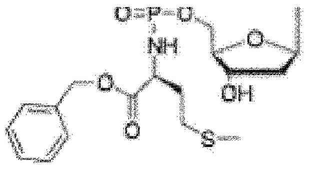
^{1}H-NMR (MeOD, 500 MHz) δ 8.15 - 8.12 (m, 1H, *H*-Ar, Naph), 7.89 - 7.87 (m, 1H, *H*-Ar, Naph), 7.72 - 7.67 (m, 2H, *H*-Ar, Naph), 7.56 - 7.46 (m, 3H, 2 x *H*-Ar, *H*-base), 7.40 - 7.37 (m, 1H, *H*-Ar), 7.20 - 7.12 (m, 4H, *H*-Ar, Ph), 6.14 - 6.08 (m, 1H, *H*-1'), 5.49 - 5.46 (m, 1H, OC*H* ester), 4.32 - 4.26 (m, 3H, 2 x *H*-5', *H*-3'), 4.04 - 3.98 (m, 1H, *H*-4', C*H*CH$_3$), 3.30 - 3.24 (m, 2H, 2 x C*H* ester), 2.99 - 2.91 (m, 2H, 2 x C*H* ester), 2.14 - 2.07 (m, 1H, *H*-2'), 1.75 - 1.64 (m, 1H, *H*-2'), 1.33 - 1.29 (m, 3H, CHC*H*$_3$)

^{13}C-NMR (MeOD, 125 MHz) δ 175.02, 174.66 (2 x d, $^{3}J_{C-P}$ = 3.75 Hz, *C*=O, ester), 159.48 ($^{2}J_{C-F}$ = 25.0 Hz, *C*=O, base), 150.57 (*C*=O, base), 147.97, 147.80 (2 x d, $^{2}J_{C-P}$ = 7.5 Hz, O*C*-Ar), 141.73, 141.68 (2 x d, $^{1}J_{C-F}$ = 232.5 Hz, *C*F-base), 141.54, 141.49, 141.48, 139.10, 136.27, 136.26 (*C*-Ar), 129.01, 128.94, 128.91, 127.91, 127.87, 128.85, 127.80, 127.77, 127.60, 127.57, 127.50, 126.20, 126.18, 125.69 (*C*H-Ar), 125.50, 125.43 (2 x d, $^{2}J_{C-F}$ = 25 Hz, *C*H-base), 122.64, 122.60, 121.85 (*C*H-Ar), 116.57, 116.26 (2 x d, $^{4}J_{C-P}$ = 2.5 Hz, *C*H-Ar), 86.96 (*C*-1'), 86.87, 86.66 (2 x d, $^{3}J_{C-P}$ = 7.50 Hz, *C*-4'), 77.85, 79. (O*C*H ester), 72.21, 72.07 (*C*-3'), 67.77, 67.75 (2 x d, $^{2}J_{C-P}$ = 6.25 Hz, *C*-5'), 51.97, 51.82 (*C*HCH$_3$), 40.91, 40.86 (*C*-2'), 40.44, 40.43, 40.38, 40.34 (2 x C*H*$_2$ ester), 20.30, 20.16 (2 x d, $^{3}J_{C-P}$ = 6.25 Hz, CH*C*H$_3$)

5-Fluoro-2'-deoxyuridine-5'-*O*-[phenyl-(benzoxy-L-methioninyl)]phosphate (CPF586) (provided as a reference example)

[0113]





[0114] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and phenyl-(benzoxy-L-methioninyl)-phosphochloridate (0.7 g, mmol) in THF (10 mL). Column purification gave the product as a yellowish solid (0.014 g, 2%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.34, 3.94

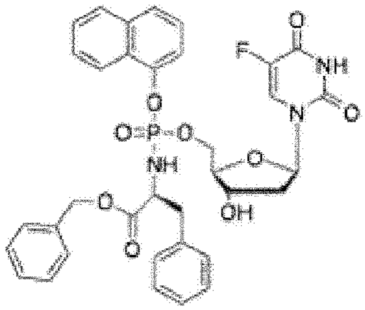
^{19}F-NMR (MeOD, 470 MHz) δ - 167.40, - 167.69

^{1}H-NMR (MeOD, 500 MHz) δ 7.83 - 7.80 (m, 1H, *H*-Ar), 7.74 - 7.72 (m, 1H, *H*-Ar), 7.64 - 7.62 (m, 1H, *H*-Ar), 7.37 - 7.32 (m, 6H, *H*-Ar, *H*-base), 7.26 - 7.17 (m, 2H, *H*-Ar), 6.25 - 6.17 (m, 1H, *H*-1'), 5.18, 5.13 (AB system, J_{AB} = 12.0 Hz, 2H, CH_2Ph), 4.40 - 4.35 (m, 1H, *H*-3'), 4.32 - 4.22 (m, 2H, *H*-5'), 4.16 - 4.03 (m, 2H, NHC*H*, *H*-4'), 2.44, 2.36 (2 x t, *J* = 7.50 Hz, CH_2S), 2.16 - 2.08 (m, 1H, 1 x *H*-2'), 1.98 - 1.82 (m, 6H, 1 x *H*-2', NHCHCH_2CH$_2$SCH_3),

MS (ES+) m/e: 646 (MNa$^+$, 100%), 624 (MH+, 10%) Accurate mass: $C_{27}H_{31}FN_3O_9PS$ required 623.56

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(benzoxy-L-phenylalaninyl)] phosphate (CPF587) (provided as a reference example)

[0115]



[0116] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl-(benzoxy-L-phenylalaninyl)-phosphochloridate (1.45 g, mmol) in THF (10 mL). Column purification gave the product as a white solid (0.007 g, 1%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.27, 4.14

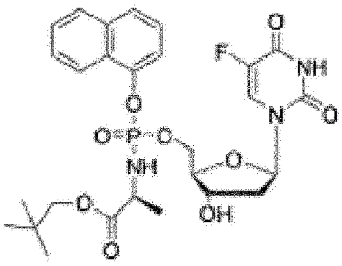
^{19}F-NMR (MeOD, 470 MHz) δ - 166.99, - 167.18

^{1}H-NMR (MeOD, 500 MHz) δ 8.11 - 8.00 (m, 1H, *H*-Ar, Ar), 7.89 - 7.85 (m, 1H, *H*-Ar), 7.69 - 7.67 (m, 1H, *H*-Ar), 7.60 - 7.49 (m, 3H, 2 x *H*-Ar, *H*-base), 7.37 - 7.33 (m, 2H, *H*-Ar), 7.25 - 7.12 (m, 10H, *H*-Ar), 6.09 - 6.04 (m, 1H, *H*-1'), 5.11 - 5.01 (m, 2H, CH_2Ph), 4.29 - 4.18 (m, 1H, C*H*CH$_3$), 4.15 - 4.08 (m, 1H, *H*-3'), 4.02 - 3.95 (m, 2H, *H*-5'), 3.86 - 3.67 (m, 1H, *H*-4'), 3.14 - 3.10 (m, 1H, 1 x

NHCHCH_2Ph), 2.91 - 2.82 (m, 1H, 1 x NHCHCH_2Ph), 2.12 - 2.06, 2.00 - 1.95 (2 x m, 1H, *H*-2'), 1.68 - 1.62, 1.42 - 1.36 (2 x m, 1H, *H*-2')

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(2,2-dimethylpropoxy-L-alaninyl)] phosphate (CPF588) (provided as a reference example)

[0117]



[0118] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl-(2,2-dimethylpropoxy-L-alaninyl)-phosphochloridate (0.77 g, mmol) in THF (10 mL). Column purification gave the product as a white solid (0.006 g, 1%).

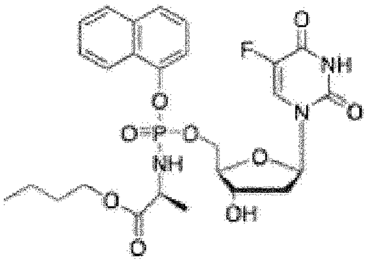
^{31}P-NMR (MeOD, 202 MHz) δ 4.56, 4.33

^{19}F-NMR (MeOD, 470 MHz) δ - 167.32, - 167.43

^{1}H-NMR (MeOD, 500 MHz) δ 8.19 - 8.16 (m, 1H, *H*-Ar, Ar), 7.91 - 7.89 (m, 1H, *H*-Ar), 7.74 - 7.71 (m, 2H, *H*-Ar), 7.57 - 7.51 (m, 3H, 2 x *H*-Ar, *H*-base), 7.46 - 7.41 (m, 1H, *H*-Ar), 6.17 - 6.10 (m, 1H, *H*-1'), 4.42 - 4.30 (m, 3H, *H*-3', 2 x *H*-5'), 4.13 - 4.07 (m, 2H, *H*-4', C*H*CH$_3$), 3.86, 3.75 (AB system, J_{AB} = 10.50 Hz, 2H, CH_2C(CH$_3$)$_3$), 2.18 - 2.10 (m, 1H, *H*-2'), 1.81 - 1.70 (m, 1H, *H*-2'), 1.41 - 1.38 (m, 3H, CHCH_3), 0.95, 0.94 (2 x s, 9H, CH$_2$C(CH_3)$_3$)

5-Fluoro-2'-deoxyuridine-5'-*O*-[1-naphthyl-(butoxy-L-alaninyl)]phosphate (CPF589) (provided as a reference example)

[0119]



[0120] Prepared according to the standard procedure **D** from 5-Fluoro-2'-deoxyuridine (0.25 g, 1.01 mmol), NMI (0.41 g, 5.07 mmol, 0.40 mL) and 1-naphthyl-(butoxy-L-alaninyl)-phosphochloridate (0.75 g, mmol) in THF (10 mL). Column purification gave the product as a white solid (0.006g, 1%).

^{31}P-NMR (MeOD, 202 MHz) δ 4.52, 4.35

^{19}F-NMR (MeOD, 470 MHz) δ - 167.36, - 167.49

^{1}H-NMR (MeOD, 500 MHz) δ 8.19 - 8.16 (m, 1H, *H*-Ar, Naph), 7.1 - 7.89 (m, 1H, *H*-Ar, Naph), 7.75 - 7.72 (m, 2H, *H*-Ar, Naph), 7.58 - 7.51 (m, 3H, 2 x *H*-Ar, *H*-base), 7.46 - 7.41 (m, 1H, *H*-Ar), 6.18 - 6.11 (m, 1H, *H*-1'), 4.42 - 4.40 (m, 1H, 1 x H-5'), 4.37 - 4.32 (m, 2H, 1 x *H*-5', *H*-3'), 4.12 - 4.01 (m, 4H, *H*-4', C*H*CH_3, OCH_2 $CH_2CH_2CH_3$), 2.20 - 2.12 (m, 1H, *H*-2'), 1.85 - 1.73 (m, 1H, *H*-2'), 1.61 - 1.54 (m, 2H, OCH_2C$H_2$$CH_2CH_3$), 1.39 - 1.31 (m, 5H, OCH_2CH_2C$H_2$$CH_3$, CHC$H_3$), 0.93 - 0.89 (m, 3H, $OCH_2CH_2CH_2$CH_3)

Biological assays

[0121] Experimental data having regard to compounds embodying the present invention are described below.

Cell cultures

[0122] Murine leukaemia L1210/0 and human T-lymphocyte CEM/0 cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Human glioblastoma U87 cells were kindly provided by Dr. E. Menue (Institut Pasteur, Paris, France). Thymidine kinase-deficient CEM/TK$^-$ cells were a kind gift from Prof. S. Eriksson (currently at Uppsala University, Uppsala, Sweden) and Prof. A. Karlsson (Karolinska Institute, Stockholm, Sweden). Thymidine kinase-deficient L1210/TK$^-$ were derived from L1210/0 cells after selection for resistance against 5-bromo-2'-dUrd (Balzarini *et al.,* 1982). Infection of relevant cell lines with *Mycoplasma hyorhinis* (ATCC) resulted in chronically-infected cell lines further referred to as L1210.Hyor and U87.Hyor. All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) with 10 % foetal bovine serum (FBS) (Biochrom AG, Berlin, Germany), 10 mM Hepes and 1 mM Sodium Pyruvate (Invitrogen). Cells were grown at 37 °C in a humidified incubator with a gas phase of 5 % CO_2.

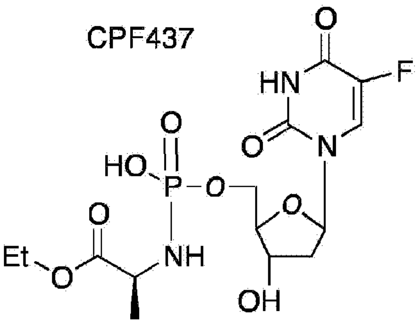
Cytostatic assays

[0123] Monolayer cells (U87 and U87.Hyor) were seeded in 48-well microtiter plates (Nunc™, Roskilde, Denmark) at 10,000 cells/well. After 24 hours, an equal volume of fresh medium containing the test compounds was added. On day 5, cells were trypsinized and counted in a Coulter counter (Analis, Suarlée, Belgium). Suspension cells (L1210/0, L1210/TK$^-$, L1210.Hyor, CEM/0, CEM/TK$^-$) were seeded in 96-well microtiter plates (Nunc™) at 60,000 cells/well in the presence of a given amount of the test compounds. The cells were allowed to proliferate for 48 h (L1210) or 72 hours (CEM) and were then counted in a Coulter counter. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce the number of viable cells by 50 %.

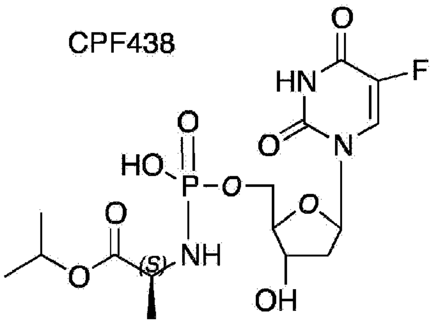
[0124] **Assay 1.** The samples were assayed for biological activity versus a range of tumour cell lines with data recorded in Table 1 below. Data are expressed as CC_{50} in µM, i.e. cytostatic concentration required to inhibit cell proliferation by 50%. The cell lines employed were L1210/0 (a leukemia cell line), FM3A/0 (a breast cancer cell line), Cem/0 (an acute lymphoblastic leukemia cell line) and HeLa (a cervical cell line).

[0125] Table 1 also contains comparative data for 5FU, 5-FdUrd and reference compounds CPF 382, CPF 437 and CPF 438. The structure of CPF 382 is given above. The structure of each of CPF 437 and CPF 438 is as follows:

CPF437



CPF438



[0126] As can be seen from the data in Table 1, compounds of the present invention can exhibit cytostatic activity that is comparable to or better than that of 5-FU, whilst exhibiting marked cytostatic activity in nucleoside kinase-deficient cells. Such a cytostatic activity in nucleotide kinase-deficient cells is in direct contrast to that of 5-FdUrd.

