(19) DANMARK

(10) DK/EP 1646639 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 07 H 19/10 (2006.01)* *A 61 K 31/7068 (2006.01)* *A 61 K 31/7072 (2006.01)* *A 61 P 35/00 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2016-09-12**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2016-08-17**

(86) Europæisk ansøgning nr.: **04743483.2**

(86) Europæisk indleveringsdag: **2004-07-20**

(87) Den europæiske ansøgnings publiceringsdag: **2006-04-19**

(86) International ansøgning nr.: **GB2004003148**

(87) Internationalt publikationsnr.: **WO2005012327**

(30) Prioritet: **2003-07-21 GB 0317009**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **NuCana BioMed Limited, 10 Lochside Place, Edinburgh EH12 9RG, Storbritannien**

(72) Opfinder: **McGUIGAN, Christopher, 2 Alfreda Road, Whitchurch, Cardiff CF4 2EH, Storbritannien**

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **NUKLEOTIDPHOSPHORAMIDATER SOM ANTICANCERMIDLER**

(56) Fremdragne publikationer:
WO-A-01/07454
WO-A1-99/37753
US-A1- 2003 109 697
LACKEY D B ET AL: "Enzyme-catalyzed therapeutic agent (ECTA) design: activation of the antitumor ECTA compound NB1011 by thymidylate synthase" BIOCHEMICAL PHARMACOLOGY, PERGAMON, OXFORD, GB, vol. 61, 2001, pages 179-189, XP002204184 ISSN: 0006-2952
S.A. HARRIS ET AL.: "Synthesis and antiviral evaluation of phosphoramidate derivatives of (E)-5-(2-bromovinyl)-2'-deoxyuridine" ANTIVIRAL CHEMISTRY AND CHEMOTHERAPY, vol. 12, 2001, pages 293-300, XP008037325
WHALEN LISA J ET AL: "Synthesis and evaluation of phosphoramidate amino acid-based inhibitors of sialyltransferases" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB LNKD- DOI:10.1016/S0960-894X(02)00735-7, vol. 13, no. 2, 1 January 2003 (2003-01-01), pages 301-304, XP002495584 ISSN: 0960-894X
JUODKA BENEDIKTAS ET AL: "Oligonucleotides and nucleotide-peptides. XXXVII. On the mechanism of hydrolysis of uridylyl-(5' .fwdarw. N)-amino acids. Intramolecular catalysisby the .alpha.-carboxyl group of amino acids" JOURNAL OF CARBOHYDRATES. NUCLEOSIDES NUCLEOTIDES, MARCEL DEKKER, BASEL, CH, vol. 8, no. 6, 1 January 1981 (1981-01-01) , pages 519-535, XP009099165 ISSN: 0094-0585
MCGUIGAN C ET AL: "SYNTHESIS AND EVALUATION OF SOME MASKED PHOSPHATE ESTERS OF THE ANTI-

Fortsættes ...

HERPESVIRUS DRUG 882C (NETIVUDINE) AS POTENTIAL ANTIVIRAL AGENTS" ANTIVIRAL CHEMISTRY & CHEMOTHERAPY, BLACKWELL SCIENTIFIC PUBL., LONDON, GB, vol. 9, 1 January 1998 (1998-01-01), pages 233-243, XP000943516 ISSN: 0956-3202

JUODKA B ET AL: "Oligonucleotides and nucleotide-peptides. XXXIV. Synthesis and some properties of complex nucleotidyl (oligonucleotidyl)-(P .fwdarw. N)-amino acids (peptides) and their ethyl esters" JOURNAL OF CARBOHYDRATES. NUCLEOSIDES NUCLEOTIDES, MARCEL DEKKER, BASEL, CH, vol. 6, no. 4, 1 January 1979 (1979-01-01) , pages 333-357, XP009099193 ISSN: 0094-0585

D.M. LEHSTEN ET AL.: "An improved procedure for the synthesis of nucleoside amidates" ORGANIC PROCESS RESEARCH AND DEVELOPMENT, vol. 6, 2002, pages 819-822, XP002584043

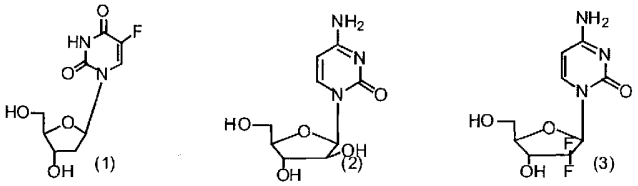
D.C. REMY ET AL.: "Studies on fluorinated pyrimidines. XIV. The synthesis of derivatives of 5-fluoro-2'-deoxyuridine 5'-phosphate and related compounds" J. ORG. CHEM., vol. 27, 1962, pages 2491-2500, XP002584044

ABRAHAM T W ET AL: "Synthesis and biological activity of aromatic amino acid phosphoramidates of 5-fluoro-2'-deoxyuridine and 1-beta-arabinofuranosylcytosine: evidence of phosphoramidase activity", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 39, 1 January 1996 (1996-01-01), pages 4569-4575, XP002147029, ISSN: 0022-2623, DOI: 10.1021/JM9603680

DESCRIPTION

[0001] The present invention relates to nucleotide derivatives and their use in the treatment of cancer.

[0002] Nucleoside analogues such as fluorodeoxyuridine (1), cytarabine (2) and gemcitabine (3) are well established as anticancer agents. They function as inhibitors of DNA synthesis after activation to their 5'-phosphate form.

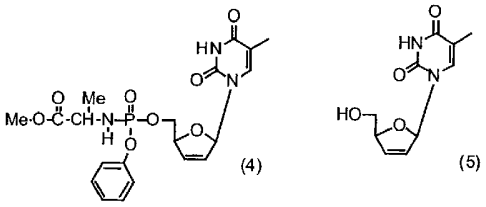


[0003] The free bioactive phosphate forms do not in general represent useful drugs due to their poor membrane permeation. In an effort to circumvent this a number of phosphate pro-drug approaches have been reported [Rosowsky et al, J. Med. Chem., 1982, 25, 171-8; Hong et al, J. Med. Chem., 1985, 28, 171-8; Kodama et al, Jpn. J. Cancer Res., 1989, 80, 679-85; Hong et al, 1979, 22, 1428-32; Ji et al, J. Med. Chem., 1990, 33, 2264-70; Jones et al, Nucleic Acids Res., 1989, 17, 7195-7201; Hunston et al, J. Med. Chem., 1984, 27, 440-4; Lorey et al, Nucleosides Nucleotides, 1997, 16, 1307-10; Farquhar et al, J. Med. Chem., 1983, 26, 1153-8; Shuto et al, Nucleosides Nucleotides, 1992, 11, 437-46; Le Bec et al, Tet. Letts., 1991, 32, 6553-6; Phelps et al, J. Med. Chem., 1980, 23, 1229-32].

[0004] In general the phosphate prodrugs have biological properties and therapeutic activities that are similar to, or somewhat lower than, the parent nucleoside analogue.

[0005] We have carried out extensive work in this area from an antiviral perspective, largely on dideoxy nucleosides, and have reported a phosphoramidate approach which has been widely adopted for the delivery of bio-active phosphates of antiviral nucleosides.

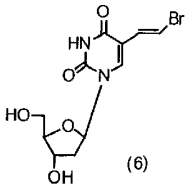
[0006] An example is the phosphoramidate (4) derived from anti-HIV d4T (5).



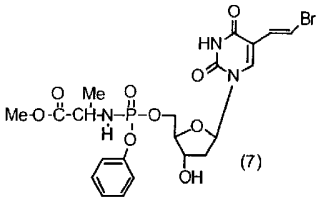
[0007] We observed the effect of variations in the ester [McGuigan et al, AVCC, 1998, 9, 473-9], amino acid [McGuigan et al, Antiviral Res., 1997, 35, 195-204; AVCC, 2000, 11, 111-6], and aryl [Siddiqui et al, J. Med. Chem., 1999, 42, 393-9] regions of the phosphoramidate, as well as the effect of amino acid stereochemistry [McGuigan et al, AVCC, 1996, 7, 184-8], phosphate stereochemistry [Allender et al, Analytica Chim. Acta, 2001, 435, 107-13] and nucleoside [Balzarini et al, BBRC, 1996, 225, 363-9; McGuigan et al, BioOrg. Med, Chem. Lett., 1996, 6, 2369-62; McGuigan et al, Bioorg. Med. Chem. Lett., 2000, 10, 645-7].

[0008] This work has lead to the optimal description of phenyl methoxyalaninyl phosphoramidate as the prototype pro-moiety for the intracellular delivery of bioactive nucleotides [Balzarini et al, PNAS, 1996, 93, 7295-9; McGuigan et al, J. Med. Chem., 1996, 39, 1748-53].

[0009] Lackey et al [Biochem Pharmacol., 2001, 61, 179-89] have reported the application of our phosphoramidate pro-drug method for antiviral nucleosides to the anti-herpetic agent bromovinyl-2'-deoxyuridine (BVDU) (6). In particular, they have found that the phenyl methoxyalaninyl phosphoramidate (7) has significant anti-cancer activity. This is in marked contrast to the parent (antiviral) nucleoside (6).



(6)



(7)

[0010] Limited SAR has been presented by this group, although in their patent applications [WO0239952, EP1200455, CA2317505, US6339151, EP116797, AU2451601] they claims a series of general variations in the base, and phosphate regions. However, based on our prior art, the phenyl methoxyalaninyl phosphoramidate (7) would be anticipated to be amongst the most optimal of strictures.

[0011] Surprisingly, it has now been found that other derivatives of oxyamino acid- phosphoramidate nucleoside analogues are significantly more potent in the treatment of cancer than the phenyl methoxyalaninyl phosphoramidate (7).

[0012] US 2003/109697 discloses phosphoramidate compounds for treating cancer, infectious disease, an autoimmune disorder or an inflammatory condition. The compounds are 5'-phosphoramidatyl, 1,5-substituted pyrimidine compounds.

[0013] Abraham TW et al. (J. Med. Chem., 1996, 39,4569-4575) discloses the synthesis and testing for anti-tumour activity against L1210 mouse lymphocytic leukaemia cells of a series of compounds which are amino acid phosphoramidate diesters of FUdR and Ara-C together with related compounds such as 5-fluoro-2'-deoxy-5'uridyl-N-(1-carbomethoxy-2-phenylethyl)phosphoramidate.

[0014] According to a first aspect of the present invention there is provided a compound of formula I as set out in claim 1.

[0015] Reference in the present specification to an alkyl group means a branched or unbranched, saturated hydrocarbyl radical. The alkyl group is preferably C_1 to C_{16}, more preferably C_1 to C_6.

[0016] Reference in the present specification to an aryl group means an aromatic group containing 5 to 14 ring atoms, for example phenyl or naphthyl. The aromatic group may be a heteroaromatic group containing one, two, three or four, preferably one, heteroatoms selected, independently, from the group consisting of O, N and S. Examples of such heteroaromatic groups include pyridyl, pyrrolyl, furanyl and thiophenyl. Preferably, the aryl group comprises phenyl or substituted phenyl.

[0017] The group Ar comprises a substituted or unsubstituted aryl group, wherein the term "aryl group" and the possible substitution of said group is as defined herein. Preferably, Ar is a substituted or unsubstituted phenyl group. Particularly preferred substituents are electron withdrawing groups such as halogen (preferably chlorine or fluorine), trihalomethyl (preferably trifluoromethyl), cyano and nitro groups. For example, Ar can be phenyl, 3,5-dichloro-phenyl, p-trifluoromethyl-phenyl, 12-cyano-phenyl, or p-nitro-phenyl. When Ar is a heteroaromatic group, preferably it is optionally substituted pyridyl.