[0127] As can also be seen from Table 1, the activity in TK^- cells of compounds embodying the present invention can be markedly greater than that of reference compounds CPF 382, CPF 437 and CPF 438.

Table 1

	L1210/0	L1210/TK^-	FM3A/0	FM3ArTK$^-$	Cem/0	Cem/TK^-	HeLa	HeLa/TK^-
5-FdUrd	0.00082 ± 0.00008	3.1 ± 0.2			0.028 ± 0.002	1.5 ± 0.1		
5-FdUrd (2)	0.0010 ± 0.0001	4.8 ± 4.0	0.0065 ± 0.0055	0.70 ± 0.02	0.026 ± 0.000	4.4 ± 2.9	0.029 ± 0.007	1.4 ± 0.5
5-FdUrd (3)	0.0011 ± 0.0002	3.0 ± 0.1			0.022 ± 0.006	3.0 ± 0.4	0.050 ± 0.011	1.4 ± 0.4
FU	0.33 ± 0.17	0.32 ± 0.31	0.18 ± 0.02		18 ± 5		0.54 ± 0.12	
CPF 382(1)	0.0255	37.8			0.346	32.7		
CPF 382(2)	0.0271	39.3			0.21	29.2		

	L1210/0	L1210/TK⁻	FM3A/0	FM3ArTK⁻	Cem/0	Cem/TK⁻	HeLa	HeLa/TK⁻
CPF 437	36 ± 5	> 100			> 100	> 100	> 100	> 100
CPF 438	0.12 ± 0.02	51 ± 9			2.1 ± 0.6	32 ± 2	3.7 ± 0.5	72 ± 0
CPF 373	0.015 ± 0.007	0.027 ± 0.004			0.089 ± 0.043	0.32 ± 0.07		
CPF 373(2)	0.0061 ± 0.0043	0.064 ± 0.028	0.059 ± 0.046	0.74 ± 0.18	0.046 ± 0.010	0.74 ± 0.63	0.065 ± 0.013	2.5 ± 1.3
CPF 381	0.028 ± 0.007	13 ± 8			0.18 ± 0.03	22 ± 7		
CPF 383	0.13 ± 0.04	0.94 ± 0.18	0.64 ± 0.57	4.1 ± 2.0	0.92 ± 0.11	14 ± 0	0.48 ± 0.19	9.8 ± 1.4
CPF 384	0.076 ± 0.022	1.1 ± 0.1	0.36 ± 0.25	13 ± 1	1.0 ± 0.1	30 ± 10	0.71 ± 0.15	25 ± 11
CPF 386	0.031 ± 0.005	0.36 ± 0.01			0.25 ± 0.04	1.6 ± 0.2	0.22 ± 0.04	2.8 ± 0.0
CPF 393	0.017 ± 0.003	0.18 ± 0.05			0.23 ± 0.04	4.8 ± 0.7		
CPF 394	0.039 ± 0.001	4.6 ± 0.0			0.65 ± 0.16	22 ± 1		
CPF 395	0.011 ± 0.005	0.13 ± 0.04			0.16 ± 0.02	2.4 ± 0.8		
CPF 396	0.064 ± 0.008	0.82 ± 0.16			0.36 ± 0.05	6.9 ± 1.8		
CPF 508	0.039 ± 0.001	0.14 ± 0.02	0.18 ± 0.00		0.17 ± 0.07		0.18 ± 0.05	
CPF 509	0.043 ± 0.023	0.15 ± 0.00	0.31 ± 0.06		0.057 ± 0.055		0.090 ± 0.014	
CPF 576	1.1 ± 0.5	35 ± 8			0.80 ± 0.28	46 ± 14	0.67 ± 0.03	27 ± 6
CPF 577	0.21 ± 0.08	25 ± 8			0.89 ± 0.35	32 ± 9	1.2 ± 0.0	26 ± 1
CPF 578	0.014 ± 0.003	0.088 ± 0.038			0.073 ± 0.018	1.5 ± 0.3	0.069 ± 0.003	1.5 ± 0.6
CPF 579	0.017 ± 0.007	0.12 ± 0.06			0.059 ± 0.017	1.1 ± 0.2	0.068 ± 0.001	1.4 ± 0.4
CPF 580	0.038 ± 0.014	27 ± 6			0.11 ± 0.02	43 ± 12	0.13 ± 0.04	15 ± 7
CPF	0.0028 ±	0.13 ±			0.015 ±	0.28 ±	0.029	0.44 ±

	L1210/0	L1210/TK⁻	FM3A/0	FM3ArTK⁻	Cem/0	Cem/TK⁻	HeLa	HeLa/TK⁻
581	0.0010	0.13			0.006	0.04	± 0.023	0.35
CPF 582	0.031 ± 0.010	0.13 ± 0.02			0.035 ± 0.025	0.92 ± 0.007	0.071 ± 0.036	2.2 ± 1.3
CPF 583	0.35 ± 0.07	31 ± 5			0.98 ± 0.40	28 ± 8	1.1 ± 0.4	20 ± 11
CPF 585	0.016 ± 0.006	0.062 ± 0.009			0.053 ± 0.021	0.19 ± 0.04	0.078 ± 0.018	1.3 ± 0.9
CPF 586	0.073 ± 0.035	4.1 ± 1.2			0.28 ± 0.03	25 ± 0	0.15 ± 0.02	11 ± 7
CPF 587	0.012 ± 0.007	5.6 ± 1.3			0.10 ± 0.03	7.2 ± 0.1	0.16 ± 0.08	6.8 ± 1.5
CPF 588	0.27 ± 0.11	1.2 ± 0.7			0.49 ± 0.05	6.7 ± 1.0	0.70 ± 0.11	32 ± 26
CPF 589	0.022 ± 0.004	0.11 ± 0.06			0.064 ± 0.007	0.84 ± 0.60	0.12 ± 0.02	2.7 ± 1.5

[0128] Assay 2. Samples were also assayed for their % retention of activity in mycoplasma infected cells. The results are set out in Table 2 below. The results show that compounds of the present invention can retain high activity in mycoplasma infected cells, in contrast to the activity shown by 5-FdURD. Administration of a Thymidine Phosphorylase (TP) inhibitor restores the cytostatic activity of 5-FdUrd in myocoplasma infected cell cultures, providing evidence of the deteriorating role of TP in the eventual cytostatic activity of 5-FdUrd. As mycoplasma infection of cells is known to greatly reduce the activity of nucleosides, including 5-FdUrd, the activity of some nucleosides in mycoplasma infected cells provides a potential benefit in patients that are mycoplasma infected.

Table 2.

CC50 values in μM for 5-FdUrd and compounds embodying the present invention in mycoplasma negative and positive cells, and % retention of activity on mycoplasma infection. "% retention" is a measure of the ratio of the CC50 values measured with respect to L1210 with respect to those for L1210/Hyor and is calculated as $CC50_{L1210}$ x 100 ÷ $CC50_{L1210/Hyor}$.			
Cpd	L1210	L1210/Hyor	% Retention
5-FdUrd	0.00051	0.278	0.2
CPF 373	0.011	0.025	44
CPF 381	0.026	0.15	18
CPF 393	0.029	0.02	145
CPF 394	0.030	0.26	12
CPF 395	0.019	0.045	42
CPF 396	0.056	0.17	33
CPF 576	1.4	2.73	51

CC50 values in μM for 5-FdUrd and compounds embodying the present invention in mycoplasma negative and positive cells, and % retention of activity on mycoplasma infection. "% retention" is a measure of the ratio of the CC50 values measured with respect to L1210 with respect to those for L1210/Hyor and is calculated as $CC50_{L1210}$ x 100 ÷ $CC50_{L1210/Hyor}$.			
Cpd	L1210	L1210/Hyor	% Retention
CPF 577	0.23	0.63	36
CPF 578	0.015	0.048	31
CPF 579	0.019	0.045	42
CPF 580	0.048	0.41	12
CPF 581	0.0037	0.017	22
CPF 582	0.035	0.042	83
CPF 583	0.387	11.9	3.3
CPF 585	0.021	0.051	41
CPF 586	0.1	0.87	11
CPF 587	0.022	4.2	0.5
CPF 588	0.237	0.39	61
CPF 589	0.02	0.063	32

[0129] Further experiments (Assays 3 to 8 below) were carried out with respect to the compound CPF 373 embodying the present invention.

Assay 3. Cytostatic activity of 5-FdUrd and its prodrug CPF-373 against TK-competent and TK-deficient tumour cell lines

[0130] The cytostatic activity of 5-FdUrd and CPF-373 was determined in different TK-expressing and TK-deficient tumour cell lines. As shown in Table 3, 5-FdUrd is strongly dependent on the expression of TK for its cytostatic activity. Its IC_{50} increased by 4,000-fold for L1210/TK- cells (IC_{50}: 3.1 μM) *versus* wild-type L1210/0 cells (IC_{50}: 0.0008 μM) and by 50-fold for CEM/TK- cells (IC_{50}: 1.5 μM) *versus* CEM/0 cells (IC_{50}: 0.028 μM). In contrast, the cytostatic activity of the 5-FdUrd prodrug CPF-373 remained virtually unchanged in TK-deficient cells when compared with wild-type cells (IC_{50}: 0.027 and 0.011 μM for L1210/TK^- and L1210/0, and 0.32 and 0.089 μM for CEM/TK^- and CEM/0 cells, respectively). Although the cytostatic activity of CPF-373 was 3- to 10-fold inferior to 5-FdUrd against wild-type L1210/0 and CEM/0 cells, it proved 5- to 100-fold superior to 5-FdUrd in the TK-deficient tumour cell lines (see Table 3).

Table 3. Cytostatic activity of 5-FdUrd and CPF-373 as represented by the IC_{50} value in different cell lines

	$IC_{50}{}^{a}$ (μM)	
Cell lines	**5-FdUrd**	**CPF-373**
L1210/0	0.0008 ± 0.000095	0.011 ± 0.0065

	IC_{50}[a] (µM)	
Cell lines	**5-FdUrd**	**CPF-373**
L1210/TK-	3.1 ± 0.14	0.027 ± 0.0028
L1210.Hyor	0.24 ± 0.054	0.025 ± 0.0073
CEM/0	0.028 ± 0.0014	0.089 ± 0.030
CEM/TK-	1.5 ± 0.071	0.32 ± 0.049
U87	0.007 ± 0.001	0.035 ± 0.0005
U87.Hyor	3.0 ± 0.55	0.039 ± 0.0025
[a] 50% Inhibitory concentration or compound concentration required to inhibit tumour cell proliferation by 50 %		

Assay 4. Effect of mycoplasma infection of tumour cell cultures on the cytostatic activity of 5-FdUrd and its prodrug CPF-373

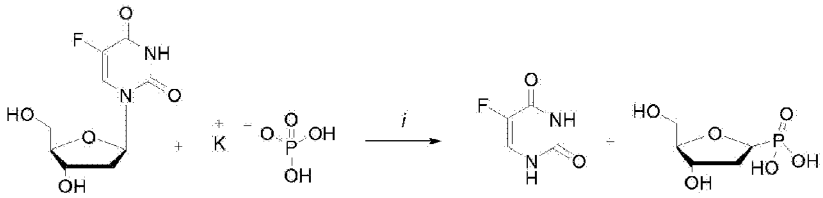
[0131] The L1210/0 cell cultures were infected with the mycoplasma species *M. hyorhinis* (cells designated: L1210.Hyor). 5-FdUrd markedly lost its cytostatic activity against the mycoplasma-infected L1210.Hyor cells by 300-fold (IC_{50}: 0.24 µM). Also, 5-FdUrd lost its cytostatic activity by 400-fold in U87.Hyor cell cultures when compared with uninfected U87 cells (see Table 3). In sharp contrast, the 5-FdUrd prodrug CPF-373 kept a similar cytostatic potential in both L1210/0 and L1210.Hyor cell cultures (IC_{50}: 0.011 and 0.025 µM, respectively). A similar observation was made for this prodrug when evaluated for its cytostatic activity in U87 and U87.Hyor cell cultures (IC_{50}: 0.035 and 0.039 µM, respectively). Thus, whereas the free nucleoside 5-FdUrd markedly lost its cytostatic potential against *Mycoplasma hyorhinis*-infected tumour cell lines, the antiproliferative potential of its prodrug CPF-373 was independent of the mycoplasma infection.

[0132] Assay 5. Experiments were carried out to assess the stability of CPF 373 in the presence of Thymidine Phosphorylase (TP). The experiments are illustrated with reference to Figures 9 to 11, each of which comprises NMR spectra, as discussed below. The present assay shows that the insensitivity of compounds embodying the present invention to the action of the catabolic enzyme TP, which is often upregulated in tumours, renders the compounds of the present invention more independent of the catabolic enzyme TP than 5-FdUrd.

Phosphorylase assay on 5-FdUrd and its ProTide compound CPF 373 by Thymidine Phosphorylase (TP) purified from *Escherichia Coli.*

[0133] Nucleoside 5-FdUrd can be degraded to its relative base 5FU by a phosphorolytic reaction, using thymidine phosphorylase purified from *Escherichia coli* as well as uridine phosphorylase purified from Ehrlich ascite tumor. This breakdown has been suggested to be one of the reasons for the limited therapeutic effectiveness of 5-FdUrd according to the following scheme:

5-FdUrd 5-FU



Scheme

[0134] The phosphorylase assay was carried out towards phosphorolysis by *Thymidine Phosphorylase* purified from *Escherichia coli* using *in situ* ^{19}F NMR. The application to the ProTide compound CPF 373 was an attempt to prevent the cleavage of the structure and thus circumvent the action of the enzyme.

[0135] Two potassium phosphate buffers at pH = 7.4, 200 nM solution and 300 nM solution respectively, were used as phosphate donor. Units of enzyme were defined as the amount of enzyme required to hydrolyse about 0.25 mg of inosine per min used as standard. Assays were conducted for 30 minutes.

Phosphorylase assay on 5-FdUrd

[0136] Initially, ^{19}F NMR (470 MHz) spectra of 5-FdUrd and 5FU previously dissolved in deuterated methanol, were recorded. 5-FdUrd showed a singlet at ~ δ-167.21 ppm and 5FU at ~ δ-169.30 ppm. Thus, the phosphorylase assay was carried out by dissolving 5-FdUrd in deuterated methanol, in the presence of potassium phosphate buffer (200 nM solution; pH = 7.4), recording the blank before of the addition of the enzyme thymidine phosphorylase (TP) (20.7 UNI). ^{19}F NMR spectra, recorded at 25 °C, showed the singlet of 5-FdUrd at ~ δ-165.17 ppm and a new peak at ~ δ-169.50ppm, attributed to 5FU, as shown in Figure 9 at spectrum **A.**

[0137] Then, to prove the cleavage of the nucleoside into the relative base, a new experiment was performed by dissolving equal moles of the nucleoside analogue 5-FdUrd and the relative base 5FU, at the same condition described above without the TS enzyme, as shown in Figure 9 at spectrum B. This spectrum showed two singlets with the same chemical shifts previously observed in Figure 9 spectrum A. These data confirmed that the 5FU has a chemical shift at ~ δ-169.50 ppm and thus the phosphorolytic action of enzyme (TP). Conversion of nucleoside 5-FdURd into the free base 5FU was 66%.

[0138] When the initial concentration of potassium phosphate buffer was increased from 200nM up to 205 nM, substrate 5-FdUrd was fully converted into the base 5-FU as shown in the Figure 10.

Phosphorylase assay on ProTide compound CPF 373

[0139] Phosphorylase assay was applied to benzyl L-alanine phenyl derivative CPF 373 in order to investigate the stability, following the procedure and the conditions above described. ProTide compound **CPF 373** proved to be completely stable as showed by comparing chemical shifts of sample analysed without TP enzyme, as shown in Figure 11 spectrum A, and in the presence of TP, as shown in Figure 11 spectrum B. ^{19}F NMR was repeated after 4 days and the ProTide compound **CPF 373** was shown once again to be stable.

[0140] These experiments confirmed that the nucleoside 5-FdUrd is rapidly degraded into its relative base 5FU by a phosphorolytic reaction, in the presence of thymidine phosphorylase, with a half-life of less than 30 minutes, while prodrug compound **CPF 373** showed an evident stability against TP enzymatic activity, at longer time exposure up to 3 days. This important result showed that 5-FdUrd ProTides derivatives embodying the present invention could favor the therapeutic effect of 5-FdUrd.

Assay 6. Exposure of 5-FdUrd and CPF-373 to E. *coli*-encoded TP and human-encoded TP and UP

[0141] The substrate specificity of thymidine phosphorylase towards natural thymidine (dThd), uridine (Urd), 5-FdUrd and CPF-373 was investigated by high pressure liquid chromatography (HPLC). Reaction mixtures containing 100 µM test compound and recombinant TP or UP (human TP: 8.6 ng/µL; *E. coli* TP: 3.0 ng/µL; human UP: 4 ng/mL) in a total volume of 500 µL reaction buffer (10 mM TrisHCl; 300 µM NaCl; 1 mM EDTA; 2 mM KH_2PO_4/K_2HPO_4) were incubated at room temperature. At different time points (i.e. 0, 20, 40 min) 100 µL aliquots of the reaction mixtures were withdrawn and heated at 95 °C for 3 min to inactivate the enzyme. The resulting reaction products were separated on a reverse-phase RP-8 column (Merck, Darmstadt, Germany) and quantified by HPLC analysis (Alliance 2690, Waters, Milford, MA). The separation of dThd from thymine was performed by a linear gradient from 98% separation buffer (50 mM NaH_2PO_4 and 5 mM heptane sulfonic acid, pH 3.2) and 2 % acteonitrile, to 20% separation buffer + 80% acetonitrile (8 min 98% separation buffer + 2% acetonitrile; 5 min linear gradient of 98% separation buffer + 2% acetonitrile to 20% separation buffer + 80% acetonitrile; 10 min 20% separation buffer + 80% acetonitrile, followed by equilibration at 98% separation buffer + 2% acetonitrile). UV-based detection was performed at 267 nm. The separation of Urd from uracil was performed by a linear gradient from 100% separation buffer (see above) to 60% separation buffer + 40% acetonitrile (3 min 100% separation buffer; 6 min linear gradient of 100% separation buffer to 60% separation buffer + 40% acetonitrile; 6 min 60% separation buffer + 40% acetonitrile, followed by equilibration at 100% separation buffer). UV-based detection was performed at 258 nm.

Phosphorolysis of 5-FdUrd and CPF-373 by thymidine and uridine phosphorylases

[0142] 5-FdUrd and its prodrug CPF-373 were exposed to purified thymidine phosphorylase derived from *E.coli* or human erythrocytes, and to purified uridine phosphorylase derived from human tumors. Whereas *E.coli* and human TP rapidly converted dThd and 5-FdUrd to their free bases, CPF-373 kept fully stable in the presence of these enzymes (Fig. 2). Under similar experimental conditions, uridine was converted to uracil by human UP, but not by *E.coli* TP, or human TP. When both compounds were exposed to UP, dThd and CPF-373 were not affected by the enzyme, whereas 5-FdUrd was slightly hydrolysed (Fig. 2, panel C).

Assay 7. Thymidylate synthase (TS) activity measurements

[0143] The activity of TS in intact L1210/0 and L1210/TK- cells was measured by evaluation of tritium release from [5-^{3}H]dUMP (formed in the cells from [5-^{3}H]dUrd or [5-^{3}H]dCyd) in the reaction catalysed by TS. This method has been described in detail by Balzarini & De Clercq (1984). Shortly, cell cultures (500 µL DMEM culture medium) were prepared containing ~ 3 x 10^6 L1210 cells and appropriate amounts of the test compounds (5-FdUrd and CPF-373). After 30 min, 2 h and 4 h preincubation of the cells with the compounds at 37°C, 1 µCi of [5-^{3}H]dUrd or [5-^{3}H]dCyd was added to the cell cultures. After 30 min incubation, 100 µL of the cell suspensions were withdrawn and added to a cold suspension of 500 µL activated charcoal (VWR, Haasrode, Belgium) (100 mg/ml in TCA 5%). After 10 min, the suspension was centrifuged at 13,000 rpm for 10 min, after which the radioactivity in 400 µL supernatant was counted in a liquid sinctillator using OptiPhase HiSafe (Perkin Elmer, Waldham, MA).

Inhibition of thymidylate synthase (TS) by 5-FdUrd and CPF-373

[0144] The major target for the cytostatic activity of 5-FdUrd is thymidylate synthase (TS). The activity of TS in intact tumour cells can be directly monitored by measuring the tritium release in intact L1210/0 cell cultures that were exposed to [5-^{3}H]deoxyuridine ([5-^{3}H]dUrd) or [5-^{3}H]deoxycytidine ([5-^{3}H]dCyd). Indeed, after intracellular conversion of [5-^{3}H]dUrd or [5-^{3}H]dCyd to [5-^{3}H]dUMP, the C-5 tritium atom on the pyrimidine base is released during the TS-catalysed reductive methylation. The ability of 5-FdUrd and its prodrug CPF-373 to inhibit tritium release from [5-^{3}H]dUrd and [5-^{3}H]dCyd was therefore evaluated in L1210/0 cell cultures at a variety of compound concentrations. 5-FdUrd proved to be a potent inhibitor of TS in situ. Its IC_{50} for tritium release from [5-^{3}H]dCyd and [5-^{3}H]dUrd was around 0.0007 - 0.0009 µM (see Table 4).

Table 4. IC_{50} values of 5-FdUrd and CPF-373 against TS in intact L1210/0 tumour cells (as determined by tritium release from [5-^{3}H]dUrd and [5-^{3}H]dCyd after 30 min exposure to the drugs).

	IC_{50}[a] (µM)	
Compound	**Tritium release from dUrd***	**Tritium release from dCyd***
5-FdUrd	0.0009 ± 0.0002	0.0007 ± 0.003
CPF-373	0.16 ± 0.05	0.19 ± 0.08

[a] 50% Inhibitory concentration or compound concentration required to inhibit tritium release from [5-^{3}H]dUrd or [5-^{3}H]dCyd in drug-exposed L1210/0 cell cultures by 50%.The inhibitory activity of CPF-373 on tritium release was much less (~ 200-fold) pronounced than that of 5-FdUrd, especially after only 30 min preincubation of the cells with the drugs (IC_{50}: 0.16-0.19 µM). However, longer preincubation times of the cells (up to 4 hr) with 5-FdUrd and CPF-373 before measuring TS activity in the intact tumour cells revealed a much more pronounced inhibitory activity of the prodrug against TS in situ (Fig. 3).

Indeed, whereas the inhibition of ^{3}H release was only 2-fold increased upon longer preincubation times of 5-FdUrd, the inhibitory potential of CPF-373 increased 10-fold (Fig. 3, panels A and B, and C and D).

[0145] Preincubation of the tumour cells with 5-FdUrd and CPF-373 for at least 4 hrs results in TS inhibition in the intact tumour cells at drug concentrations that are very comparable with the 50% cytostatic activity concentrations of these drugs.

[0146] The present observations thus indicate that the 5-FdUrd prodrug needs several metabolic conversion steps before reaching TS as the target enzyme for inhibition, and support the view that CPF-373 acts as an efficient prodrug of 5-FdUrd to exert its eventual cytostatic activity.

[0147] The activity of TS in the presence of 5-FdUrd and CPF-373 was also measured in intact L1210/TK- cells using [5-^{3}H]dCyd as an externally supplied substrate (due to TK deficiency, [5-^{3}H]dUrd cannot be used). As demonstrated in Table 5 and Fig. 3 (panels E and F), the concentration of 5-FdUrd required to cause 50 % inhibition of TS decreased by a factor 5,700 in TK-deficient L1210/TK- cells (IC_{50}: 1.4 µM) when compared to wild-type L1210/0 cells (IC_{50}: 0.0003 µM). In contrast, the inhibitory activity of CPF-373 against TS remained virtually unchanged in L1210/TK- cells (IC_{50}: 0.053 µM in L1210/TK- cells *versus* 0.013 µM in L1210/0 cells).

Table 5. IC_{50} values of 5-FdUrd and CPF-373 against TS in intact L1210/0 and L1210/TK- cells (as determined by tritium release from [5-^{3}H]dCyd after 4 hours of preincubation with the products)

	IC_{50}[a] (µM)	
Compound	**L1210/0**	**L1210/TK-**
5-FdUrd	0.0003 ± 0.00003	1.42 ± 0.09
CPF-373	0.013 ± 0.008	0.053 ± 0.0009

[a] 50% Inhibitory concentration or compound concentration required to inhibit tritium release from [5-^{3}H]dCyd in drug-exposed L1210 cells by 50% upon pre-exposure of the tumour cells for 4 hrs to the drugs.

Assay 8. Stability assays

Carboxypeptidase Y (EC 3.4.16.1) assay

[0148] The enzymatic stability of the prodrug CPF-373 towards carboxypeptidase Y was studied using *in situ* ^{31}P NMR (202 MHz). The experiment was carried out by dissolving CPF-373 (3.0 mg) in d_6-acetone (150 µL) and adding TRIZMA buffer pH 7.6 (300 µL). The resulting solution was placed in an NMR tube and a ^{31}P-NMR experiment at 25 °C was recorded as the blank experiment. The enzyme Carboxypeptidase Y (0.2 mg) was solubilised in TRIZMA (150 µL) and added to the solution of the phosphoramidate derivative in the NMR tube. Next, the tube was placed in the NMR machine, which was set to run a ^{31}P-NMR experiment (64 scans) every 4 minutes for 14 hours at 25 °C. Data were processed and analysed with the Bruker Topspin 2.1 program. Carboxypeptidase Y and TRIZMA buffer were purchased from Sigma-Aldrich.

Human serum

[0149] The stability of the prodrug CPF-373 in the presence of human serum was studied using *in situ* ^{31}P NMR (202 MHz). The ProTide CPF-373 (**1**) (5.0 mg) was dissolved in DMSO (0.05 mL) and D_2O (0.15 mL). Then the sample was transferred into an NMR tube, which was inserted in the NMR chamber at 37 °C (with enough solvent to obtain a control NMR reading of the blank). Then 0.3 ml human serum was quickly added to the sample in the NMR tube. NMR experiments were programmed to record data every 15 minutes for 12 hours and 30 minutes. Because of excess noise and poor shimming profiles (most likely due to the biological media and concentration), individual spectra were further processed. After normal Fourier transform processing, each spectrum was deconvoluted (Lorentz-Gauss deconvolution) to reveal solely the frequency and area of spectral peaks without the baseline. Data recorded were processed and analysed with the Bruker Topspin 2.1 program.

Buffer at pH 1

[0150] The stability of the prodrug CPF-373 towards hydrolysis at pH = 1 was studied using *in situ* ^{31}P NMR (202 MHz). The ProTide CPF-373 (**1**) (2.6 mg) was dissolved in MeOD (0.1 mL) after which 0.5 mL buffer (pH = 1) (prepared from equal parts of 0.2 M HCl and 0.2 M KCl) was added. Then the sample was transferred into an NMR tube, and a ^{31}P NMR experiment was performed at 37 °C recording the data every 12 minutes for 14 hours. Data were processed and analysed with the Bruker Topspin 2.1 program.

Buffer at pH 8

[0151] The stability of the prodrug CPF-373 towards hydrolysis at pH = 8 was studied using *in situ* ^{31}P NMR (202 MHz). The ProTide CPF-373 (**1**) (4.9 mg) was dissolved in MeOD (0.1 mL) after which 0.5 mL buffer (pH = 8) (prepared from a solution of 0.1 M Na_2HPO_4, which was adjusted by 0.1 M HCl) was added. Then the sample was transferred into an NMR tube, and a ^{31}P NMR experiment was performed at 37 °C recording the data every 12 minutes for 14 hours. Data were processed and analysed with the Bruker Topspin 2.1 program.

Stability studies

[0152] Chemical stability studies on the prodrug CPF-373 (**1**) have been performed by exposing the compound to human serum and to aqueous buffers (pH 1.0 and 8.0) using in situ ^{31}P NMR. Each experiment has been carried out dissolving the ProTide in the suitable deuterated solvent and analysing the samples at 37 °C for about 14 hours, acquiring scans at the regular time intervals. For a better resolution original spectra (lower graphs) and deconvoluted ones (upper graphs) are reported. The stability assay of the phosphoramidate CPF-373 (**1**), after incubation in human serum, showed 73% of unchanged compound after 12 hours and 30 minutes as shown in Figure 6.

[0153] The spectra displayed a singlet peak inherent to the human serum at ~ δ2.00 and the double peak of the parent at ~ δ4.50 which after 4 hours and 15 minutes was hydrolyzed to the aminoacyl phosphoramidate intermediate shown as a singlet peak at δ7.20.