[0018] Preferably, R is methyl ($-CH_3$), ethyl ($-C_2H_5$), *n*- or *i*- propyl ($-C_3H_7$), *n*- or *i*- butyl ($-C_4H_9$) or benzyl ($-CH_2C_6H_5$). Most preferably, R is benzyl. Particularly, R is preferably benzyl when one of R' and R" is H and one of R' and R" is methyl ($-CH_3$), especially when Ar is unsubstituted phenyl, n is 0 and each of X and Y is F.

[0019] R' and R" are, independently, H, methyl ($-CH_3$), secondary butyl ($-CH_2-CH-(CH_3)_2$), benzyl ($-CH_2C_6H_5$), or, together with the C atom to which they are attached, provide a C_{5-6} ring.

[0020] Preferred compounds include those where R' and R" are both methyl, one of R' and R" is H and one of R' and R" is methyl, and R' and R", together with the C atom to which they are attached, provide a pentyl ring.

[0021] When R and R" are different, the C atom to which they are attached is chiral. The present compounds can be L or D or a mixture of stereoiosomers. Preferably they are L.

[0022] It will be appreciated that the moiety -O-C(O)-CR'R"-NH- corresponds to a carboxy-protected α-amino acid. R' and R" can thus correspond to the side chains of a naturally occurring amino acid.

[0023] For example, when one of R' and R" is H and one of R' and R" is Me or $PhCH_2$, the moiety corresponds to alanine or phenylalanine, respectively.

[0024] Preferably, the stereochemistry at the asymmetric centre -CR'R" corresponds to an L-amino acid. The stereochemistry at the asymmetric centre -CR'R" can, however, correspond to a D-amino acid. Alternatively, mixtures of of compounds can be employed having asymmetric centres corresponding to L and D amino acids.

[0025] In the present specification by "naturally occurring amino acid" we mean Alanine, Arginine, Asparagine, Aspartic Acid, Cysteine, Cystine, Glycine, Glutamic Acid, Glutamine, Histidine, Hydroxylysine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine and Valine.

[0026] The present invention is not, however, limited to compounds having a moiety corresponding to a naturally occurring amino acid. The present invention specifically includes compounds having a moiety which corresponds to a non-naturally occurring amino acid, such as, for example, those where R'=R"=alkyl, or, where together with the C atom to which they are attached, R' and R" provide a cyclic moiety. Preferably with respect to the compound of formula I, the moiety ROCOCR'R"NH- corresponds to or is derived from a non-naturally occurring amino acid.

[0027] The moiety ROCOCR'R"NH- preferably neither corresponds to nor is derived from alanine, more preferably neither corresponds to nor is derived from either of alanine or trytophan, even more preferably neither corresponds to nor is derived from any naturally occurring amino acid.

[0028] When Z is H, Q is O, n is 0 and X and Y are each F, the base moiety of the compound of formula I corresponds to that of gemcitabine i.e. compound (3) above.

[0029] Compounds of formula I wherein n is 0 and X and Y are F are preferred. Particularly preferred are compounds of formula I wherein n is 0, X and Y are F, Q is O and Z is H, corresponding to phosphoramidated gemcitabine.

[0030] Preferably, Ar is a carbomonocyclic aromatic ring moiety. More preferably, Ar is a C_6 monocyclic aromatic ring moiety, ie is optionally substituted phenyl.

[0031] One, two, three or four substituents, which may be the same or different, may be present on Ar and are selected from the group comprising halogen, which may -F, -Cl, -Br or -I; $-NO_2$; -CN; The optional substitutents are one or more up to six, preferably three, members selected from the group comprising halogen which may be F, Cl, Br and I and NO_2. Preferred substituents on Ar include F, Cl, CF_3, and NO_2.

[0032] The substituents may be at any position on the ring moiety. Where the ring moiety is C_6 ie phenyl, a single substituent at the 2 (*ortho*) or 4 (*para*) position is preferred. Where Ar is phenyl, a single substituent at the 4 position is more preferred.

[0033] Preferably, Ar is an optionally substituted phenyl moiety. More preferably, Ar is selected form the group comprising: Ph-, $pCF_3C_6H_4$-, pFC_6H_4-, $pNO_2C_6H_4$-, $pClC_6H_4$- and $oClC_6H_4$-.

[0034] According to a further aspect of the present invention there is provided a compound having formula I according to the present invention for use in a method of treatment, preferably in the prophylaxis or treatment of cancer.

[0035] According to a further aspect of the present invention there is provided a pharmaceutical composition comprising a compound having formula I of the present invention in combination with a pharmaceutically acceptable excipient, carrier or diluent.

[0036] According to a further aspect of the present invention there is provided a method of preparing a pharmaceutical composition comprising the step of combining a compound having formula I of the present invention with a pharmaceutically acceptable excipient, carrier or diluent.

[0037] The present invention is particularly applicable for the treatment of a patient having breast cancer, colon cancer or prostate cancer. Examples of such cancers include breast MDA MB231, colon HT115 and prostate PC-3.

[0038] The compound having formula I or pharmaceutical composition according to the present invention can be administered to a patient, which may be human or animal, by any suitable means.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

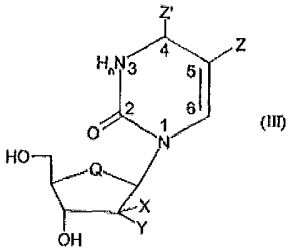
[0044] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[0045] 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.

[0046] The compounds of the invention may also be presented as liposome formulations.

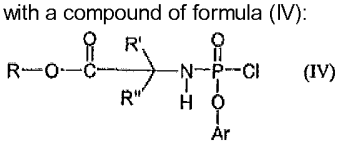
[0047] In general a suitable dose will be in the range of 0.1 to 300 mg per kilogram body weight of the recipient per day. A preferred lower dose is 0.5 mg per kilogrm body weight of recipient per day, a more preferred lower dose is 6 mg per kilogram body weight of recipient per day, an even more preferred 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.

[0048] According to a further aspect of the present invention there is provided a process for the preparation of a compound having formula I according to claim 1, the process comprising reacting a compound of formula (III):



(III)

with a compound of formula (IV):



(IV)

wherein Ar, n, Q, R, R', R", X, Y, Z' and Z have the meanings defined in claim 1 with respect to formula (I) and a double bond exist between position 3 and position 4.

[0049] Embodiments of the present invention will now be described, by way of example only, with reference to the following examples, experimental procedures and experimental data.

[0050] Data are presented for a range of structures against tumour cell types representing a range of common cancers in man with un-met clinical need: breast MDA MB231, colon HT115, prostate PC-3. Data from these assays are presented as Table I.

Experimental Procedure

General methods

[0051] The following anhydrous solvents and reagents were bought from Aldrich with sure stopper: dichloromethane (DCM), diethyl ether (Et_2O), tetrahydrofuran THF), N-methylimidazole (NMI), methanol (MeOH), dimethylformamide (DMF), 1,4-dioxane, triethylamine was dried on molecular sieves of 4 Angstrom.

Thin Layer Chromatography

[0052] Thin layer chromatography (TLC) was performed on commercially available Merck Kieselgel 60 F_{254} plates and separated components were visualized using ultraviolet light (254 nm and 366 nm).

Column Chromatography

[0053] Columns were performed using (Kieselgel 60, 35-70µm, Fluka) as the stationary phase. Samples were applied as a concentrated solution in the same eluent, or pre-adsorbed onto silica gel.

NMR Spectroscopy

[0054] ^{1}H, ^{13}C and ^{31}P-NMR were recorded on a Bruker Avance DPX300 spectrometer with operating frequencies of 300MHz, 75MHz and 121MHz respectively. ^{31}P-NMR spectra are reported in units of δ relative to 85% phosphoric acid as external standard, positive shifts are downfield. The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad signal), dd (doublet of doublet), dt (doublet of triplet). Starred signal signal are splitted due to stereoisomeric mixtures.

Standard procedures

[0055] For practical purposes, standard procedures are given where applicable.

Standard procedure 1: Synthesis of Amino ester hydrochloride salts.

[0056] To a stirring solution of anhydrous alcohol (10 mol eq.) was added thionyl chloride (2 mol eq.) at 0° C, and the resulting solution stirred for 1 hr. After warming to room temperature, the appropriate amino acid (1 mol eq) was added and the reaction heated at reflux for 6-16 hrs. Removal of solvent and recrystallisation from methanol/ether gave the amino ester hydrochloride salts.

Standard procedure 2: Synthesis of Amino benzyl ester hydrochloride salts.

[0057] The appropriate amino acid (1.0 mol eq.), p-toluene sulfonic acid (1.0 mol eq.) and anhydrous benzyl alcohol (4.1 mol

eq.) were heated at reflux in toluene (10 mol eq.) with Dean-Stark trap for 24 hrs. On cooling to room temperature, Et_2O was added and the mixture was left in ice bath for 1hr then filtrated and washed with Et_2O. The solid was dissolved in DCM and washed with 10% K_2CO_3 and water. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give an oil. This was solubilized in acetone and neutralized with 1 M HCl. Et_2O was added and the solid was filtered and washed with Et_2O to give a white solid.

Standard procedure 3: Synthesis of Phosphorodichloridate species.

[0058] Phosphorus oxychloride (1.0 mol eq.) and the appropriate substituted phenol (1.0 mol) were stirred with anhydrous diethylether (31 mol eq.). To this was added anhydrous triethylamine (1.0 mol eq) at -80 °C and left to rise to room temperature over 16 hrs. the triethylamine hydrochloride salt was filtered off, and the filtrate reduced to dryness to give the crude product as a clear liquid.

Standard procedure 4: Synthesis of Phosphochloridate species.

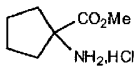
[0059] Phosphodichloridate (1.0 mol eq.) and the appropriate amino ester hydrochloric salt (1.0 mol eq.) were suspended in anhydrous DCM. Anhydrous triethylamine was added dropwise at -80 °C and after 1hr the reaction was left to rise to room temperature. The formation of phosphochloridate was monitored by ^{31}P-NMR. After 2-5 hrs the solvent was removed under reduced pressure and the solid obtained washed with anhydrous ether (2x20 ml), filtered, and the filtrate reduced to dryness to give the products as crude oil. These oils were usually used without further purification.

Standard procedure 5: Synthesis of Phosphoroamidate derivatives.

[0060] To a stirring solution of (E)-5-(2-bromovinyl)-2'-deoxyuridine (1.0 mol eq.) and the appropriate phosphochloridate (2.0-3.0 mol eq) in anhydrous THF at -80°C was added dropwise over 1 min NMI (5.0 mol eq.). After 15 mins the reaction was left to rise to room temperature and stirred at room temperature for 2-19 hrs. The solvent was removed under reduced pressure and the yellow oil obtained was dissolved in DCM, washed with 0.5 M HCl, and water. The organic layer is dried over $MgSO_4$, filtered, reduced to dryness and purified by flash chromatography (Chloroform/Methanol 97/3, Dichloromethane/Methanol 97/3).

Synthesis of Methyl-1-amino-1-cyclopentanoate hydrochloride salt. $C_6H_{14}ClNO_3$, MW=179.68.

[0061]



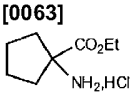
[0062] This was synthesised according to ***Standard Procedure 1,*** using 1-amino-1-cyclopentanecarboxylic acid (3.876 g, 30 mmol) with thionyl chloride (4.44 mL, 45 mmol,) and anhydrous methanol (15.5 mL). The product was isolated as a white solid (4.81 g, yield 89%).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.1 (3H, bs, $N\underline{H_3}^+Cl^-$), 3.85 (3H, s, $OC\underline{H_3}$), 2.3-2.2 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.6 ($2CH_2$ cyclopent), 38.1 ($2CH_2$ cyclopent), 54.8 ($\underline{C}H_3O$), 66.6 ($\underline{C_q}$ cyclopentane), 174.1 ($\underline{C}OOMe$).