[0154] When chemical hydrolysis was evaluated at extreme experimental conditions, i.e. at pH 1.0 and pH 8.0 at 37 °C, a full stability of prodrug CPF-373 (**1**) in both acidic and basic buffer conditions was observed. Spectra were recorded for 14 hours acquiring scans every 12 minutes at regular intervals as shown in the Figures 7 and 8. The ProTide (**1**) examined at pH 1.0 showed constant doublet peaks of diastereoisomers at δ4.35; 4.50 throughout the time of the assay (Figure 7).
Also, at pH 8.0 the spectra displayed a persistent peak of the prodrug (**1**) at δ4.48 and a singlet peak at δ2.55 corresponding to a buffer peak (Figure 8).

Metabolism of 5-FdUrd phosphoramidates

[0155] As shown in Fig. 4, the putative mechanism of activation of the ProTides inside the cell, after uptake, involves a first enzymatic activation step mediated by a carboxypeptidase-type enzyme which hydrolyzes the ester of the aminoacyl moiety (step a) followed by spontaneous cyclization and subsequent spontaneous displacement of the aryl group (step b) and opening of the unstable ring mediated by water (step c). The last step involves a hydrolysis of the P-N bond mediated by a phosphoramidase-type enzyme (step d) with release of the nucleoside monophosphate in the intact cell (Fig 4) (McGuigan et al., 2009; Mehellou et al., 2010).

[0156] To prove the proposed metabolic scheme for CPF-373 (**1**) and whether the ester motif of the 5-FdUrd phosphoramidate derivative is cleaved-off or not, an enzyme incubation experiment was carried out that was designed to mimic the first stages of the putative activation in the intact tumour cells. The compound (**1**) was incubated with carboxypeptidase Y (also known as cathepsin A) in TRIZMA buffer and the conversion of (**1**) was monitored by ^{31}P NMR. Spectra were recorded for 14 h acquiring scans at the periodic intervals every 4 minutes as shown in Figure 5. For a better resolution original spectra (lower graphs) and deconvoluted ones (upper graphs) are shown.

[0157] At the ^{31}P NMR the prodrug CPF-373 (**1**) appeared as two peaks δ4.07; 4.23 corresponding with the two diastereoisomers noted as parent with the characteristic doubling-up of the chiral phosphate centre of the phosphoramidate. After the addition of cathepsin A the compound was quickly hydrolyzed after 4 minutes to intermediates δ4.95; 5.16 which lack the ester motif and this intermediate did not persist as it was in turn quickly metabolized to the aminoacyl phosphoramidate intermediate, the final product in this assay, via the loss of the aryl group (steps a to c in Fig. 4). The intermediate appeared as a singlet peak at δ6.85 due to the achiral phosphate centre. Thus, the enzymatic assay spectra showed a fast metabolism of the parent ~ δ4.00 with complete conversion to the putative intermediate within 26 minutes, which further stayed consistently present throughout the 14 h of the assay. The cleavage of the P-N bond releasing the nucleoside monophosphate was not detected in the enzyme experiment, as expected. This experiment indicates that the first activation step of ProTide CPF-373 (**1**) may be sufficiently efficient, and therefore, may allow the eventual delivery of the nucleoside monophosphate metabolite in the intact tumour cells.

Conclusion

[0158] In conclusion, the present invention provides novel phosphoramidate nucleotide prodrugs of the anticancer nucleoside analogue 5-fluoro-2'-deoxyuridine (5-FdUrd), which were synthesized and evaluated for their cytostatic activity. Whereas 5-FdUrd substantially lost its cytostatic potential in thymidine kinase (TK)-deficient murine leukaemia L1210 and human lymphocyte CEM cell cultures, compounds which form part of this disclosure, such as CPF-373, markedly kept their antiproliferative activity in both the wild-type and TK-deficient tumour cells and are thus largely independent of intracellular TK activity to exert their cytostatic action. CPF-373, for example, was found to inhibit thymidylate synthase (TS) in the TK-deficient and wild-type cell lines at drug concentrations that correlated well with its cytostatic activity in these cells. CPF-373 does not seem to be susceptible to inactivation by catabolic enzymes such as thymidine phosphorylase (TP) and uridine phosphorylase (UP). These findings are in line with the observations that 5-FdUrd, but not CPF-373, substantially loses its cytostatic potential in the presence of TP-expressing mycoplasmas in the tumour cell cultures. Therefore, compounds of the present invention are novel 5-FdUrd phosphoramidate prodrugs that (i) may circumvent potential resistance mechanisms of tumour cells (e.g. decreased TK activity) and (ii) is not degraded by catabolic enzymes such as TP whose activity is often upregulated in tumour cells or expressed in mycoplasma-infected tumour tissue. See Vande Voorde, J. et al Biochemical Pharmacology 82 (2011) 441-452.

[0159] See also McGuigan, C. et al J. Med. Chem. 2011, 54 7247-7258 (published September 05,2011).

[0160] Table 6 below records the cytostatic activity of 5-FU, 5-FdUrd, reference example CPF382 and compounds embodying the present invention against tumour cell lines in terms of IC_{50} or compound concentration required to inhibit tumour cell proliferation by 50%. Data are the mean (±SD) of at least two to four independent experiments. Table 6 identifies the phosphoramidate motif of reference example CPF382 and of compounds embodying the present invention with respect to: "aryl", which corresponds to Ar of Formula I and is either phenyl (Ph) or 1-naphthyl (Nap); "ester", which corresponds to R_3 of Formula I; and "AA", which sets out the amino acid whose alpha C atom and substituents on the alpha C atom correspond to CR_1R_2 of Formula I. Table 6 discloses compounds embodying the present invention not previously mentioned above in Table 1, as well as additional data for some of the compounds mentioned in Table 1.

Table 6.

				IC_{50} (*μ*M)					
Compd	**aryl**	**Ester**	**AA**	**L1210/0**	**L1210/TK$^-$**	**Cem/0**	**CemfTK$^-$**	**HeLa**	**HeLafTK$^-$**
5-FU				0.33 ± 0.17	0.32 ± 0.31	18 ± 5	12 ± 1	0.54 ± 0.12	0.23 ± 0.01

				IC_{50} (*µ*M)					
Compd	**aryl**	**Ester**	**AA**	**L1210/0**	**L1210/TK$^-$**	**Cem/0**	**CemfTK$^-$**	**HeLa**	**HeLafTK$^-$**
5-FdUrd				0.0011 ± 0.0002	3.0 ± 0.1	0.022 ± 0.006	3.0 ± 0.4	0.050 ± 0.011	1.4 ± 0.4
	Ph	Me	Ala	0.022 ± 0.007	41 ± 3	0.70 ± 0.37	35 ± 12	0.28 ± 0.14	4.7 ± 0.4
	Ph	Et	Ala	0.13 ±0.04	0.94 ± 0.18	0.92 ± 0.11	14 ± 0	0.48 ±0.19	9.8 ±1.4
	Ph	*i*-Pr	Ala	0.076 ± 0.022	1.1 ± 0.1	1.0 ± 0.1	30 ± 10	0.71 ± 0.15	25 ±11
	Ph	*c*-Hex	Ala	0.039 ± 0.001	0.14 ± 0.02	0.17 ± 0.07	1.2 ±0.01	0.18 ± 0.05	5.9 ± 0.4
	Ph	Bn	Ala	0.028 ± 0.007	13 ± 8	0.18 ± 0.03	22 ± 7	0.13 ± 0.01	19 ± 2
	Ph	Et	Val	0.16 ± 0.05	42 ± 2	1.0 ± 0.1	>250	1.2 ± 0.3	27 ± 7
	Ph	Bn	Leu	0.044 ± 0.025	2.0 ± 0.3	0.24 ± 0.04	16 ± 1	0.067 ± 0.042	5.6 ± 0.3
	Ph	Bn	Ile	0.076 ± 0.022	1.1 ± 0.1	1.0 ± 0.1	30 ± 10	0.71 ± 0.15	25 ± 11
	Ph	Bn	Phe	0.036 ± 0.010	39 ± 4	0.25 ± 0.02	11 ± 3	0.014 ± 0.007	12 ± 2
	Ph	Pnt	Met	0.11 ± 0.06	2.2 ± 0.5	0.35 ± 0.13	13 ± 1	0.15 ± 0.00	7.1 ± 1.2
	Ph	Bn	Met	0.073 ± 0.035	4.1 ± 1.2	0.28 ± 0.03	25 ± 0	0.15 ± 0.02	11 ± 7
	Ph	Bn	Pro	0.35 ± 0.07	31 ± 5	0.98 ± 0.40	28 ± 8	1.1 ± 0.4	20 ± 11
	Ph	Et	DMG	0.039 ± 0.001	4.6 ± 0.0	0.65 ± 0.16	22 ± 1	0.59 ± 0.09	17 ± 2
	Ph	Bn	DMG	0.017 ± 0.003	0.18 ± 0.05	0.23 ± 0.04	4.8 ± 0.7	0.24 ± 0.07	3.7 ± 0.1
	Nap	Et	Ala	0.031 ± 0.005	0.36 ± 0.01	0.25 ± 0.04	1.6 ± 0.2	0.22 ± 0.04	2.8 ± 0.0

				IC_{50} (µM)					
Compd	**aryl**	**Ester**	**AA**	**L1210/0**	**L1210/TK⁻**	**Cem/0**	**CemfTK⁻**	**HeLa**	**HeLafTK⁻**
	Nap	Pr	Ala	0.021 ± 0.012	0.16 ± 0.07	0.14 ± 0.01	1.1 ± 0.2	0.11 ± 0.03	2.5 ± 0.1
	Nap	Butyl	Ala	0.022 ± 0.004	0.11 ± 0.06	0.064 ± 0.007	0.84 ± 0.60	0.12 ± 0.02	2.7 ± 1.5
	Nap	Pnt	Ala	0.0028 ± 0.0010	0.13 ± 0.13	0.015 ± 0.006	0.28 ± 0.04	0.029 ± 0.023	0.44 ± 0.35
	Nap	Hex	Ala	0.0072 ± 0.0000	0.076 ± 0.015	0.080 ± 0.020	0.65 ± 0.34	0.039 ± 0.018	1.8 ± 0.1
	Nap	*c*-Bu	Ala	0.014 ± 0.003	0.088 ± 0.038	0.073 ± 0.018	1.5 ± 0.3	0.069 ± 0.003	1.5 ± 0.6
	Nap	*c*-Pnt	Ala	0.031 ± 0.010	0.13 ± 0.02	0.35 ± 0.025	0.92 ± 0.007	0.071 ± 0.036	2.2 ± 1.3
	Nap	*c*-Hex	Ala	0.043 ± 0.023	0.15 ± 0.00	0.057 ± 0.055	1.0 ± 0.1	0.090 ± 0.014	ND
	Nap	CH_2-*t*-Bu	Ala	0.27 ± 0.11	1.2 ± 0.7	0.49 ± 0.05	6.7 ± 1.0	0.70 ± 0.11	32 ± 26
	Nap	CH_2CH_2-*t*-Bu	Ala	0.016 ± 0.006	0.062 ± 0.009	0.053 ± 0.021	0.19 ± 0.04	0.078 ± 0.018	1.3 ± 0.9
	Nap	CH_2-*c*-Pr	Ala	0.017 ± 0.007	0.12 ± 0.06	0.059 ± 0.017	1.1 ± 0.2	0.068 ± 0.001	1.4 ± 0.4
	Nap	2-Ind	Ala	0.021 ± 0.002	40 ± 0	0.079 ± 0.018	1.0 ± 0.2	0.10 ± 0.06	7.1 ± 2.1
	Nap	Bn	Ala	0.011 ± 0.007	0.045 ± 0.027	0.068 ± 0.035	0.31 ± 0.06	0.065 ± 0.013	2.5 ± 1.3
	Nap	THP	Ala	0.038 ± 0.014	27 ± 6	0.11 ± 0.02	43 ± 12	0.13 ± 0.04	15 ± 7
	Nap	*c*-Hex	Val	1.1 ± 0.5	35 ± 8	0.80 ± 0.28	46 ± 14	0.67 ± 0.03	27 ± 6
	Nap	Pnt	Leu	0.017 ± 0.001	1.2 ± 0.4	0.071 ± 0.008	15 ± 4	0.039 ± 0.014	7.5 ± 0.4

				IC$_{50}$ (μM)					
Compd	**aryl**	**Ester**	**AA**	**L1210/0**	**L1210/TK$^-$**	**Cem/0**	**CemfTK$^-$**	**HeLa**	**HeLafTK$^-$**
	Nap	Bn	Leu	0.028 ± 0.004	1.5 ± 0.6	0.13 ± 0.00	30 ± 6	0.080 ± 0.022	9.4 ± 1.4
	Nap	Pnt	Ile	0.22 ± 0.12	12 ± 2	0.46 ± 0.11	17 ± 1	0.30 ± 0.02	11 ± 1
	Nap	Pnt	Phe	0.026 ± 0.001	2.9 ± 1.2	0.10 ± 0.00	8.3 ± 1.0	0.040 ± 0.000	6.6 ± 0.5
	Nap	Bn	Phe	0.012 ± 0.007	5.6 ± 1.3	0.10 ± 0.03	7.2 ± 0.1	0.16 ± 0.08	6.8 ± 1.5
	Nap	Bn	Met	0.072 ± 0.001	1.9 ± 0.2	0.19 ± 0.10	11 ± 1	0.087 ± 0.017	8.3 ± 0.0
	Nap	Bn	Pro	0.21 ± 0.08	25 ± 8	0.89 ± 0.35	35 ± 9	1.2 ± 0.0	26 ± 1
	Nap	Et	DMG	0.064 ± 0.008	0.82 ± 0.16	0.36 ± 0.05	6.9 ± 1.8	0.20 ± 0.12	3.2 ± 0.0
	Nap	Pnt	DMG	0.037 ± 0.010	0.30 ± 0.13	0.14 ± 0.00	5.4 ± 1.1	0.12 ± 0.03	2.3 ± 0.1
	Nap	Bn	DMG	0.011 ± 0.005	0.13 ± 0.04	0.16 ± 0.02	2.4 ± 0.8	0.078 ± 0.020	3.1 ± 0.6

[0161] Table 7 below records the cytostatic activity of 5-FdUrd, reference example CPF382 and compounds embodying the present invention in wild type murine leukemia L1210 cell cultures (L1210/0) and L1210 cell cultures, infected with *Mycoplasma hyorhinis* (L1210.Hyor) in terms of IC$_{50}$ or compound concentration to inhibit cell proliferation by 50%. Data are mean (±SD) of at least two to four independent experiments. Table 7 identifies the phosphoramidate motif of reference example CPF382 and of compounds embodying the present invention, as discussed above with respect to Table 6, but with "Naph" standing for 1-naphthyl. Table 7 discloses compounds embodying the present invention not previously mentioned above in Table 2, as well as additional data for some of the compounds mentioned in Table 2.

Table 7.

				IC$_{50}$ (μM)		
Compd	**aryl**	**ester**	**AA**	**L1210/0**	**L1210.Hyor**	**IC$_{50}$(L1210.Hyor)/IC$_{50}$(L1210/0)**
5-FdUrd				0.0009 ± 0.0003	0.34 ± 0.13	378
	Ph	Me	Ala	0.040 ± 0.016	0.87 ± 0.28	22

				IC$_{50}$ (μM)		
Compd	**aryl**	**ester**	**AA**	**L1210/0**	**L1210.Hyor**	**IC$_{50}$(L1210.Hyor)/IC$_{50}$(L1210/0)**
	Ph	Et	Ala	0.11 ± 0.0021	0.54 ± 0.12	5
	Ph	*i*-Pr	Ala	0.050 ± 0.013	0.70 ± 0.10	14
	Ph	*c*-Hex	Ala	0.032 ± 0.0050	0.040 ± 0.016	1.25
	Ph	Bn	Ala	0.026 ± 0.008	0.15 ± 0.006	5.8
	Ph	Et	Val	0.20 ± 0.033	4.4 ± 1.1	22
	Ph	Bn	Leu	0.054 ± 0.0021	0.17 ± 0.047	3.2
	Ph	Bn	Ile	0.98 ± 0.39	2.2 ± 0.031	2.2
	Ph	Bn	Phe	0.016 ± 0.0014	0.56 ± 0.023	35
	Ph	Pnt	Met	0.13 ± 0.0078	0.41 ± 0.21	3.2
	Ph	Bn	Met	0.058 ± 0.035	0.76 ± 0.18	13
	Ph	Bn	Pro	0.35 ± 0.022	18 ± 0.71	51
	Ph	Et	DMG	0.030 ± 0.0005	0.26 ± 0.01	8.7
	Ph	Bn	DMG	0.029 ± 0.001	0.02 ± 0.002	0.69
	Naph	Et	Ala	0.028 ± 0.0021	0.095 ± 0.0028	3.4
	Naph	Pr	Ala	0.030 ± 0.00035	0.036 ± 0.0064	1.2
	Naph	butyl	Ala	0.0095 ± 0.0021	0.021 ± 0.0071	2.2
	Naph	Pnt	Ala	0.0021 ± 0.00007	0.006 ± 0.0014	2.9
	Naph	Hex	Ala	0.0032 ± 0.00035	0.0022 ± 0.00028	0.69
	Naph	*c*-Bu	Ala	0.011 ± 0.0014	0.024 ± 0.00014	2.2
	Naph	*c*-Pnt	Ala	0.016 ± 0.0007	0.024 ± 0.005	1.5
	Naph	*c*-Hex	Ala	0.036 ± 0.017	0.049 ± 0.004	1.4

				IC50 (μM)		
Compd	**aryl**	**ester**	**AA**	**L1210/0**	**L1210.Hyor**	**IC_{50}(L1210.Hyor)/IC_{50}(L1210/0)**
	Naph	CH_2-*t*-Bu	Ala	0.093 ± 0.033	0.18 ± 0.069	1.9
	Naph	CH_2 CH_2-*t*-Bu	Ala	0.012 ± 0.0018	0.032 ± 0.0088	2.7
	Naph	CH_2-*c*-Pr	Ala	0.014 v 0.0042	0.031 ± 0.0064	2.2
	Naph	2-Ind	Ala	0.039 ± 0.019	0.042 ± 0.040	1.08
	Naph	Bu	Ala	0.011 ± 0.009	0.025 ± 0.01	2.27
	Naph	THP	Ala	0.041 ± 0.0028	0.48 ± 0.11	11.7
	Naph	*c*-Hex	Val	1.2 ± 0.17	1.29 ± 0.29	1.08
	Naph	Pnt	Leu	0.031 ± 0.0020	0.035 ± 0.010	1.13
	Naph	Bn	Leu	0.029 ± 0.0021	0.048 ± 0.020	1.7
	Naph	Pnt	Ile	0.42 ± 0.021	0.70 ± 0.074	1.67
	Naph	Pnt	Phe	0.030 ± 0.0039	0.14 ± 0.007	4.67
	Naph	Bn	Phe	0.021 ± 0.0061	0.23 ± 0.078	11
	Naph	Bn	Met	0.054 ± 0.013	0.20 ± 0.098	3.7
	Naph	Bn	Pro	0.26 ± 0.055	0.65 ± 0.070	2.5
	Naph	Et	DMG	0.056 ± 0.04	0.17 ± 0.03	3
	Naph	Pnt	DMG	0.045 ± 0.0021	0.019 ± 0.0028	0.42
	Naph	Bn	DMG	0.019 ± 0.004	0.045 ± 0.004	2.4

[0162] Table 8 below records the cytostatic activity of 5-FdUrd and compounds embodying the present invention in CEM cell cultures containing (Cem/hEnt-1) or lacking (Cem/hEnt-0) the hEnt1 transporter in terms of IC_{50} or compound concentration required to inhibit tumour cell proliferation by 50%. Data are mean (±SD) of at least two to four independent experiments. Table 8 identifies the phosphoramidate motif of compounds embodying the present invention, as discussed above with respect to Table 6, but with "Naph" standing for 1-naphthyl. The data of Table 8 show that compounds embodying the present invention are less dependent on the presence of the hENT1 transporter, than 5-FdUrd, since they lose only 7- to 15-fold antiproliferative activity against the hENT1-deficient CEM cells. These observations are in agreement with an only 2- to 7-fold decreased cytostatic activity of compounds embodying the present invention in the presence of transport inhibitors (i.e. dipyridamole and NBMPR), compared to a 20- to 60-fold loss of antiproliferative activity of 5-FuDrd and FdUMP under similar experimental conditions.

Table 8.

				IC_{50} (μM)			
compd	aryl	Ester	AA	Cem/hEnt-1	Cem/hEnt-0	Cem/hEnt-1 + dipyridamole	Cem/hEnt-1 + NBMPR
5-FdUMP				0.05 ± 0.02	3.6 ± 0.69	1.74	1.06
5-FdUrd				0.04 ± 0.02	2.5 ± 0.65	1.36	0.80
	Ph	Bn	Ala	0.13 ± 0.05	1.4 ± 0.65	0.66	0.72
	Ph	Et	DMG	0.37 ± 0.14	5.8 ± 0.50	2.35	2.56
	Ph	Bn	DMG	0.17 ± 0.06	1.2 ± 0.11	0.26	0.61
	Naph	Bn	Ala	0.05 ± 0.02	0.6 ± 0.11	0.13	0.26
	Naph	Et	DMG	0.21 ± 0.07	1.4 ± 0.20	0.52	0.62
	Naph	Bn	DMG	0.05 ± 0.03	0.4 ± 0.13	0.16	0.28

[0163] Studies were performed on compound CPF 381 as follows:
An enzymatic phosphorylase assay was carried out using thymidine phosphorylase (TP, purified from *Esherichia coli*) in the presence of potassium phosphate buffer (300 nM solution, pH 7.4). The ^{19}F NMR spectrum after 5 min, 14 h and 72 h did not show any evidence of phosphorolysis. In contrast to 5-FdUrd, CPF 381 is at best a very poor, if any, substrate for thymidine phosphorylase.

[0164] A chemical hydrolysis was evaluated under experimental conditions at pH 1 and pH 8 and monitored by ^{31}P NMR. During the assay (14 h) under acidic conditions (pH 1) only two peaks representing the two diastereoisomers were recorded. Lack of formation of new signals in the ^{31}P NMR spectrum indicates that compound CPF 381 is highly stable in acidic medium. The same result was observed when compound CPF 381 was subjected to the assay under mild basic conditions (pH 8).

[0165] Studies were performed on compound CPF 581 as follows:
A enzymatic study using a carboxypeptidase Y assay was performed in which compound CPF 581, carboxypeptidase Y, and Trizma buffer (pH 7.6) were dissolved in acetone-d_6 and ^{31}P NMR spectrum

(202 MHz) spectra were recorded at regular intervals (every 7 min) over 14 h. Compound CPF 581 was rapidly hydrolyzed to a first metabolite lacking the ester (R_3) moiety, both diastereoisomers being processed at roughly similar rate. Further processing of the first metabolite led to the formation of an anionic second metabolite, lacking Ar, within about 45 min with an estimated half life of less than 5 min. The rate of the initial activation step might thus be considered in general as one of requirments for good biological actrivity of phosphoramidates. Chemical hydrolysis of compound CPF 373 in the presence of triethylamine and water produced the diammonium salt of the anionic second metabolite, which was added to the final assay sample derived from compound CPF 373, i.e. containing only the enzymatic second metabolite derived from compound CPF 581 in Trizma. The sample had a ^{31}P NMR spectrum showing only a single peak at δ_P 6.85 ppm, strongly supporting this part of the metabolic pathway and activation of the phosphoramidate compounds of the present invention.

[0166] Studies were performed on compound CPF 386 as follows:
The stability of compound CPF 386 in the presence of human serum was investigated using in situ ^{31}P NMR. A control ^{31}P NMR data of compound CPF 386 in DMSO and D_2O were recorded. The NMR sample was then treated with human serum and immediately subjected to further ^{31}P NMR experiments at 37°C. The ^{31}P NMR data were recorded every 15 minutes over 14 h. The spectra displayed a single peak inherent to human serum at ~δ_P 2.00 ppm and two peaks corresponding to compound CPF 386 at ~δ_P 4.59 and 4.84 ppm. After about 6 h and 45 min the compound was hydrolyzed partly to an intermediate, lacking R_3 (Et), as a single peak at δ_P 5.79 ppm. After 11 h and 30 min, the formation of the second metabolite, lacking Ar (1-naphthyl), shown as single peak at δ_P 7.09 ppm was observed. After 13 h and 30 min the reaction mixture contained 96% of the parent compound CPF 386 together with the proposed first metabolite (3%) and second metabolite (1%).

References

[0167]

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PATENTKRAV

1. Forbindelse valgt fra gruppen omfattende:

5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-isoleucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-phenylalaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(pentoxy-L-methioninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(benzoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-isoleucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-phenylalaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(benzoxy-L-methioninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-α,α-dimethylglycin)]phosphat;

eller et farmaceutisk acceptabelt derivat deraf, hvor det farmaceutisk acceptable derivat er et hvilket som helst farmaceutisk acceptabelt salt, hydrat, solvat eller en hvilken som helst krystallinsk form.

2. Forbindelse ifølge krav 1, hvor forbindelsen er valgt fra gruppen omfattende:

5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-isoleucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(benzoxy-L-phenylalaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[phenyl(pentoxy-L-methioninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(benzoxy-L-leucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-isoleucinyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-phenylalaninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(benzoxy-L-methioninyl)]phosphat
5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-α,α-dimethylglycin)]phosphat.

3. Forbindelse ifølge krav 1, hvor forbindelsen er 5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-leucinyl)]phosphat eller et farmaceutisk acceptabelt derivat deraf, hvor det farmaceutisk acceptable derivat er et hvilket som helst farmaceutisk acceptabelt salt, hydrat, solvat eller en hvilken som helst krystallinsk form.

4. Forbindelse ifølge krav 3, hvor forbindelsen er 5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(pentoxy-L-leucinyl)]phosphat.

5. Forbindelse ifølge krav 1, hvor forbindelsen er 5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphat eller et farmaceutisk acceptabelt derivat deraf hvor det farmaceutisk acceptable derivat er et hvilket som helst farmaceutisk acceptabelt salt, hydrat, solvat eller en hvilken som helst krystallinsk form.

6. Forbindelse ifølge krav 5, hvor forbindelsen er 5-fluor-2'-deoxyuridin-5'-*O*-[1-naphthyl(hexoxy-L-alaninyl)]phosphat.

7. Forbindelse ifølge et hvilket som helst af kravene 1 til 6, hvor forbindelsen er til anvendelse i en fremgangsmåde til profylakse eller behandling af cancer hos homo sapiens.

8. Forbindelse til anvendelse ifølge krav 7, hvor canceren er valgt fra gruppen omfattende leukæmi, pankreas-, prostata-, lunge-, bryst-, cervixcancer; øsofageal-; gastrointestinal-, herunder gastrisk, tyndtarms-, colon- og rectumcancer; cancer i hoved og hals og ovariecancer.

9. Forbindelse til anvendelse ifølge krav 7 eller krav 8 til anvendelse i en fremgangsmåde til profylakse eller behandling af cancer hos en patient valgt fra:

A) en patient, der har udviklet eller har potentiale for at udvikle resistens i tumorceller med hensyn til aktiviteten af 5-fluoracil eller 5-fluor-2'-deoxyuridin i profylaksen eller behandlingen af cancer;

B) en patient med celler med et sænket niveau af nukleosid-transporterproteiner;

C) en patient med nukleosidkinase-deficiente celler;

D) en patient med mykoplasma-inficerede celler; og/eller

E) en patient med celler med et forhøjet niveau af thymidylat-syntase (TS).

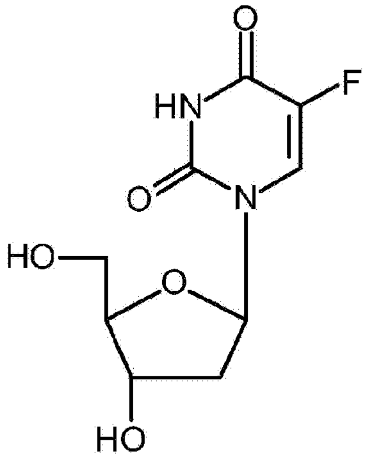
10. Forbindelse til anvendelse ifølge et hvilket som helst af kravene 7 til 9 til anvendelse i sammen med anden anti-cancerteerapi.

11. Farmaceutisk sammensætning, der omfatter en forbindelse ifølge et hvilket som helst af kravene 1 til 6 i kombination med en farmaceutisk acceptabel bærer, fortynder eller excipiens.