Synthesis of Ethyl-1-amino-1-cyclopentanoate hydrochloride salt. $C_8H_{16}ClNO_2$, MW=193.71.

[0063]



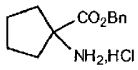
[0064] This was synthesised according to ***Standard Procedure 1,*** using 1-amino-1-cyclopentanecarboxylic acid (5.0 g, 38.6 mmol) with thionyl chloride (5.72 mL, 58 mmol) and anhydrous ethanol (29 mL). The product was isolated as a white solid (6.98 g, yield 93%).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.0 (3H, bs, N$\underline{H}_3^+Cl^-$), 4.3 (2H, q, ^3J=8, OC$\underline{H}_2$CH$_3$), 2.3-2.2 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane), 1.4 (3H, t, ^3J=8, OCH$_2$C$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 ($\underline{C}H_3CH_2$), 25.8 (2CH$_2$ cyclopent), 37.4 (2CH$_2$ cyclopent), 63.0 (CH$_3\underline{C}H_2$), 66.2 ($\underline{Cq}$ cyclopentane), 172.1 ($\underline{C}$OOEt).

Synthesis of Benzyl-1-amino-1-cyclopentanoate hydrochloride salt. $C_{14}H_{18}ClNO_2$, MW=255.78.

[0065]

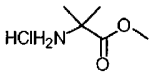


[0066] This was synthesised according to ***Standard Procedure 2,*** using 1-amino-1-cyclopentanecarboxylic acid (3.682 g, 28.5 mmol) with *p*-toluene sulfonic acid monohydrate (5.625 g, 29.55 mmol) and anhydrous benzylic alcohol (12 mL, 116 mmol), in Toluene (20 mL). The product was isolated as a white solid (6.441 g, yield 88.5%) **Hydrochloride salt.** ^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.05 (3H, bs, N$\underline{H}_3^+Cl^-$), 7.4-7.25 (5H, m, Ph), 5.15 (2H, s, CH$_2\underline{Ph}$), 2.3 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.9 (2CH$_2$ cyclopent), 37.3 (2CH$_2$ cyclopent), 66.3 ($\underline{Cq}$ cyclopentane), 68.3 ($\underline{C}$H$_2$Ph), 129.2, 129.0, 128.8 ('*o*', '*m*', CH$_2\underline{Ph}$), 135.5 ('*p*', CH$_2\underline{Ph}$), 172.1 ($\underline{C}$OOBn).

Synthesis of methyl-2-amino-2-methylpropanoate hydrochloride salt $C_5H_{12}ClNO_3$, MW 153.61.

[0067]



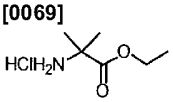
[0068] This was synthesised according to ***Standard Procedure 1,*** using 2-amino-isobutyric acid (5.102 g, 48.49 mmol) with thionyl chloride (11.538 g, 96.98 mmol, 7.04 mL) and anhydrous methanol (19.6 mL). The product was isolated as a white solid (6.636 g, yield 89.2%).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.81 (3H, bs, N$\underline{H}_3$Cl), 3.83 (3H, s, OC$\underline{H}_3$), 1.74 (6H, s, [C$\underline{H}_3$]$_2$C).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.1, 24.3 ([$\underline{C}$H$_3$]$_2$C), 57.9 ($\underline{C}$[CH$_3$]$_2$), 172.4 ($\underline{C}$OOCH$_3$).

Synthesis of ethyl-2-amino-2-methylpropanoate hydrochloride salt. $C_6H_{14}ClNO_2$, MW 167.63.

[0069]



[0070] This was synthesised according to ***Standard Procedure 1,*** using 2-amino-isobutyric acid (5.102 g, 48.49 mmol) with thionyl chloride (11.772 g, 98.95 mmol, 7.2 mL) and anhydrous ethanol (29 mL). The product was isolated as a white solid (7.159 g, yield 86.3%).

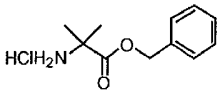
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.93 (3H, bs, N$\underline{H}_3$Cl), 4.3 (2H, q, ^3J =7.1 Hz, OC$\underline{H}_2$CH$_3$), 1.75 (6H, s, [C$\underline{H}_3$]$_2$C), 1.33 (3H, t, ^3J=7.1

Hz, $OCH_2C\underline{H}_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 ($\underline{C}H_3CH_2O$), 24.3 ($[\underline{C}H_3]_2C$), 57.9 ($\underline{C}[CH_3]_2$), 63.1 ($O\underline{C}H_2CH_3$), 171.6 ($\underline{C}OOCH_2CH_3$).

Synthesis of benzyl-2-amino-2-methylpropanoate hydrochloride salt. $C_{11}H_{16}ClNO_2$, MW 229.70.

[0071]



[0072] This was synthesised according to ***Standard Procedure 2,*** using 2-amino-isobutyric acid (1.960 g, 19.00 mmol) with *p*-toluene sulfonic acid monohydrate (3.750g, 19.7 mmol) and benzylic alcohol (8.360 g, 77.30 mmol, 8 mL), in toluene (20 mL). The product was isolated as a white solid (2.556 g, yield 87.4%)

[0073] ***p*-toluenesulfonate salt:** ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.40 (3H, bs, $N\underline{H}_3Cl$), 7.79 (2H, d, ^3J=8.0 Hz, '*m*' *p*-TSA), 7.34 (5H, m, $CH_2\underline{Ph}$), 7.14 (2H, d, ^3J=8.0 Hz, 'o' p-TSA), 5.16 (2H, s, $C\underline{H}_2Ph$), 2.38 (3H, s, $C\underline{H}_3$ *p*-TSA), 1.57 (6H, s, $[C\underline{H}_3]_2C$)

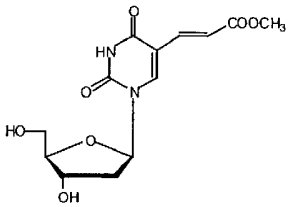
^{13}C-NMR ($CDCl_3$; 75 MHz): δ 21.8 ($\underline{C}H_3$, *p*-TSA), 23.9 ($[\underline{C}H_3]_2C$), 57.8 ($\underline{C}[CH_3]_2$), 68.3 ($\underline{C}H_2Ph$), 126.55, 128.5, 128.8, 129.0, 129.3 ($CH_2\underline{Ph}$+*p*-TSA), 135.4 ('*ipso*', $CH_2\underline{Ph}$), 140.8 ('*p*',*p*-TSA), 141.9 ('*ipso*', *p*-TSA), 171.9 ($\underline{C}OOCH_2Ph$).

Hydrochloride salt: ^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.10 (3H, bs, $N\underline{H}_3$-Cl), 7.41-7.31 (5H, m, $CH_2\underline{Ph}$), 5.27 (2H, s, $CH_2\underline{Ph}$), 1.77 ($[C\underline{H}_3]_2C$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.2 ($[\underline{C}H_3]_2C$), 58.0 ($\underline{C}[CH_3]_2$), 68.5 ($\underline{C}H_2Ph$), 128.62, 129.0, 129.1 (`*o*', `*m*', *p*', $CH_2\underline{Ph}$), 135.2 ('*ipso*', $CH_2\underline{Ph}$), 171.8 ($\underline{C}OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine **(E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine**

[0074]



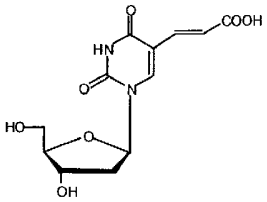
[0075] A mixture of $Pd(OAc)_2$ (0.316 g, 1.41 mmol), PPh_3 (0.741 g, 2.82 mmol), and triethylamine (4.9 mL) in 1,4-dioxane (50 mL) was stirred at 70°C until an intense red colour had developed. To this 5-iodo-2'-deoxyuridine (10 g, 28.24 mmol) and methylacrilate (4.862 g, 56.48 mmol, 5.1 mL) in 1,4-dioxane (20 mL) were added and the mixture stirred at refluxed for 30 mins. The reaction was filtered while still hot and the filtrate cooled over night at 4°C. The resulting pale yellow precipitate was filtered, washed with DCM and dried *in vacuo* to give the product as white solid (6.2 g, yield 70.7%).

^{1}H-NMR (DMSO-d_6; 300 MHz) δ 11.64 (1H, bs, N$\underline{H}$-3), 8.42 (1H, s, H-6), 7.37 (1H, d, ^3J=15.8 Hz, H vinylic), 6.86 (1H, d, ^3J=15.8 Hz, H vinylic), 6.13 (1H, t, ^3J=6.5 Hz, H-1'), 5.27-5.20 (2H, 2bs, OH-3', OH-5'), 4.27 (1H, m, H-3'), 3.81 (1H, m, H-4'), 3.68 (3H, s, $C\underline{H}_3$), 3.60 (2H, m, H-5'), 2.18 (2H, m, H-2').

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.4 (C-2'), 51.6 (CH_3), 66.7 (C-5'), 70.0 (C-3'), 85.2 (C-4'), 88.0 (C-1'), 108.5 (C-5), 116.5 (C-5b), 138.5 (C-5a), 144.4 (C-6), 149.6, 162.1 (C-2, C-4), 167.6 (COO).

(E)-5-(2-Carboxyvinyl)-2'-deoxyuridine

[0076]



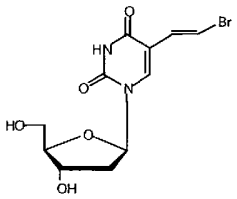
[0077] (E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (6.0 g, 19.33 mmol) was dissolved in 300 mL of 1 M NaOH and the mixture stirred at room temperature for 3 hrs, filtered and the filtrate adjusted to pH 2 with 1M HCl. On cooling at 4°C a white precipitate formed. This was filtered off and washed with cold water (2x 20 ml) and acetone (2x20 mL) and dred to give a white solid (4.441 g, yield 77.1 %).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 12.18 (1H, bs, $CO_2\underline{H}$), 11.64 (1H, s, N$\underline{H}$-3), 8.40 (1H, s, H-6), 7.30 (1H, d, ^3J=15.6 Hz, H vinylic), 6.78 (1H, d, ^3J=15.8 Hz, H vinylic), 6.14 (1H, t, ^3J=6.4 Hz, H-1'), 5.38 -5.08 (2H, bs, OH-3', OH-5'), 4.26 (1H, m, H-3'), 3.80 (1H, m, H-4'), 3.64 (2H, m, H-5'), 2.18 (2H, m, H-2').

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.1 (C-2'), 61.2 (C-5'), 70.1 (C-3'), 85.1 (C-4'), 88.0 (C-1'), 108.7 (C-5), 118.0 (C-5b), 137.9 (C-5a), 143.9 (C-6), 149.6, 162.1 (C-2, C-4), 168.4 (COOH).

(E)-5-(2-bromovinyl)-2'-deoxyuridine

[0078]



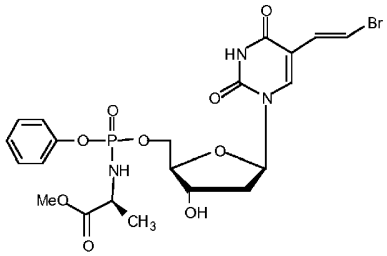
[0079] To a solution of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine (5.777 g, 19.37 mmol) in dimethylforamide (29 mL) was added K_2CO_3 (5.890 g, 42.61 mmol) and the suspension stirred at room temperature for 15 mins. A solution of N-bromosuccinimide (3.655 g, 20.53 mmol) was added dropwise over 30 mins at 20°C. The resulting suspension was filtered and the solid washed with DMF. The combined filtrate and washings were evaporated to dryness *in vacuo* and the residue dissolved in MeOH. To this silica gel was added and the suspension evaporated to dryness and the solid applied to the top of chromatographic column. The column was eluted with chloroform/methanol 92/8 to give a white solid (5787g, 71.9%). Crystallisation from water gave a white powder.