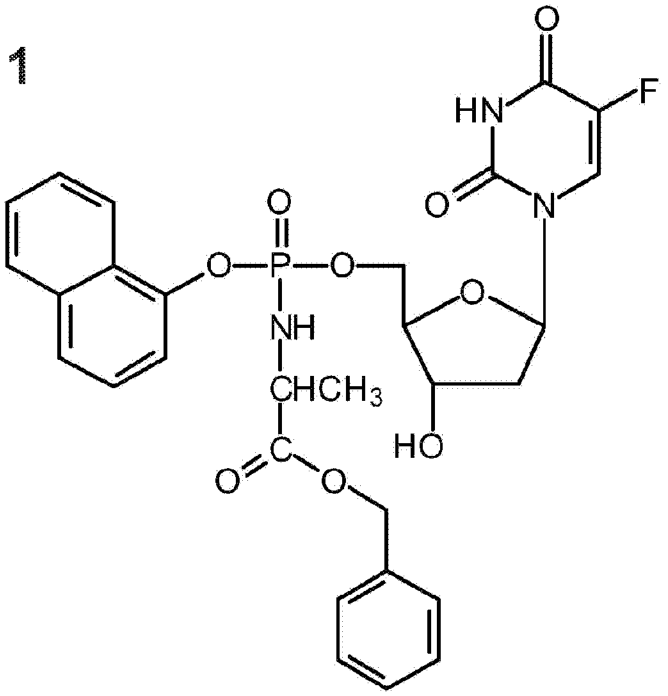
12. Fremgangsmåde til fremstilling af en farmaceutisk sammensætning, der omfatter trinnet med kombinering af en forbindelse ifølge et hvilket som helst af kravene 1 til 11 med en farmaceutisk acceptabel excipiens, bærer eller fortynder.

DRAWINGS

FIG. 1



5-FdUrd



CPF-373

FIG. 2(A)

E. coli TP

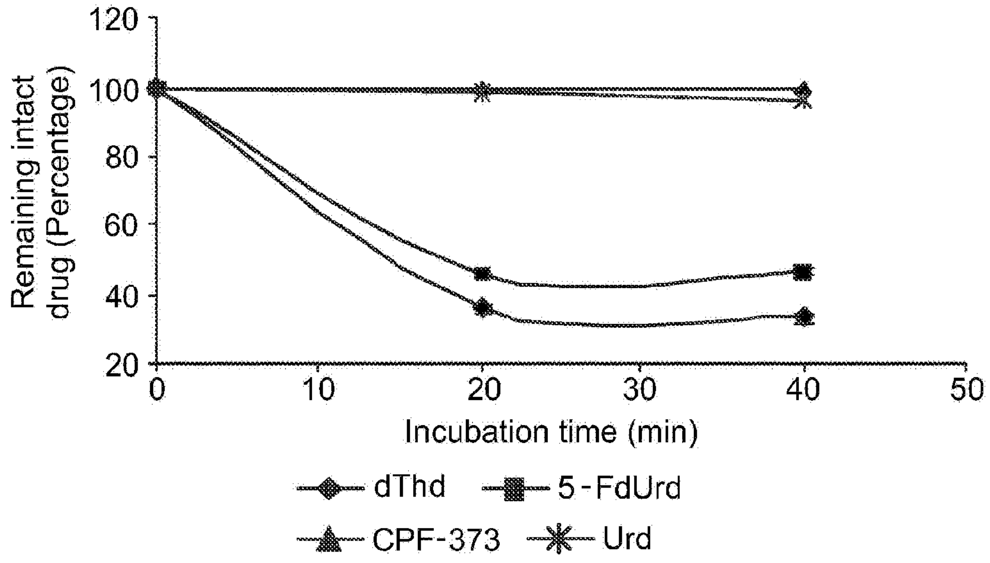


FIG. 2(B)

Human TP

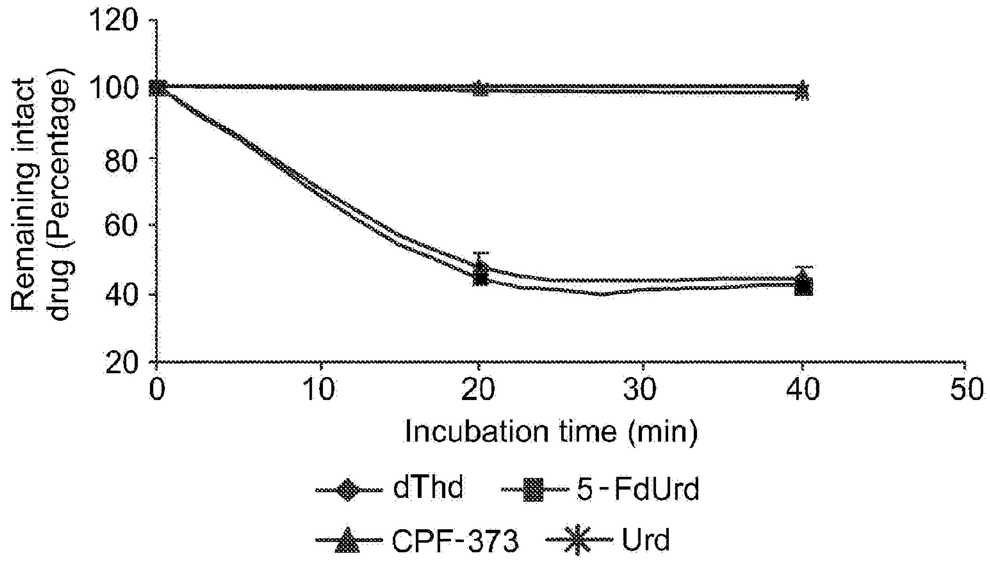


FIG. 2(C)

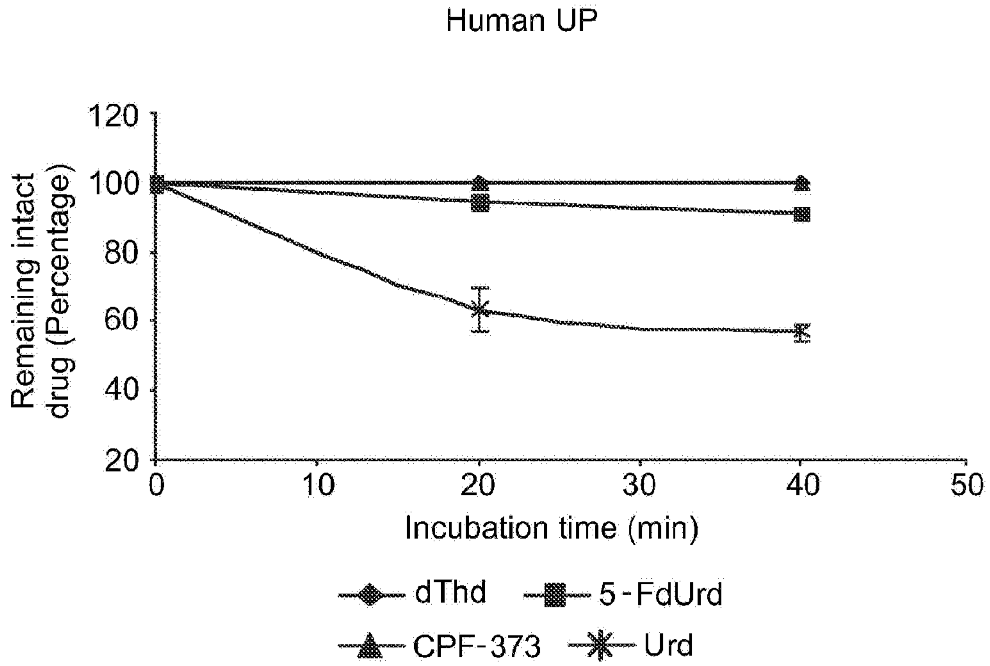

Human UP
Remaining intact drug (Percentage)
120
100
80
60
40
20
0
10
20
30
40
50
Incubation time (min)
dThd
5-FdUrd
CPF-373
Urd

FIG. 3(A)

Inhibition of TS in L1210/0 cells by 5-FdUrd
(substrate: [5-^{3}H]dUrd)

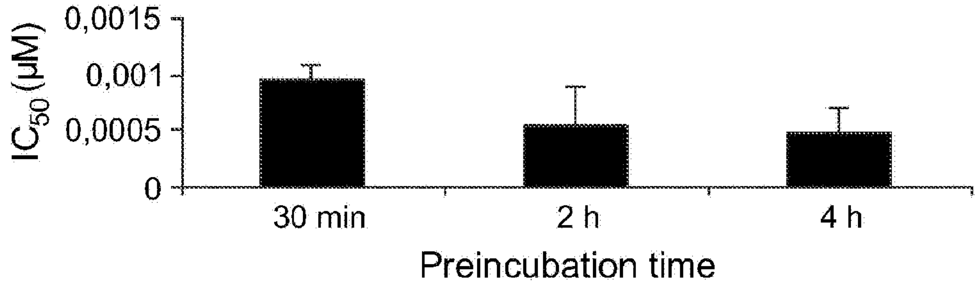


FIG. 3(B)

Inhibition of TS in L1210/0 cells by CPF-373
(substrate: [5-^{3}H]dUrd)

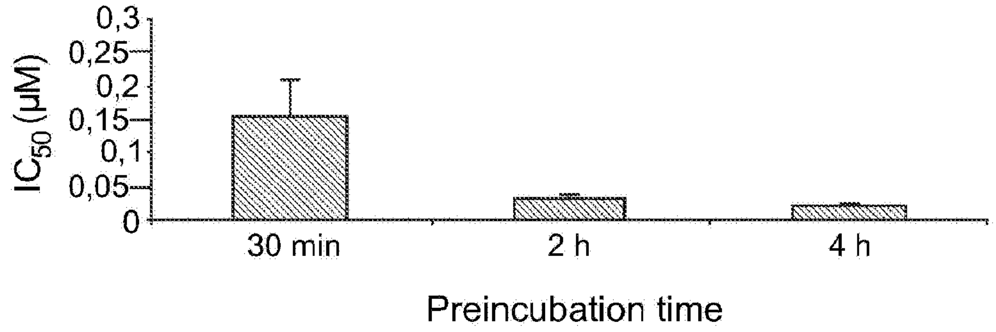


FIG. 3(C)

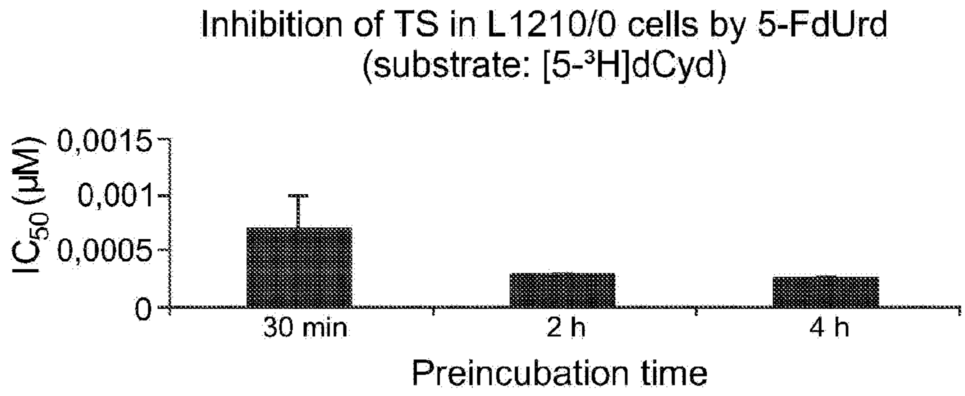

Inhibition of TS in L1210/0 cells by 5-FdUrd
(substrate: [5-³H]dCyd)
IC50 (µM)
0,0015
0,001
0,0005
0
30 min
2 h
4 h
Preincubation time

FIG. 3(D)

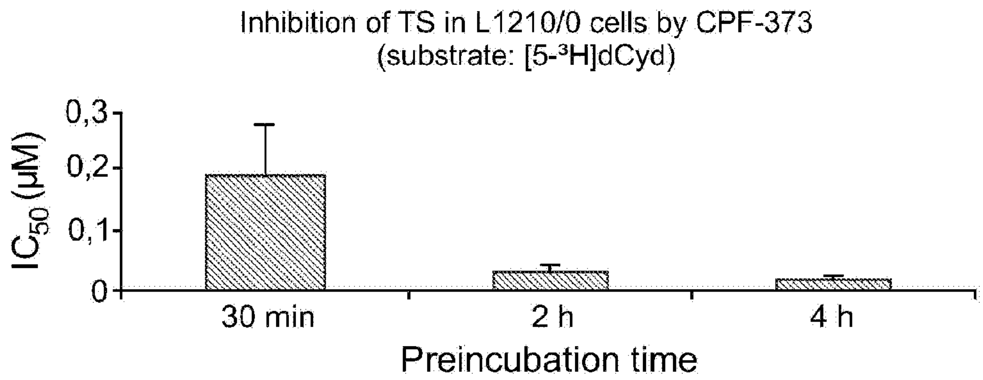

Inhibition of TS in L1210/0 cells by CPF-373
(substrate: [5-³H]dCyd)
IC50 (µM)
0,3
0,2
0,1
0
30 min
2 h
4 h
Preincubation time

FIG. 3(E)

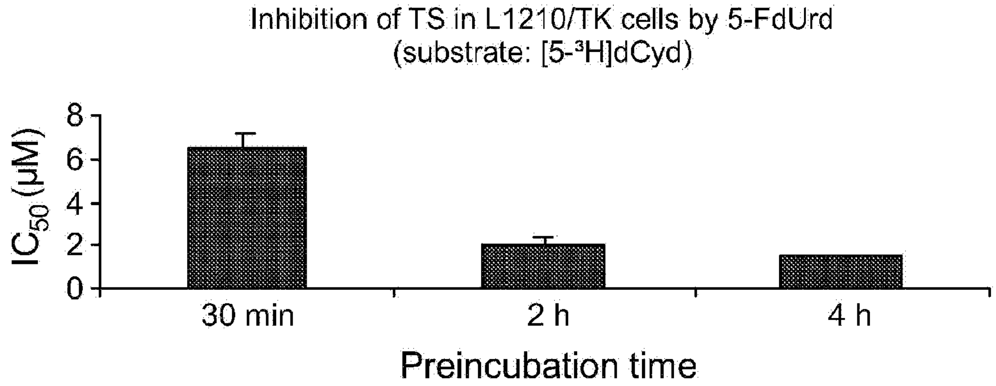

Inhibition of TS in L1210/TK cells by 5-FdUrd
(substrate: [5-³H]dCyd)
IC50 (µM)
8
6
4
2
0
30 min
2 h
4 h
Preincubation time

FIG. 3(F)

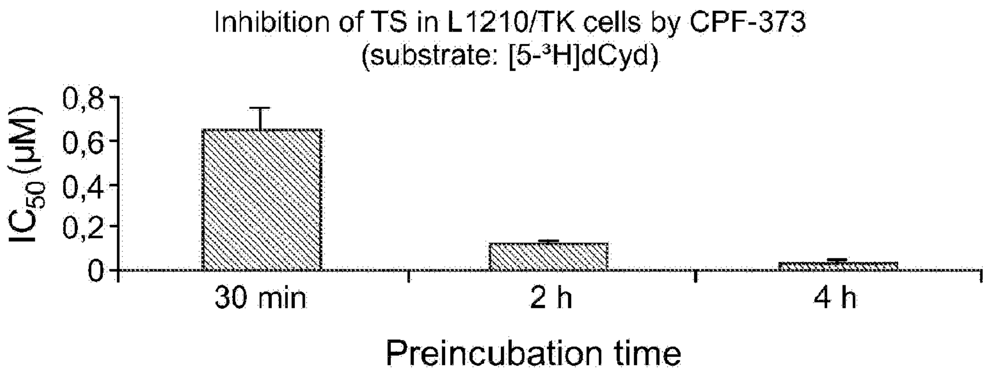
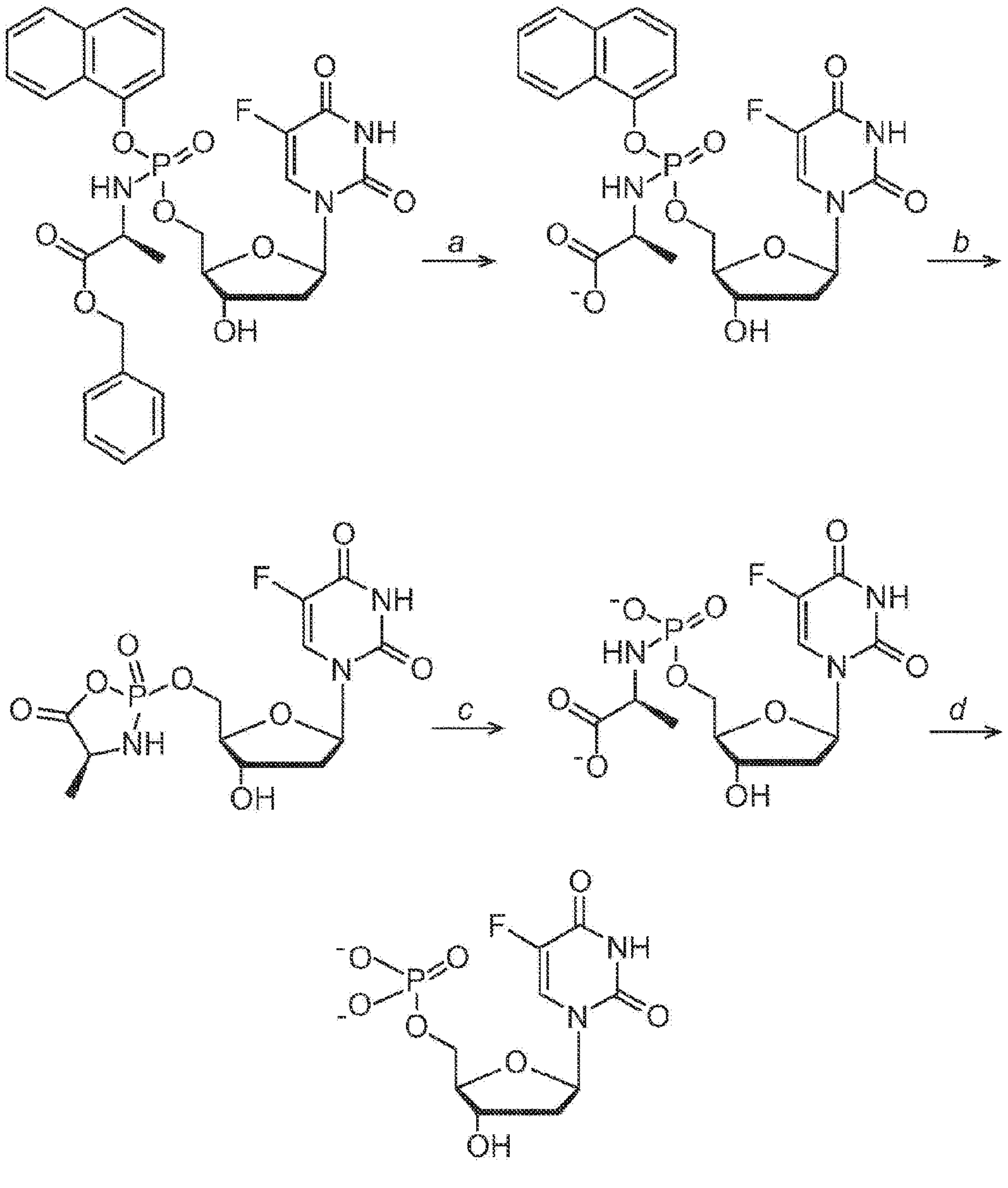

Inhibition of TS in L1210/TK cells by CPF-373
(substrate: [5-³H]dCyd)
IC50 (µM)
0,8
0,6
0,4
0,2
0
30 min
2 h
4 h
Preincubation time

FIG. 4



a: esterase or carboxypeptidase-type enzyme b: spontaneous
c: spontaneous d: phosphoramidase-type enzyme

FIG. 5

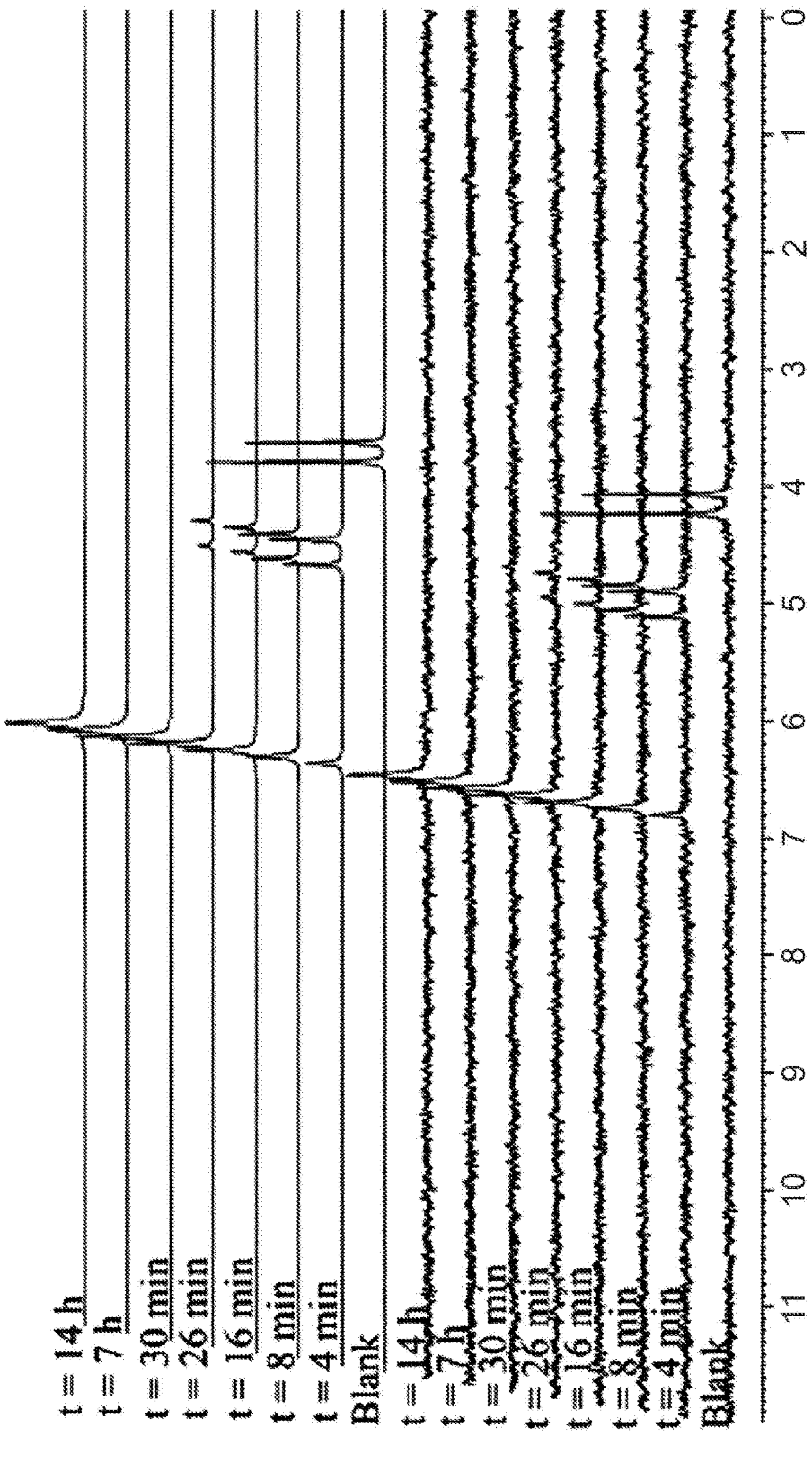

t = 14 h
t = 7 h
t = 30 min
t = 26 min
t = 16 min
t = 8 min
t = 4 min
Blank
t = 14 h
t = 7 h
t = 30 min
t = 26 min
t = 16 min
t = 8 min
t = 4 min
Blank
0
1
2
3
4
5
6
7
8
9
10
11

FIG. 6

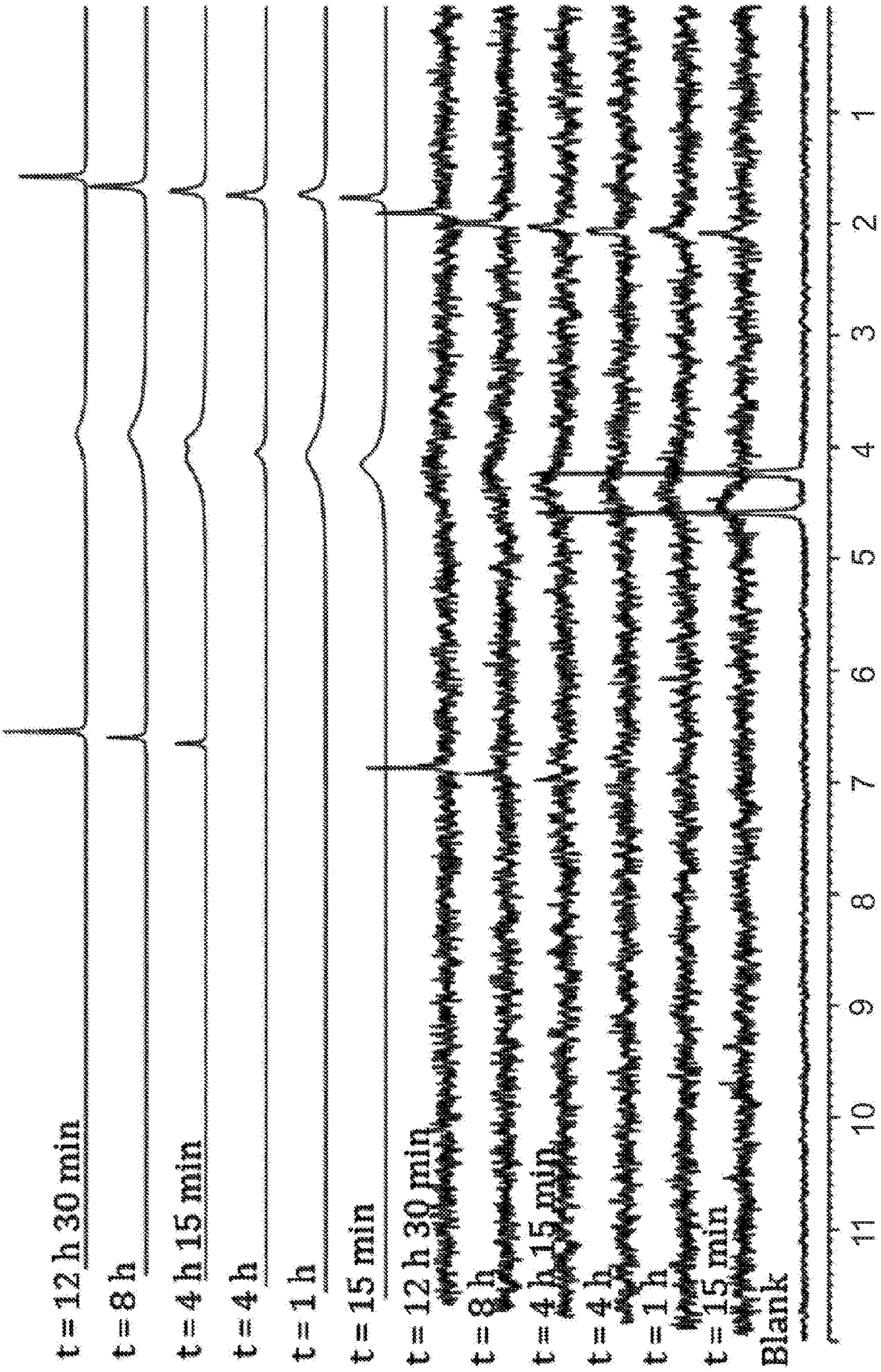

t = 12 h 30 min
t = 8 h
t = 4 h 15 min
t = 4 h
t = 1 h
t = 15 min
t = 12 h 30 min
t = 8 h
t = 4 h 15 min
t = 4 h
t = 1 h
t = 15 min
Blank
1
2
3
4
5
6
7
8
9
10
11