^{1}H-NMR (DMSO-d_6; 300 MHz) δ 11.59 (1H, bs, NH-3), 8.08 (1H, s, H-6), 7.25 (1H, d, ^3J=13.6 Hz, H-5b), 6.85 (1H, d, ^3J=13.6 Hz, H-5a), 6.13 (1H, t, ^3J=6.5 Hz, H-1'), 5.29 (1H, bs, OH-3'), 5.13 (1H, bs, OH-5'), 4.24 (1H, m, H-3'), 3.79 (1H, m, H-4'), 3.66 (2H, m, H-5'), 2.51 (1H, m, H-2'), 2.14 (1H, m, H-2').

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.2 (C-2'), 61.3 (C-5), 70.3 (C-4'), 84.8 (C-3'), 87.8 (C-1'), 108.9 (C-5b), 110.0 (C-5), 130.3 (C-5a), 149.6, 162.1 (C-2, C4).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-L-alaninyl)]-phosphate (CPF 1). (Reference example) $C_{21}H_{25}BrN_3O_9P$, MW 574.32.

[0080]



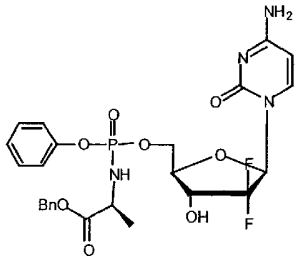
[0081] This was synthesised according to ***Standard procedure 5***, using BVdU (300 mg, 0.90 mmol), Phenyl-(methoxy-L-alaninyl)-phosphorochloridate (472 mg, 1.7 mmol), NMI (4.5 mmol, 378 µL) in THF (9 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_2Cl_2/Methanol 97:3 to give the pure product as a white foamy solid (356 mg, yield 69%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.72, 4.40.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.9 (1H, bs, H-3), 7.64 (1H, 2xs, H-6), 7.44-7.39 (1H, 2d, ^{3}J=14 Hz, H-5b), 7.37-7.15 (5H, m, O*Ph*), 6.75-6.67 (1H, 2d, ^{3}J=14 Hz, H-5a), 6.30-6.21 (1H, 2t, ^{3}J=6 Hz, H1'), 4.57-4.29 (3H, m, H-5'+H-3'), 4.2-3.96 (3H, H-4', NH, CHala), 3.72 (3H, s, CH_3O), 2.49-2.40 (1H, m, one of H-2'), 2.12-2.01 (1H, m, one of H-2'), 1.38 (3H, d, ^{3}J=7 Hz, $CH_{3\ ala}$). ^{13}C-NMR (DMSO; 75 MHz): δ 22.4 ($CH_{3\ ala}$), 41.9, 41.8 (C-2'), 51.9 (*CH*[CH_3]), 54.3 (*C*H_3O), 67.5 (C-5'), 72.3, 71.9 (C-3'), 87.3, 87.2, 86.9, 86.8 (C-1', C-4'), 110.6 (C-5b), 113.1 (C-5), 121.7 ('*o*', O*Ph*), 127.0 (`*p*', O*Ph*), 130.1 (C-5a), 131.5 ('*m*', O*Ph*), 139.2 (C-6), 150.9 ('*ipso*', O*Ph*) 151.9 (C-4), 163.2(C-2), 175.7 (*C*OOCH$_3$).

Synthesis of Gemcitabine-[phenyl-(benzoxy-L-alaninyl)]-phosphate. $C_{25}H_{27}F_2N_4O_8P$, MW=580.47 (CPF 31).

[0082]



[0083] This was synthesised according to ***Standard procedure 5,*** using gemcitabine (131 mg, 0.5 mmol), Phenyl-(benzoxy-L-alaninyl)-phosphorochloridate (529 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/2 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_2Cl_2/Methanol 95:5 to give the pure product as a white foamy solid (46 mg, yield 16%).

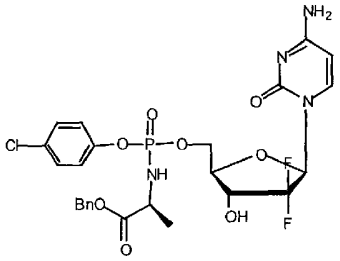
^{31}P-NMR (MeOD, 121 MHz): δ 5.05, 4.94.

^{1}H-NMR (MeOD, 300 MHz): δ 7.6-7.5 (1H, 2d, ^{3}J=7Hz H-6), 7.4-7.2 (10H. m, O*Ph*+CH_2*Ph*), 6.25 (1H, m, H-1'), 5.95 (1H, 2d, ^{3}J=7Hz, H-5), 5.19 (1H, 2s, C*H*$_2$Ph), 4.55-4.1(3H, m, H-3', H-4', CHala), 4.05 (2H, m, H-5'), 1.20 (3H, 2t, ^{3}J=6 Hz, CH_3ala).

^{13}C-NMR (MeOD, 75 MHz): δ 20.8, 20.7 (CH_3ala), 52.2, 52.0 (*C*Hala), 66.1 (C-5'), 68.4 (*C*H_2Ph), 71.9, 71.3 (C-3'), 80.6 (C-4'), 85.9 (C-1'), 97.1 (C-5), 121.8, 121.6 ('o', O*Ph*), 123 (C-2'), 126.2 ('*p*', O*Ph*), 131.8, 130.0, 129.7 ('*m*' OPh, Bn), 137.9('*ipso*', CH_2*Ph*), 142.7, 142.6 (C-6), 152.5, 152.4 (*'ipso'*, O*Ph*), 158.2 (C-2), 168.0 (C-4), 175.3, 174.9 (*C*OOBn).

Synthesis of Gemcitabine-[para-chlorophenyl-(benzoxy-L-alaninyl)]-phosphate. $C_{25}H_{26}ClF_2N_4O_8P$, MW=614.92 (CPF 40).

[0084]



[0085] This was synthesised according to ***Standard procedure 5,*** using gemcitabine (131 mg, 0.5 mmol), para-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate (582 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/2 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_2Cl_2/Methanol 95:5 to give the pure product as a white foamy solid (76 mg, yield 25%).

^{31}P-NMR (MeOD, 121 MHz): δ 5.08.

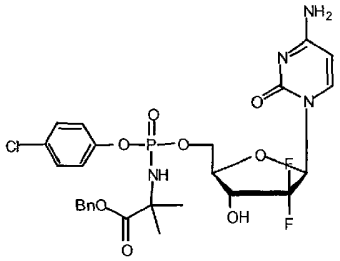
^{1}H-NMR (MeOD, 300 MHz): δ 7.65 (1H, 2d, ^{3}J=7Hz H-6), 7.5-7.2 (9H. m, O*Ph*+CH_2*Ph*), 6.2 (1H, m, H-1'), 5.9 (1H, 2d, ^{3}J=7Hz, H-5), 5.12 (1H, 2s, C*H*$_2$Ph), 4.6-4.1 (3H, m, H-3', H-4', CHala), 4.05 (2H, m, H-5'), 1.45-1.35 (3H, 2t, ^{3}J=6 Hz, CH_3ala).

^{13}C-NMR (MeOD, 75 MHz): δ 20.9, 20.7 (CH_3ala), 52.2, 52.0 (*C*Hala), 66.4, 66.2 (C-5'), 68.5 (*C*H_2Ph), 71.5 (C-3'), 80.7 (C-4'), 86.4 (C-1'), 97.2 (C-5), 123.5 ('o', O*Ph*), 126.9 (C-2'), 131.2, 130.6, 130.3 ('*m*' OPh, Bn), 131.9 (`*p*', O*Ph*) 137.5 ('*ipso'*, CH_2*Ph*), 142.8, 142.7 (C-6), 151.4, 151.0 ('*ipso'*, O*Ph*), 158.2 (C-2), 166.9 (C-4), 175.1, 174.9 (*C*OOBn).

Synthesis of Gemcitabine-[para-chlorophenyl-(benzoxy-α,α-dimethylglycinyl)]-phosphate (CPF 41).

$C_{26}H_{28}ClF_2N_4O_8P$, MW=628.95.

[0086]



[0087] This was synthesised according to ***Standard procedure 5,*** using gemcitabine (131 mg, 0.5 mmol), para-chlorophenyl-(benzoxy-α,α-dimethylglycinyl)-phosphorochloridate (603 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/3 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_2Cl_2/Methanol 95:5 to give the pure product as a white foamy solid (163 mg, yield 52%).

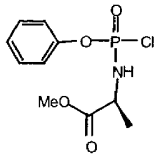
^{31}P-NMR (MeOD, 121 MHz): δ 3.56, 3.52.

^{1}H-NMR (MeOD, 300 MHz): δ 7.55 (1H, 2d, ^{3}J=7Hz, H-6), 7.4-7.15 (9H. m, O*Ph*+CH_2*Ph*), 6.25 (1H, m, H-1'), 5.85 (1H, 2d, ^{3}J=7Hz, H-5), 5.15 (1H, 2s, C*H*$_2$Ph), 4.55-4.1(3H, m, H-3', H-4'), 4.05 (2H, m, H-5'), 1.50 (6H, m, ^{3}J=6 Hz, 2CH_3dimethygly). ^{13}C-NMR (MeOD, 75 MHz): δ 28.2, 28.0 (CH_3 dimethygly), 58.6 (*C*q dimethygly), 66.2, 66.1 (C-5'), 66.7 (*C*H_2Ph), 71.5 (C-3'), 80.6 (C-4'), 86.4 (C-1'), 97.0 (C-5), 123.9, 123.6 ('o', O*Ph*), 127.3 (C-2'), 130.0, 129.7 ('*m*' OPh, Bn), 131.8 ('*p*', O*Ph*), 137.6 ('*ipso*', CH_2*Ph*), 142.8, 142.7 (C-6), 151.2, 151.1 *('ipso', OPh*), 158.1 (C-2), 167.9 (C-4), 176.8, 176.7 (*C*OOBn).

Synthesis of Phenyl-(methoxy-L-alaninyl)-phosphorochloridate.

$C_{10}H_{13}ClNO_4P$, MW=277.64.

[0088]



[0089] This is synthesised according to ***Standard procedure 4,*** using L-alanine methyl ester hydrochloride (2 g, 14.3 mmol), phenyldichlorophosphate (3.02 g, 2.14 ml, 14.3 mmol), and TEA (2.9 g, 4.0 ml, 28.7 mmol) in DCM (60 mL), to yield 3.91 g (98%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.28, 8.97.

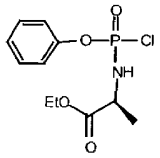
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.39-7.34 (2H, m ,'o' O*Ph*), 7.29-7.20 (2H, m ,'m+p' O*Ph*), 4.98 (1H, bs, N*H*), 4.27-4.09 (1H, m, CHala), 3.78 (3H, s, OC*H*$_3$), 1.52-1.49 (3H, 2xd, ^{3}J=7Hz, C*H*$_3$ala).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.9 (*C*H$_3$ala), 51.0 (*C*Hala), 53.6 (OC*H*$_3$), 120.9 ('*o*' OPh), 126.4 ('*p*', OPh), 130.2 ('*m*', OPh), 150.1 ('*ipso*', OPh), 173.6(*C*OOCH$_3$).

Synthesis of Phenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

$C_{11}H_{15}ClNO_4P$, MW=291.67.

[0090]



[0091] This is synthesised according to ***Standard procedure 4,*** using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), phenyldichlorophosphate (1.12g, 5.01 mmol, 749 µL), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.02 (69%) of oil. ^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.49, 9.07.

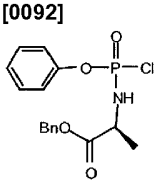
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.39-7.34 (2H, m,'o' O*Ph*), 7.29-7.20 (2H, m, 'm+p' O*Ph*), 4.95 (1H, bs, N*H*), 4.3-4.1 (3H, m, OC*H*$_2$CH$_3$, CHala), 1.50 (3H, 2xd, ^{3}J=7Hz, C*H*$_3$ala), 1.30 (3H, t, ^{3}J=7.1 Hz, OCH$_2$CH$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (*C*H$_3$CH$_2$), 20.9 (*C*H$_3$ala), 51.0 (*C*Hala), 62.6 CH$_3$*C*H$_2$), 120.9 ('*o*' OPh), 126.5 ('*p*', OPh), 130.1 ('*m*', OPh), 150.1 ('*ipso*', OPh), 175.1 (*C*OOCH$_2$CH$_3$).

Synthesis of Phenyl-(benzoxy-L-alaninyl)-phosphorochloridate.

$C_{16}H_{17}ClNO_4P$, MW= 353.74.

[0092]



[0093] This is synthesised according to ***Standard procedure 4,*** using L-alanine benzyl ester hydrochloride (1.0 g, 4.64 mmol), phenyl-dichlorophosphate (980 mg, 0.69 ml, 4.64 mmol), and TEA (0.94 g, 1290 µL, 9.27 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 1.61 (98%) of oil.

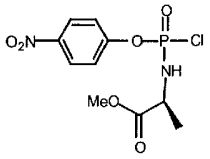
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.41, 9.23.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41-7.21 (10H, m, O*Ph*+*CH$_2$Ph*), 5.24 (2H, s, *CH$_2$*Ph), 4.95-4.88 (1H, bs, N*H*), 4.36-4.15 (1H, m, CHala), 1.52-1.49 (3H, 2xd, ^{3}J=7Hz, C*H$_3$*ala). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8 (*C*H$_3$ala), 51.1 (*C*Hala), 68.0 (*C*H$_2$Ph), 121.0 ('*o*' OPh), 126.4 ('*p*', OPh), 130.3, 129,0, 128.7 ('*m*' OPh, CH$_2$*Ph*), 135.5 ('*ipso*', CH$_2$*Ph*), 150.2 ('*ipso*', OPh), 172.9 (*C*OOCH$_2$Ph).

Synthesis of p-nitrophenyl-(methoxy-L-alaninyl)-phosphorochloridate.

$C_{10}H_{12}ClN_2O_6P$, MW=322.64.

[0094]



[0095] This is synthesised according to ***Standard procedure 4,*** using L-alanine methyl ester hydrochloride (0.70 g, 5.01 mmol), p-nitrophenyldichlorophosphate (1.362 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.60 g (99%) of crude product used without further purification.

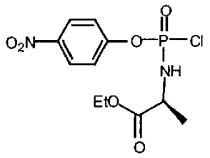
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.13, 9.03.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.1 (2H, 2d, ^{3}J=8Hz, O*Ph*), 7.3 (2H, 2d, ^{3}J=8Hz, O*Ph*), 5.0 (1H, bs, N*H*), 4.1 (1H, m, CHala), 3.75 (3H, s, OC*H$_3$*), 1.5-1.45 (3H, m, C*H$_3$*ala). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8, 20.7 (*C*H$_3$ala), 51.1, 50.9 (*C*Hala), 53.2, 53.2 (O*C*H$_3$), 121.8, 121.6 ('*o*' OPh), 126.5 *('m',* OPh), 145.7 ('*ipso*', OPh), 154.7, 154.6 ('*p*', OPh), 173.4, 173.2 (*C*OOCH$_3$).

Synthesis of p-nitrophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

$C_{11}H_{14}ClN_2O_6P$, MW=336.67.

[0096]



[0097] This is synthesised according to ***Standard procedure 4,*** using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), p-nitrophenyldichlorophosphate (1.362g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL), to yield 1.64 g (98%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.06, 8.81.

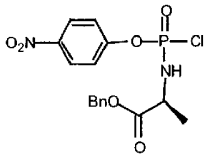
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.1 (2H, m, O*Ph*), 7.4 (2H, m, O*Ph*), 4.9-4.7 (1H, bs, N*H*), 4.3-4.1 (3H, m, OC*H$_2$*CH$_3$, CHala), 1.55-1.45 (3H, 2xd, ^{3}J=7Hz, C*H$_3$*ala), 1.40 (3H, t, ^{3}J=7Hz, OCH$_2$C*H$_3$*).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (<u>C</u>H_3CH_2), 21.1, 20.9 (<u>C</u>H_3ala), 51.2, 51.0 (<u>C</u>Hala), 62.6_CH_3<u>C</u>H_2), 121.7, 121.3 ('*o*' OPh), 126.2, 126.0 ('*m*', OPh), 145.7 ('*ipso*', OPh), 154.5 ('*p*', OPh), 173.4, 173.3 (<u>C</u>OOCH_2CH_3).

Synthesis of p-nitrophenyl-(benzoxy-L-alaninyl)-phosphorochloridate.

$C_{16}H_{16}ClN_2O_6P$, MW= 398.04.

[0098]



[0099] This is synthesised according to ***Standard procedure 4,*** using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-nitrophenyl-dichloro phosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL), to yield 1.85 g (93%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.15, 9.06.

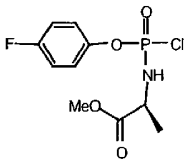
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.15 (2H, m , O<u>*Ph*</u>), 7.45 (2H ,m ,O<u>*Ph*</u>), 7.35-7.25 (5H, m, CH_2<u>*Ph*</u>), 5.2 (2H, 2s, <u>CH_2</u>Ph), 5.00 (1H, bs, N<u>*H*</u>), 4.2 (1H, m, CHala), 1.64 (3H, 2xd, ^{3}J=7Hz, C<u>H_3</u>ala).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8 (<u>C</u>H_3ala), 51.1 (<u>C</u>Hala), 68.0 (<u>C</u>H_2Ph), 121.4 ('*o*' OPh), 126.1 ('*m*'OPh), 130.3, 129.0 (CH_2<u>*Ph*</u>), 145.7 ('*ipso*', CH_2Ph), 150.2 ('*iso*', OPh), 154.6 ('*p*', OPh), 172.9 (<u>C</u>OOCH_2Ph).

Synthesis of p-fluorophenyl-(methoxy-L-alaninyl)-phosphorochloridate.

$C_{10}H_{12}ClFNO_4P$, MW=295.63.

[0100]



[0101] This is synthesised according to ***Standard procedure 4,*** using L-alanine methyl ester hydrochloride (0.70 g, 5.01 mmol), p-fluorophenyldichlorophosphate (1.210 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.11 g (75%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.98, 9.96.

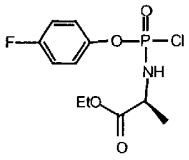
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.1 (2H, m, O<u>*Ph*</u>), 6.95 (2H, m, O<u>*Ph*</u>), 5.0 (1H, bs, N<u>*H*</u>), 4.25-4.1 (1H, m, CHala), 3.78 (3H, 2s, OC<u>*H_3*</u>), 1.55 (3H, m, C<u>*H_3*</u>ala).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8 (<u>C</u>H_3ala), 51.1, 50.9 (<u>C</u>Hala), 53.3 (O<u>C</u>H_3), 117.1, 117.0 ('*o*' OPh), 122.6, 122.5 ('*m*', OPh), 146.0 ('*iso*', OPh), 159.1, 159.0 ('*p*', OPh), 173.4, 173.2 (<u>C</u>OOCH_3).

Synthesis of p-fluorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

$C_{11}H_{14}ClFNO_4P$, MW=309.66.

[0102]



[0103] This is synthesised according to ***Standard procedure 4,*** using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), p-fluorophenyldichlorophosphate (1.210g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.07 (69%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.04, 9.95.

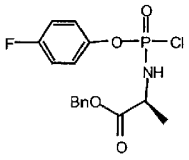
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.1 (2H, m, O*Ph*), 6.95 (2H , m, O*Ph*), 5.0 (1H, bs, N*H*), 4.25-4.1 (3H, m, O*C*$H_2$$CH_3$, CHala), 1.55 (3H, m, C$H_3$ala), 1.40 (3H, t, ^{3}J=7Hz, OCH_2CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (CH_3CH_2), 21.1, 21.0 (CH_3ala), 51.2, 51.1 (*C*Hala), 62.6_CH_3*C*H_2), 117.3 ('*o*' OPh), 122.2, 122.0 ('*m*', OPh), 145.9, 145.8 ('*ipso*', OPh), 159.0 ('*p*', OPh), 173.6, 173.5 (*C*$OOCH_2CH_3$).

Synthesis of p-fluorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate.

$C_{16}H_{16}ClFNO_4P$, MW= 371.73.

[0104]



[0105] This is synthesised according to ***Standard procedure 4,*** using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-fluorophenyl-dichloro phosphate (1.210 mg, 5.01 mmol), and TEA (1.4mL, 1.4 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.599 (86%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.15, 9.06.

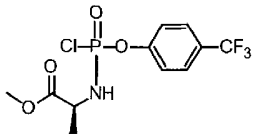
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.35-7.25 (5H, m, CH_2*Ph*), 7.1 (2H, m, O*Ph*), 6.95 (2H ,m , O*Ph*), 5.2 (2H, 2s, *C*H_2Ph), 5.00 (1H, bs, N*H*), 4.25-4.1 (1H, m, CHala), 1.55 (3H, m, CH_3ala).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8 (*C*H_3ala), 51.1, 51.0 (*C*Hala), 68.1 (*C*H_2Ph), 117.0, 116.9 ('*o*' OPh), 122.6 ('*m*'OPh), 130.3, 129.0 (CH_2*Ph*), 135.7 ('*iso*', CH_2*Ph*), 146.1, 146.0('*ipso*', OPh), 158.9 ('*p*', OPh), 173.1 (*C*$OOCH_2$Ph).

Synthesis of 4-(trifluoromethyl)-phenyl-(methoxy-L-alaninyl)-phosphorochloridate.

$C_{11}H_{12}ClF_3NO_4P$, MW=345.64.

[0106]



[0107] This is synthesised according to ***Standard procedure 4,*** using L-alanine methyl ester hydrochloride (1.0 g, 7.16 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (1.998 g, 7.16 mmol), and TEA (1.449 g, 14.32 mmol, 1916 µL) in DCM (30 mL), to yield 2.202 g (89.0%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.36, 9.22.

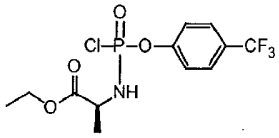
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.66 (2H, d, ^{3}J=8.1 Hz, *OPh),* 7.44-7.33 (2H, m, O*Ph*), 5.10 (1H, bs, N*H*), 3.81-3.78 (3H, 2s, CH_3O), 3.77-3.68 (1H, m, CH_3CH), 1.56-1.52 (3H, m, $CHCH_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.6, 20.7 (CH_3CH), 50.9, 51.1 ($CHCH_3$), 53.2 (CH_3O), 121.4 ('*o*', O*Ph*), 124.1 (CF_3, *J*=270 Hz), 128.0 ('*m*', O*Ph*), 128.6 (*'p', J*=34 Hz), 152.4, 152.6 ('*ipso*', O*Ph*), 173.4, 173.5 ($COOCH_3$).

Synthesis of 4-(trifluoromethyl)-phenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

$C_{12}H_{14}ClF_3NO_4$, MW=359.67.

[0108]



[0109] This is synthesised according to *Standard procedure 4*, using L-alanine ethyl ester hydrochloride (1.0 g, 6.50 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (1.813 g, 6.50 mmol), and TEA (1.316 g, 13.00 mmol, 1740 µL) in DCM (30 mL), to yield 2.150 g (92.2%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.33, 9.28.