FIG. 7

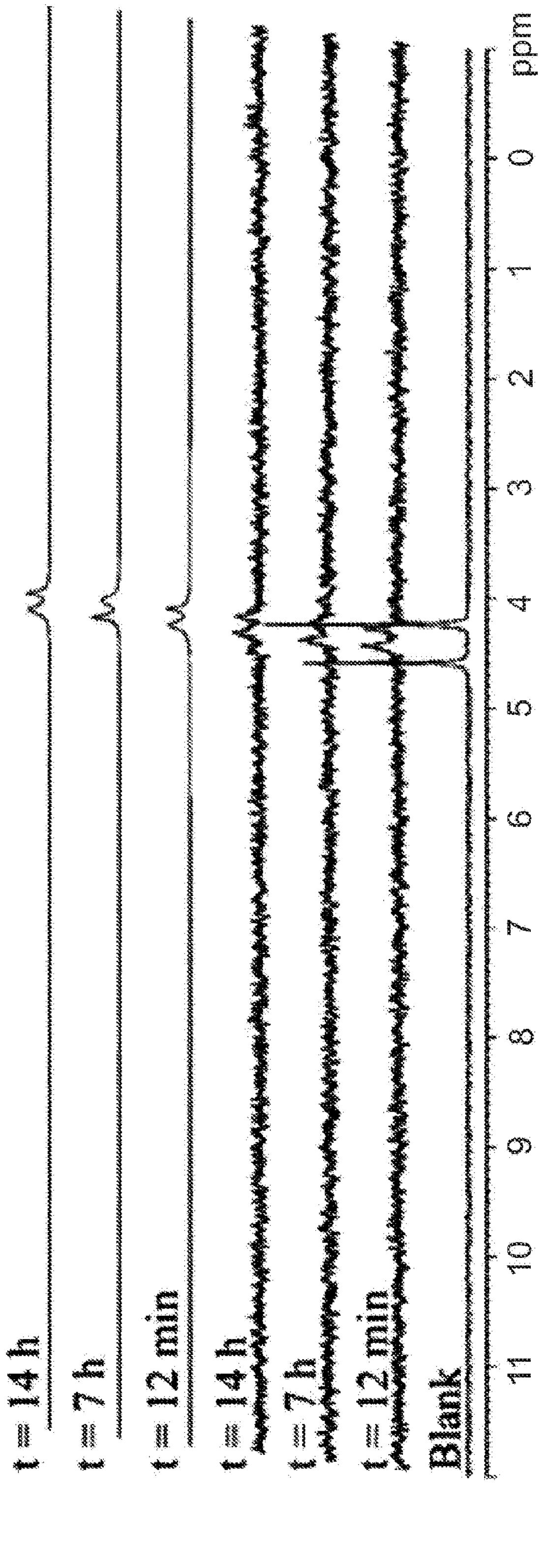


FIG. 8

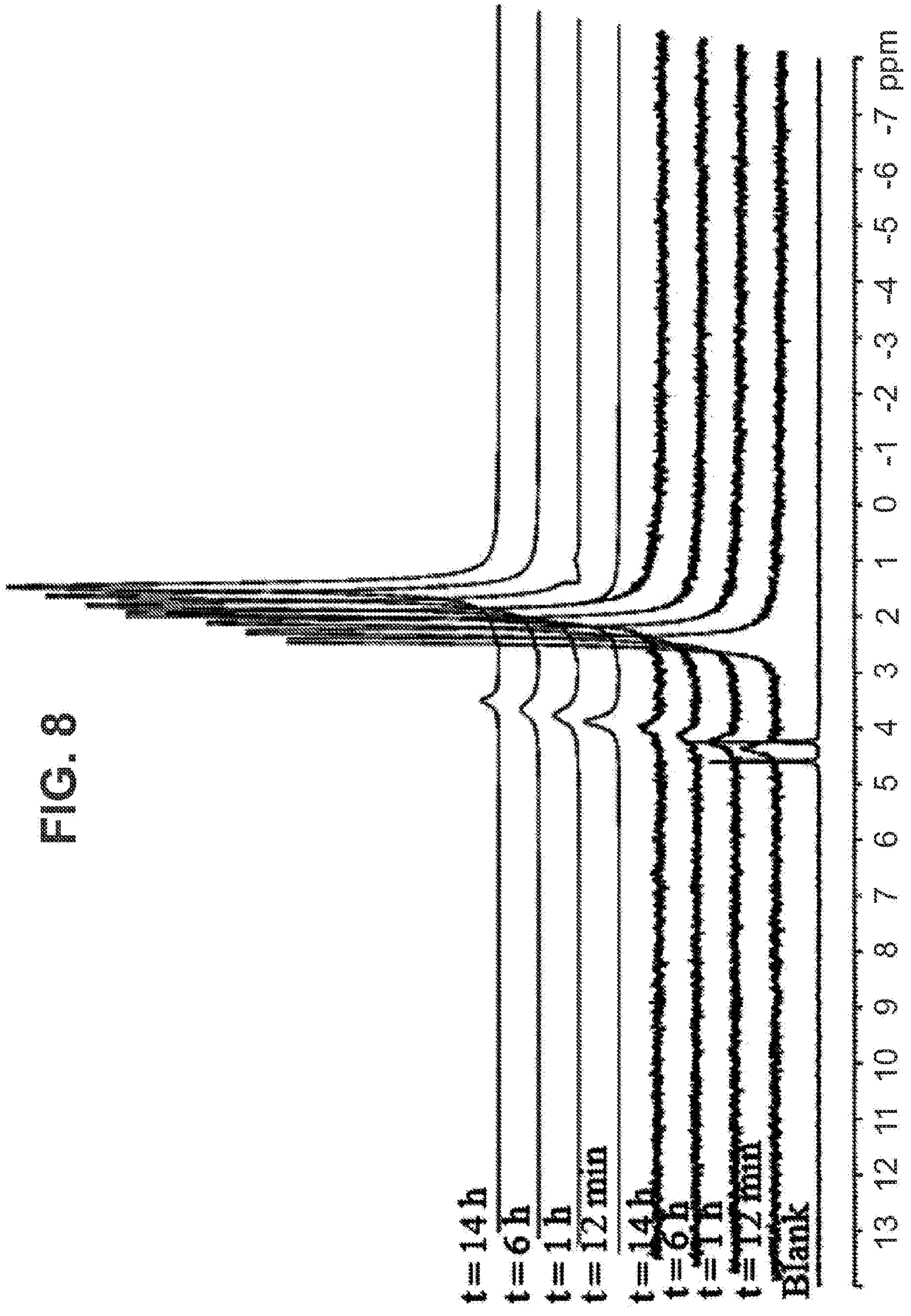

t = 14 h
t = 6 h
t = 1 h
t = 12 min
t = 14 h
t = 6 h
t = 1 h
t = 12 min
Blank
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 ppm

FIG. 9(A)

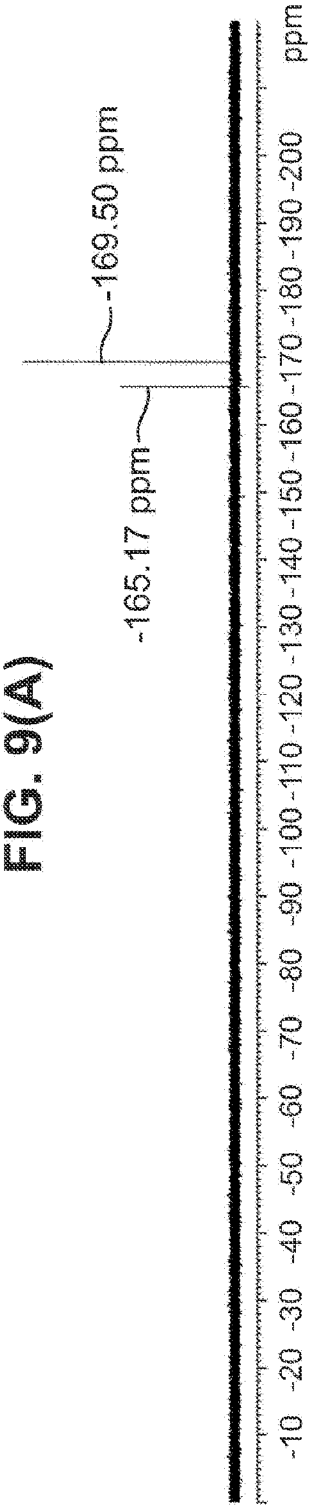
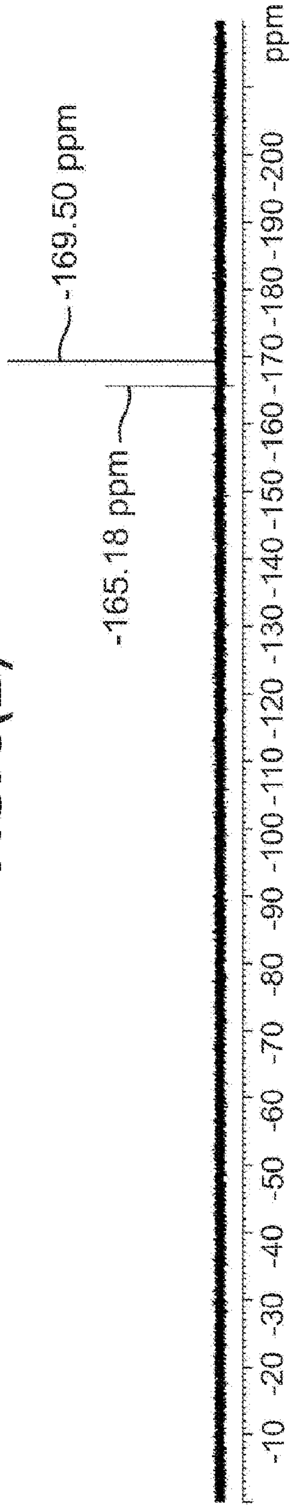

-169.50 ppm
-165.17 ppm
-10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
ppm

FIG. 9(B)


-169.50 ppm
-165.18 ppm
-10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
ppm

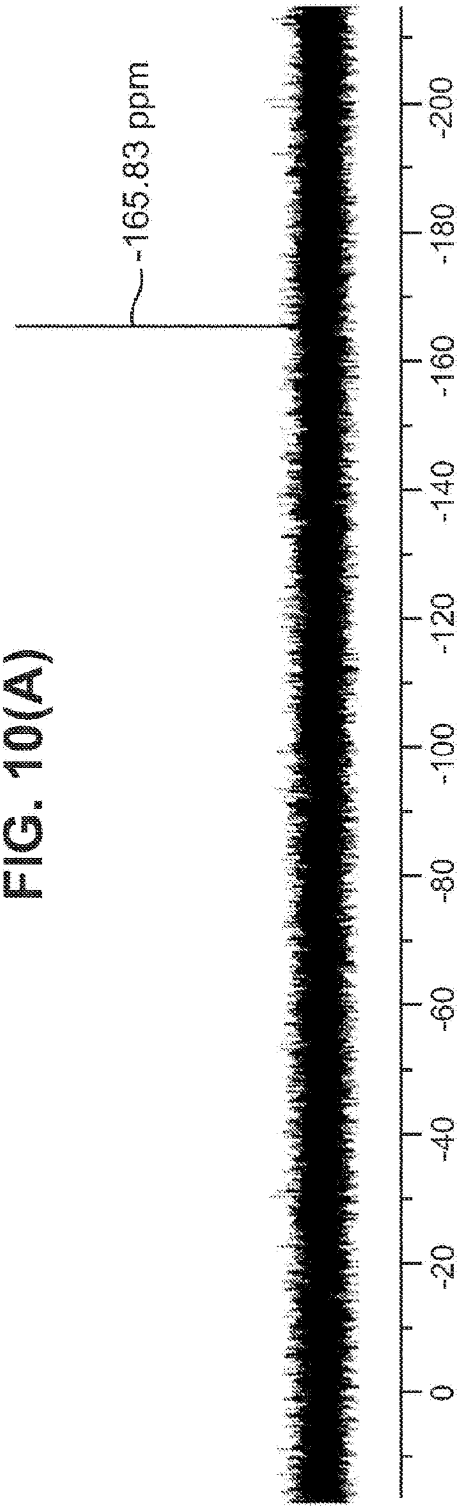


FIG. 10(A)

FIG. 10(B)

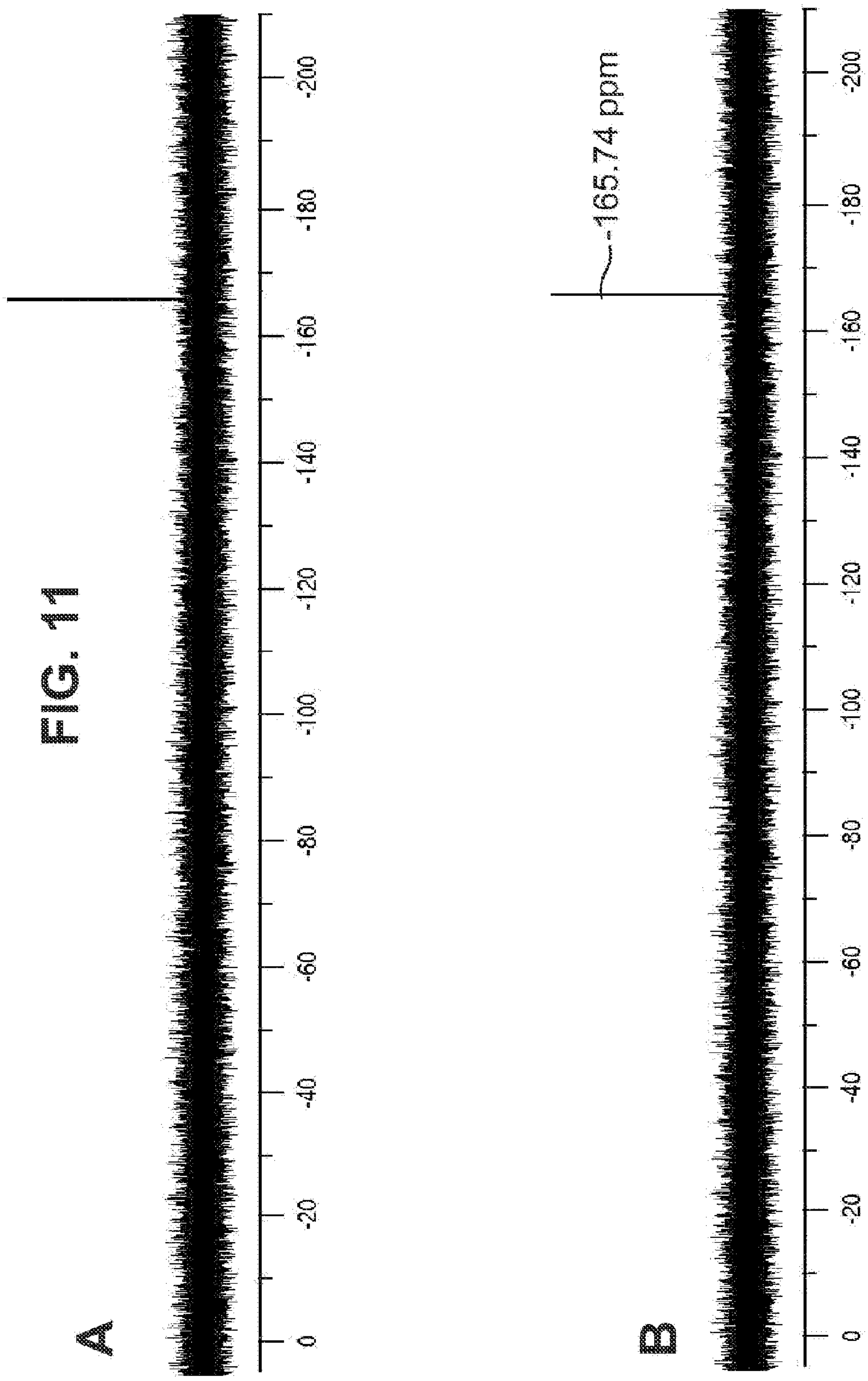


FIG. 11