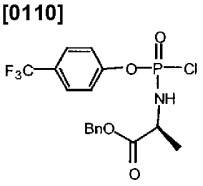
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.70 (2H, d, ^{3}J=8.2 Hz, O*Ph*), 7.46-7.39 (2H, m, O*Ph*), 4.78 (1H, bs, N*H*), 4.33-4.17 (3H, m, CH_3CH_2O + $CHCH_3$), 1.59-1.55 (1H, m, $CHCH_3$), 1.56-1.52 (3H, m, CH_2CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (CH_3CH_2O), 20.8, 20.9 (CH_3CH), 50.3, 50.9 ($CHCH_3$), 62.3, 62.5 (CH_3CH_2O), 121.4 (*'o'*, O*Ph*), 124.1 (CF_3, *J*=270 Hz), 127.7 (*'m'*, O*Ph*), 128.7 (*'p', J*=33 Hz), 152.4 (*'ipso'*, O*Ph),* 172.9 ($COOCH_2CH_3$).

Synthesis of p-trifluorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate.

$C_{17}H_{16}ClF_3NO_4P$, MW = 421.73.

[0110]



[0111] This is synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-trifluorophenyl-dichloro phosphate (1.490 mg, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL). The crude was

purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 1.80 (85%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.11, 8.84.

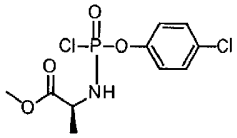
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.65 (2H, m, *OPh)*, 7.4-7.2 (7H, m, *CH_2Ph* + 2H O*Ph*), 5.25 (2H, 2s, CH_2Ph), 4.75-4.55 (1H, bs, N*H*), 4.25-4.1 (1H, m, CHala), 1.60-1.55 (3H, 2d, ^{3}J=7Hz, CH_3ala).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.9 (CH_3ala), 51.3, 51.0 (CHala), 68.2, 68.1 (CH_2Ph), 121.4, 120.9 ('o', O*Ph)*, 125.2 (d, J=270Hz, CF_3), 126.6 (*'m'*, O*Ph*), 129.1, 128.8, 127.8 (Bn), 130.0 ('*p*', q, J=32Hz, O*Ph*), 135.4 (*'ipso'*, CH_2Ph), 153.0 (*'ipso'*, OPh), 172.8 (COOCH_2Ph).

Synthesis of 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate.

$C_{10}H_{12}Cl_2NO_4P$, MW=312.09.

[0112]



[0113] This is synthesised according to ***Standard procedure 4,*** using L-alanine methyl ester hydrochloride (1.0 g, 7.16 mmol), 4-chlorophenylphosphorodichloridate (1.757 g, 7.16 mmol), and TEA (1.449 g, 14.32 mmol, 1995 µL) in DCM (30 mL), to yield 1.621 g (72.5%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.36, 9.07.

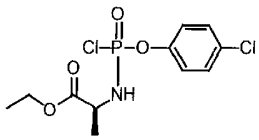
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.35-7.15 (4H, m, O*Ph*), 4.48-4.36 (1H, bs, N*H*), 4.22-4.04 (1H, m, C*H*CH_3), 3.76-3.74 (3H, 2s, CH_3O), 1.49-1.46 (3H, m, CHCH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 21.0 (CH_3CH), 50.8, 51.1 (CHCH_3), 53.4 (CH_3O),121.9, 122.1, 122.3, 122.4 ('*o*', O*Ph*), 130.6, 130.4, 130.2 (*'m'*, O*Ph*), 132.0 (*'p'*, OPh), 148.6 (*'ipso'*, O*Ph*), 173.5 (COOCH_3).

Synthesis of 4-chlorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

$C_{11}H_{14}Cl_2NO_4P$, MW=326.11.

[0114]



[0115] This is synthesised according to ***Standard procedure 4,*** using L-alanine ethyl ester hydrochloride (1.000 g, 6.50 mmol), 4-chlorophenylphosphorodichloride (1.595 g, 6.50 mmol), and TEA (1.315 g, 13.00 mmol, 1810 µL) in DCM (20 mL), to yield 1.794 mg (yield 84.7%) of product.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.54, 9.25.

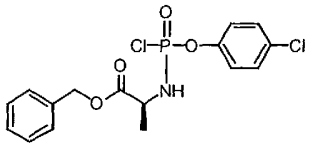
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.44-7.21 (4H, m, O*Ph*), 4.59 (1H, bs, N*H*), 4.33-4.13 (3H, m, OCH_2CH_3+ CHCH_3), 1.57-1.56 (3H, m, CH_3CH), 1.43-1.21 (3H, m, OCH$_2$CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5, 14.6 (OCH_2CH_3), 21.0, 21.5 (CH_3CH), 50.9, 51.2 (CHCH_3), 62.4, 62.5 (OCH_2CH_3), 122.04, 122.3, 122.4 ('*o*', O*Ph*), 130.4 (*'m'*, O*Ph*), 131.9 (*'p'*, O*Ph*), 148.5, 148.6 (*'ipso'*, O*Ph*), 173.0,173.1 (COOCH_2CH_3).

Synthesis of 4-nitrophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{16}H_{16}Cl_2NO_4P$, MW=388.18.

[0116]



[0117] This is synthesised according to ***Standard procedure 4,*** using L-alanine benzyl ester hydrochloride (1.000 g, 4.63 mmol), 4-chlorophenylphosphodichloride (1.136 g, 4.63 mmol), and TEA (937.0 mg, 9.26 mmol, 1290 µL) in DCM (40 mL), to yield 1534 mg (yield 86.5%) of crude product used without further purification.

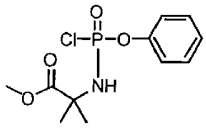
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.43, 9.16.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.08 (9H, m, O*Ph*+ CH_2Ph), 5.19 (2H, s, C*H*$_2$Ph), 4.61-4.54 (1H, bs, N*H*), 4.26-4.10 (1H, m, C*H*CH_3), 1.42-1.38 (3H, m, C*H*$_3$CH). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.9, 21.0 (*C*H_3CH), 51.0, 51.2 (*C*HCH_3), 68.1, 68.2 (O*C*H_2Ph), 122.3, 122.4 (*'o', OPh),* 128.8, 129.1, 130.4 (*'o', 'm', 'p',* CH_2*Ph*+O*Ph*), 131.9 (*'ipso',* CH_2*Ph*), 135.3 (*'p',* O*Ph*), 148.5 (*'ipso',* O*Ph*), 172.7, 172.8 (*C*OOCH_2Ph).

Synthesis of phenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{11}H_{15}ClNO_4P$, MW=291.67.

[0118]



[0119] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate methyl ester hydrochloride (583.5 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (20 mL), to yield 1.041 g (95.2%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.99 (s).

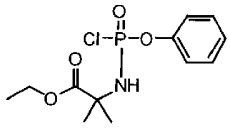
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41-7.17 (5H, m, O*Ph*), 4.98 (1H, bs, N*H*), 3.80 (3H, s, OC*H*$_3$), 1.71-1.69 (6H, 2s, [C*H*$_3$]$_2$C).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.3, 27.2, 27.0 ([*C*H_3]$_2$C), 53.6 (O*C*H_3), 58.8 (*C*[CH_3]$_2$), 120.0, 121.1 ('*o*' O*Ph*), 126.2 (*'p',* O*Ph*), 130.3 (*'m',* O*Ph*) 145.7 (*'p',* O*Ph*), 150.2, 150.3 ('*ipso*', O*Ph*), 175.6, 175.7 (*C*OOCH_3).

Synthesis of phenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{12}H_{17}ClNO_4P$, MW=305.69.

[0120]



[0121] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate ethyl ester hydrochloride (628.6 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (20 mL), to yield 1.018 g (88.8%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.02 (s)

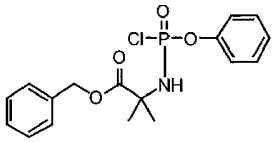
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.23-7.37 (5H, m, *OPh),* 4.98 (1H, bs, N-*H*), 4.24 (2H, q, ^{3}J=7.1 Hz, OCH_2CH_3), 1.70, 1.68 (6H, 2s, $[CH_3]_2C$), 1.30 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (CH_3CH_2O), 27.3, 26.9 ($[CH_3]_2C$), 58.7 ($C[CH_3]_2$), 62.7 (OCH_2CH_3), 121.1, 121.0 (*'o'*, OPh), 127.6 (*'p'*, OPh), 130.7 (*'m'*, OPh), 150.4 (*'ipso'*, O*Ph*), 175.2, 175.1 ($COOCH_2CH_3$).

Synthesis of phenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C17H_{19}ClNO_4P$, MW= 367.76.

[0122]



[0123] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate benzyl ester hydrochloride (861.4 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (30 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 580 mg (42.2%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.79 (s)

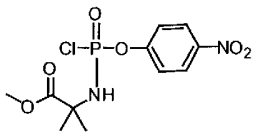
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45-7.27 (10H, m, O*Ph*+CH_2*Ph*), 5.28 (2H, s, CH_2*Ph*), 4.81, 4.78 (1H, 2bs, N*H*), 1.78, 1.75 (6H, 2s, $[CH_3]C$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.3, 26.9 ($[CH_3]C$), 53.9 ($C[CH_3]_2$), 60.9 (CH_2Ph), 121.0, 126.3, 128.6, 129.0, 129.1, 130.3, 135.5 (O*Ph*, CH_2*Ph*), 135.5 ('*ipso*', CH_2*Ph*), 150.3, 150.2 (*'ipso'*, OPh), 175.0, 175.2 ($COOCH_2Ph$).

Synthesis of 4-nitrophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{11}H_{14}ClN_2O_6P$, MW=336.67.

[0124]



[0125] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate methyl ester hydrochloride (290.0mg, 1.89 mmol), 4-nitrophenylphosphodichloride (483.3 mg, 1.89 mmol), and TEA (382.5 mg, 3.78 mmol, 526.9 µL) in DCM (15 mL), to yield 486 mg (yield 76.4%) of crude product used without further purification.

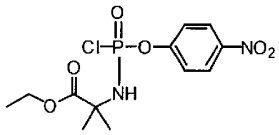
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.61 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.25 (2H, d , ^{3}J=9.0 Hz, OPh), 7.43 (2H, d, ^{3}J=9.0 Hz, OPh), 4.91-4.87 (1H, 2bs, NH), 3.79 (3H, s, OCH_3), 1.69-1.66 (6H, 2s, $[CH_3]_2C$). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.1, 27.3 ($[CH_3]_2C$), 53.8 (OCH_3), 59.2 ($C[CH_3]_2$), 121.7, 121.8 (*'o'* OPh), 126.2 (*'m', OPh),* 145.7 (*'p',* OPh), 154.8, 154.7 (*'ipso',* OPh), 175.4, 175.6 ($COOCH_3$).

Synthesis of 4-nitrophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{12}H_{16}ClN_2O_6P$, MW=350.69.

[0126]



[0127] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate ethyl ester hydrochloride (270.0 mg, 1.61 mmol), 4-nitrophenylphodichloride (412.3 mg, 1.61 mmol), and TEA (325.8 mg, 3.22 mmol, 448.8 µL) in DCM (15 mL), to yield 500 mg (yield 88.5%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.64 (s)

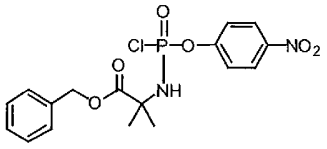
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.35 (2H, d, ^{3}J=9.0 Hz, OPh), 7.53 (2H, d, ^{3}J=9.0 Hz, OPh), 4.99-4.96 (1H, 2bs, NH), 4.34 (2H, q, ^{3}J=7.1 Hz, OCH_2CH_3), 1.79-1.76 (6H, 2s, $[CH_3]_2C$), 1.40 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (OCH_2CH_3), 27.0, 27.3 ($[CH_3]_2C$), 59.1, 59.2 ($C[CH_3]_2$), 62.9, 63.0 (OCH_2CH_3), 121.7, 121.8 (*'o'* OPh), 126.2 (*'m',* OPh), 145.7 (*'p',* OPh), 154.7, 154.8 (*'ipso', OPh),* 175.4, 175.6 ($COOCH_2CH_3$).

Synthesis of 4-nitrophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{17}H_{18}ClN_2O_6P$, MW=412.76.

[0128]



[0129] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate benzyl ester hydrochloride (578 mg, 2.52 mmol), 4-nitrophenylphosphodichloride (645 mg, 2.52 mmol), and TEA (510 mg, 5.04 mmol, 702.5 µL) in DCM (20 mL), to yield 936 mg (yield 90.0%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.56 (s)

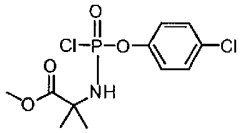
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.29 (2H, d, ^{3}J=9.0 Hz, OPh), 7.47 (2H, d , ^{3}J=9.0 Hz, OPh), 7.40-7.37 (5H, m, CH_2Ph), 5.27 (2H, s, CH_2Ph), 5.04-5.01 (1H, 2bs, NH), 1.77-1.74 (6H, 2s, $[CH_3]_2C$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.3, ($[CH_3]_2C$), 59.2 ($C[CH_3]_2$), 68.5 (OCH_2Ph), 121.6, 121.7, 126.2, 128.6, 129.1, (*'o', 'm', 'p',* CH_2Ph+ OPh), 135.7 (*'ipso',* CH_2Ph), 145.7 (*'p', OPh),* 154.7, 154.8 (*'ipso',* OPh), 175.8, 175.9 ($COOCH_2Ph$).

Synthesis of 4-chlorophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{11}H_{14}Cl_2NO_4P$, MW=326.11

[0130]



[0131] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate methyl ester hydrochloride (280.0 mg, 1.82 mmol), 4-chlorophenylphosphodichloride (447.4 mg, 1.82 mmol), and TEA (368.3 mg, 3.64 mmol, 507.3 µL) in DCM (20 mL), to yield 554 mg (yield 91.1 %) of crude product used without further purification.

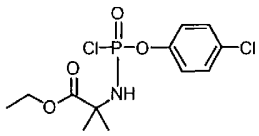
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.05 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38 (2H, d, ^{3}J=9.0 Hz, *OPh),* 7.28-7.24 (2H, 2d, ^{3}J=9.0 Hz, O*Ph*), 4.87-4.83 (1H, 2bs, N*H*), 3.84 (3H, s, OC*H*$_3$), 1.73-1.71 (6H, 2s, [C*H*$_3$]$_2$C). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.3, ([*C*H$_3$]$_2$C), 53.7 (O*C*H$_3$), 58.9 (*C*[CH$_3$]$_2$), 122.5 (*'o'* O*Ph*), 129.7 ('*m*', O*Ph*), 131.8 ('*p*', O*Ph*) 148.7, 148.9 (*'ipso',* O*Ph*), 175.5, 175.7 (*C*OOCH$_3$).

Synthesis of 4-chlorophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{12}H_{16}Cl_2NO_4P$, MW=340.14.

[0132]



[0133] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate ethyl ester hydrochloride (293.4 mg, 1.75 mmol), 4-chlorophenylphosphodichloride (430.0 mg, 1.75 mmol), and TEA (354.2 mg, 3.50 mmol, 488.0 µL) in DCM (15 mL), to yield 571.7 mg (yield 96.1 %) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.09 (s)

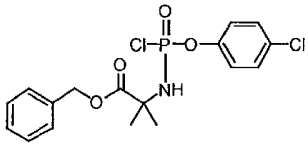
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38 (2H, d, ^{3}J=9.1 Hz, O*Ph*), 7.26 (2H, d, ^{3}J=9.1 Hz, O*Ph*), 4.88-4.84 (1H, 2bs, N*H*), 4.29 (2H, q, ^{3}J=7.1 Hz, OC*H*$_2$CH$_3$), 1.74-1.70 (6H, 2s, [C*H*$_3$]C), 1.35 (3H, t, ^{3}J=7.1 Hz, OCH$_2$C*H*$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (OCH$_2$*C*H$_3$), 27.0, 27.3 ([*C*H$_3$]$_2$C), 58.9 (*C*[CH$_3$]$_2$), 62.8 (O*C*H$_2$CH$_3$), 122.5 (*'o'*, O*Ph*), 130.4 (*'m'*, O*Ph*), 131.8 (*'p'*, O*Ph*), 148.7, 148.8 (*'ipso',* O*Ph),* 175.1, 175.3 (*C*OOCH$_2$CH$_3$).

Synthesis of 4-chlorophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{17}H_{18}Cl_2NO_4P$, MW=402.21.

[0134]



[0135] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate benzyl ester hydrochloride (402.0 mg, 1.75 mmol), 4-chlorophenylphosphodichloride (430 mg, 1.75 mmol), and TEA (354.2 mg, 3.50 mmol, 488.0 µL) in DCM (15 mL), to yield 657.9 mg (yield 93.5%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.00 (s)

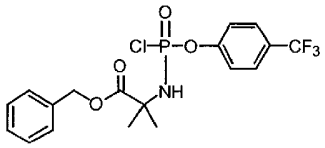
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.39-7.12 (9H, m, CH_2*Ph*+ O*Ph*), 5.18 (2H, s, CH_2Ph), 4.75-4.72 (1H, 2bs, N*H*), 1.68-1.65 (6H, 2s, [CH_3]$_2$C).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.3, ([*C*H_3]$_2$C), 59.0 (*C*[CH_3]$_2$), 68.4 (O*C*H_2Ph), 122.5, 128.6, 129.1, 130.7 (*'o' , 'm', 'p',* CH_2*Ph*+ O*Ph*), 131.8 (*'p',* CH_2*Ph*), 135.4 (*'p',* O*Ph*), 148.6, 148.7 (*'ipso',* O*Ph*), 174.9, 175.1 (*C*OOCH_2Ph).

Synthesis of 4-(trifluoromethyl)-phenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{18}H_{18}ClF_3NO_4P$, MW=435.76.

[0136]



[0137] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate benzyl ester hydrochloride (341.0 mg, 1.49 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (414.3 mg, 1.49 mmol), and TEA (300.5 mg, 2.97 mmol, 413.9 µL) in DCM (15 mL), to yield 623.9 mg (96.4%) of crude product used without further purification.

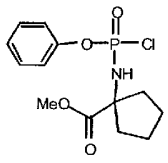
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.74 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.66 (2H, d, ^{3}J=8.8 Hz, O*Ph*), 7.42-7.30 (7H, m, O*Ph*+ CH_2*Ph*), 5.25 (2H, s, CH_2Ph), 4.95-4.91 (1H, 2bs, N*H*), 1.75-1.72 (6H, 2s, [CH_3]$_2$C)-^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.3 ([*C*H_3]$_2$C), 59.1 (*C*[CH_3]$_2$), 68.4 (*C*H_2Ph), 121.1, 121.4, 127.7, 128.4, 128.5, 128.6, 128.9 (*'o', 'm', 'p',* O*Ph*+CH_2*Ph*), 124.2 (CF_3, *J*=265 Hz), 135.4 (*'ipso',* CH_2*Ph*), 152.6, 152.7 (*'ipso',* O*Ph*), 174.9, 175.0 (*C*OOCH_2Ph).

Synthesis of Phenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{13}H_{17}ClNO_4P$, MW = 317.70.

[0138]



[0139] This is synthesised according to ***Standard procedure 4,*** using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), phenyldichlorophosphate (1.12 g, 0.749 ml, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.266 g (81 %) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.90.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.4-7.2 (5H, m ,O*Ph*), 4.3 (1H, bs, N*H*), 3.75 (3H, 2s, OCH_3), 2.15 (4H, m, 4H cyclopentane), 1.9-1.7

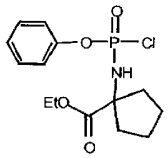
(4H, m, 4H cyclopentane)..

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.4 ($2CH_2$ cyclopent), 38.8, 38.7, 38.6 ($2CH_2$ cyclopent), 53.3, 53.2 (<u>C</u>H_3O), 66.6 (<u>Cq</u> cyclopentane), 121.1, 121.0 ('*o*' OPh), 126.3 ('*p*', OPh), 130.3, 130.2 ('*m*', OPh), 150.2 ('*ipso*', OPh), 174.8 (<u>C</u>$OOCH_3$).

Synthesis of Phenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{14}H_{19}ClNO_4P$, MW=331.73.

[0140]



[0141] This is synthesised according to ***Standard procedure 4,*** using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (955 mg, 5.01 mmol), phenyldichlorophosphate (1.12g, 5.01 mmol, 749 µL), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.457 g (89%) of oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.04, 7.97.

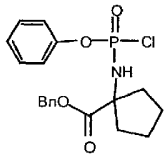
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.4-7.1 (5H, m, *<u>OPh</u>*), 4.7 (1H, bs, N<u>*H*</u>), 4.2 (2H, 2q, ^{3}J=7.1 Hz, O<u>*C*</u>H_2CH_3), 2.15 (4H, m, 4H cyclopentane), 1.9-1.7 (4H, m, 4H cyclopentane), 1.30 (3H, t, ^{3}J=7.1 Hz, OCH_2<u>*C*</u>H_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (<u>C</u>H_3CH_2), 24.5 ($2CH_2$ cyclopent), 38.8, 38.7, 38.6, 38.5 ($2CH_2$ cyclopent), 62.0 CH_3<u>C</u>H_2), 68.3 (<u>Cq</u> cyclopentane), 120.9 ('*o*' OPh), 126.3 ('*p*', O<u>*Ph*</u>), 130.3 ('*m*', OPh), 150.3-150.2 ('*ipso*', OPh), 174.9-174.8 (<u>C</u>$OOCH_2CH_3$).

Synthesis of Phenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{19}H_{21}ClNO_4P$, MW=393.80.

[0142]



[0143] This is synthesised according to ***Standard procedure 4,*** using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (0.984 g, 3.84 mmol), phenyl-dichlorophosphate (0.577 ml, 3.84 mmol), and TEA (1.08 mL, 7.69 mmol) in DCM (30 mL), to yield 1.485 g (98%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.85.

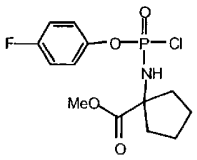
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.3-7.0 (10H, m, O<u>*Ph*</u>+CH_2<u>*Ph*</u>), 5.2 (2H, s, <u>*C*</u>H_2Ph), 4.95-4.65 (1H, bs, N<u>*H*</u>), 2.25-2.1 (4H, m, 4H cyclopentane), 1.9-1.7 (4H, m, 4H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.4, 24,3 ($2CH_2$ cyclopent), 38.8, 38.7, 38.5 ($2CH_2$ cyclopent), 67.3 (<u>Cq</u> cyclopentane), 68.0 (<u>C</u>H_2Ph), 121.0 ('*o*' OPh), 126.4 ('*p*', OPh), 130.1, 129.0, 128.8 ('*m*' OPh, CH_2<u>*Ph*</u>), 135.4 ('*ipso*', CH_2*Ph*), 150.1 ('*ipso*', OPh), 173.4 (<u>C</u>$OOCH_2$Ph).

Synthesis of p-fluorophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{13}H_{16}ClNO_4P$, MW=335.70.

[0144]



[0145] This is synthesised according to ***Standard procedure 4,*** using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), para-fluorophenyldichlorophosphate (1.21 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.65 g (99%) of crude product used without further purification. ^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.61.

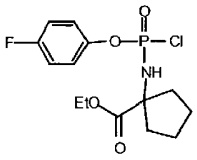
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.3-7.2 (2H, m, O*Ph*), 7.1-7.0 (2H, m, O*Ph*), 4.7 (1H, bs, N*H*), 3.78 (3H, 2s, OC*H*$_3$), 2.25-2.15 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane)..

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.4 ($2CH_2$ cyclopent), 38.7, 38.6, 38.5 ($2CH_2$ cyclopent), 53.3 (*C*H_3O),66.3-66.2 (*Cq* cyclopentane), 117.1-116.8 ('*o*' OPh), 122.6-122.5 ('*m*', OPh), 146.1-145.9 (*'ipso',* OPh), 159.0 (*'p'*, OPh), 175.3-175.2 (*C*OOCH$_3$).

Synthesis of p-fluorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{14}H_{18}ClFNO_4P$, MW=349.72.

[0146]



[0147] This is synthesised according to ***Standard procedure 4,*** using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (955 mg, 5.01 mmol), para-fluorophenyldichlorophosphate (1.21g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL), to yield 1.64 g (94%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.70.

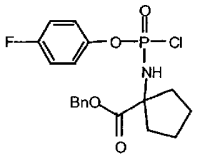
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.3-7.2 (2H, m, O*Ph*), 7.1-7.0 (2H, m, O*Ph*), 4.8 (1H, bs, N*H*), 4.2 (2H, 2q, ^{3}J=7.1 Hz, OC*H*$_2$CH$_3$), 2.25-2.1 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane), 1.4 (3H, t, ^{3}J=7.1 Hz, OCH$_2$*CH*$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 (*C*H_3CH$_2$), 24.4 ($2CH_2$ cyclopent), 38.8, 38.7, 38.6, 38.5 ($2CH_2$ cyclopent), 62.3_CH$_3$*C*H$_2$), 68.3 (*Cq* cyclopentane), 117.4, 117.0 ('*o*' OPh), 122.7, 122.6 (*'m',* OPh), 146.1, 146.0 (*'ipso',* OPh), 159.0 (*'p'*, OPh), 174.9 (*C*OOCH$_2$CH$_3$).

Synthesis of p-fluorophenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{19}H_{20}ClFNO_4P$, MW= 411.79.

[0148]



[0149] This is synthesised according to ***Standard procedure 4,*** using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (1.281 g, 5.01 mmol), para-fluorophenyl-dichlorophosphate (1.21 g, 5.01 mmol), and TEA (1.4 mL, 10 mmol) in DCM (40 mL), to yield 1.85 g (90%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.85.

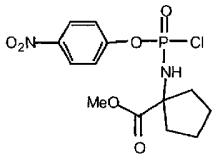
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.65-7.4 (5H, m, CH_2*Ph*), 7.3-7.2 (2H, m, O*Ph*), 7.1-7.0 (2H, m , O*Ph*), 5.2 (2H, s, CH_2Ph), 4.6 (1H, bs, N*H*), 2.2-2.1 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.5 ($2CH_2$ cyclopent), 38.9, 38.8, 38.6, 38.5 ($2CH_2$ cyclopent), 68.1 (*Cq* cyclopentane), 68.4 (CH_2Ph), 117.0, 116.8 ('*o*' OPh), 122.6, 122.5 ('*m*' OPh) 129.1, 129.0, 128.8, 128.7 (CH_2Ph), 135.7 (*'ipso'*, CH2Ph), 146.1, 145.9 (*'ipso'*, OPh), 159.0 (*'p'*, OPh), 174.6 ($COOCH_2$Ph).

Synthesis of p-nitrophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{13}H_{16}ClN_2O_6P$, MW=362.70.

[0150]



[0151] This is synthesised according to ***Standard procedure 4,*** using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), para-nitrophenyldichlorophosphate (1.632 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.601 g (90%) of crude product used without further purification. ^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.02.

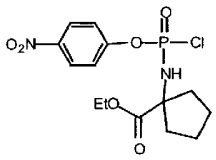
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.2 (2H, 2d, ^{3}J=8 Hz, O*Ph*), 7.32 (2H, 2d, ^{3}J=8 Hz O*Ph*), 4.9 (1H, bs, N*H*), 3.71 (3H, s, OCH_3), 2.25-2.00 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane)..

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.3 ($2CH_2$ cyclopent), 38.7, 38.6 ($2CH_2$ cyclopent), 53.3 (CH_3O),68.6 (*Cq* cyclopentane), 121.8, 121.7 (*'o'* OPh), 126.0 (*'m'*, OPh), 145.6 (*'ipso'*, OPh), 154.8, 154.7 (*'p'*, OPh), 175.1-175.0 ($COOCH_3$).

Synthesis of p-nitrophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{14}H_{18}ClN_2O_6P$, MW=376.73.

[0152]



[0153] This is synthesised according to ***Standard procedure 4,*** using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (955

mg, 5.01 mmol), para-nitrophenyldichlorophosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL), to yield 1.669 g (90%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.95.

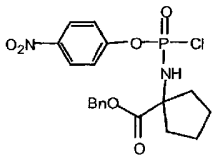
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.1 (2H, 2d, ^{3}J=8 Hz, O*Ph*), 7.28 (2H, 2d, ^{3}J=8 Hz O*Ph*), 4.8 (1H, bs, N*H*), 4.2 (2H, 2q, ^{3}J=7.1 Hz, O$CH_2$$CH_3$), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane), 1.27 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 (CH_3CH_2), 24.4 ($2CH_2$ cyclopent), 38.8, 38.7 ($2CH_2$ cyclopent), 62.4_CH_3CH_2), 68.5 (*Cq* cyclopentane), 121.8, 121.1 ('*o*' OPh), 126.1, 125.9 ('*m*', OPh), 145.6 ('*ipso*', O*Ph*), 154.8 ('*p*', OPh), 174.9 ($COOCH_2CH_3$).

Synthesis of p-nitrophenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{19}H_{20}ClN_2O_6P$, MW= 438.80.

[0154]



[0155] This is synthesised according to ***Standard procedure 4***, using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (0.835 g, 3.25 mmol), para-nitrophenyl-dichlorophosphate (0.85 g, 3.25 mmol), and TEA (0.91 mL, 6.7 mmol) in DCM (30 mL), to yield 1.215 g (85%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.99, 7.90.

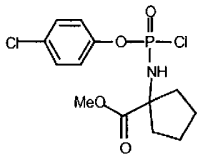
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.1 (2H, 2d, ^{3}J=8 Hz, O*Ph*), 7.4-7.2 (7H, m, O*Ph*+ CH_2*Ph*), 5.18 (2H, s, CH_2Ph), 5.0 (1H, bs, N*H*), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.4 ($2CH_2$ cyclopent), 38.8, 38.7, 38.6, 38.5 ($2CH_2$ cyclopent), 68.0 (CH_2Ph), 68.6 (*Cq* cyclopentane). 121.8, 121.7 ('*o*' OPh), 126.1, 125.9 ('*m*'OPh) 129.1, 129.0, 128.8, 128.6 (CH_2Ph), 135.7 ('*ipso*', CH_2Ph), 145.6 ('*ipso*', OPh), 154.8,154.7 ('*p*', OPh), 174.5, 174.4 ($COOCH_2$Ph).

Synthesis of p-chlorophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{13}H_{16}Cl_2NO_4P$, MW=352.15.

[0156]



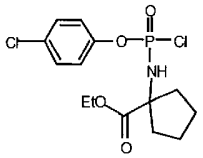
[0157] This is synthesised according to ***Standard procedure 4,*** using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.443 g, 2.5 mmol), para-chlorophenyldichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 ml, 5 mmol) in DCM (20 mL), to yield 0.852 g (98%) of crude product used without further purification. ^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.55, 9.5.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.35-7.15 (4H, m, *OPh),* 4.95 (1H, bs, N*H*), 3.78 (3H, s, OCH_3), 2.2-2.00 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.3 ($2CH_2$ cyclopent), 38.7 ($2CH_2$ cyclopent), 53.3 (CH_3O),68.6 (*Cq* cyclopentane), 122.0 ('*o*' OPh), 130.1 ('*m*', OPh), 133.2 ('*p*', OPh), 149.9 ('*ipso*', O*Ph*), 175.1-175.0 ($COOCH_3$).

Synthesis of p-chlorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{14}H_{18}Cl_2NO_4P$, MW=366.18.

[0158]



[0159] This is synthesised according to ***Standard procedure 4,*** using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (0.477 g, 2.5 mmol), para-chlorophenyldichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.880 g (97%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.85, 9.70.

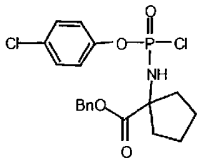
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.35-7.15 (4H, m, *OPh)*, 4.9 (1H, bs, N*H*), 4.22 (2H, 2q, ^{3}J=7.1 Hz, O*CH*$_2$CH$_3$), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane), 1.27 (3H, t, ^{3}J=7 Hz, OCH$_2$*CH*$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 (*C*H$_3$CH$_2$), 24.4 (2CH$_2$ cyclopent), 38.8, 38.7 (2CH$_2$ cyclopent), 62.5, 62.4 CH$_3$CH$_2$), 68.1 (*Cq* cyclopentane), 122.2, 122.1 ('*o*' OPh), 130.1 ('*m*', OPh), 133.2 ('*p*', OPh), 149.8 ('*ipso*', OPh), 174.8 (*C*OOCH$_2$CH$_3$).

Synthesis of p-chlorophenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{19}H_{20}Cl_2NO_4P$, MW= 428.25.

[0160]



[0161] This is synthesised according to ***Standard procedure 4,*** using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (0.640 g, 2.5 mmol), para-chlorophenyl-dichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 1.041 g (97%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.39, 8.95.

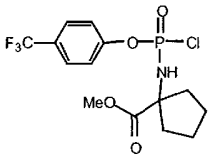
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.4-7.15 (9H, m, O*Ph* + CH$_2$*Ph*), 5.20 (2H, s, *CH*$_2$Ph), 5.0 (1H, bs, N*H*), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.4 (2CH$_2$ cyclopent), 38.8, 38.7, 38.6 (2CH$_2$ cyclopent), 68.1, 68.0 (*C*H$_2$Ph), 68.2 (*Cq* cyclopentane), 121.9, 121.8 ('*o*' OPh), 130.5, 130.4, 129.3, 129.2 ('*m*' OPh, CH$_2$*Ph*), 133.2 ('*p*', OPh), 135.7 ('*ipso*', CH$_2$Ph), 149.9 ('*ipso*', OPh), 174.3, 174.2 (*C*OOCH$_2$Ph).

Synthesis of p-trifluorophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{14}H_{16}ClF_3NO_4P$, MW=385.70.

[0162]



[0163] This is synthesised according to ***Standard procedure 4,*** using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.443 g, 2.5 mmol), para-trifluorophenyldichlorophosphate (0.700 g, 2.5 mmol), and TEA (0.7 ml, 5 mmol) in DCM (20 mL), to yield 0.931 g (97%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.80, 8.62.

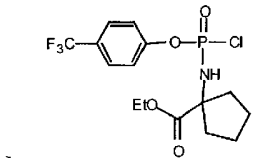
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.65 (2H, 2d, ^{3}J=8 Hz, *OPh),* 7.35 (2H, 2d, ^{3}J=8 Hz O*Ph*), 5.02 (1H, bs, N*H*), 3.78 (3H, s, OC*H*$_3$), 2.25-2.05 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane)..

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 22.8 (2CH_2 cyclopent), 37.5, 37.2 (2CH_2 cyclopent), 51.5 (*C*H_3O),68.4 (*Cq* cyclopentane), 120.0 ('*o*', O*Ph*), 124.8 (d, J=270Hz, CF_3), 126.6 (*'m',* O*Ph*), 129.5 ('*p*', q, J=32Hz, O*Ph*), 152.8 (*'ipso',* OPh), 175.2 (*C*OOCH_3).

Synthesis of p-trifluorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{15}H_{18}ClF_3NO_4P$, MW=399.73.

[0164]



[0165] This is synthesised according to ***Standard procedure 4,*** using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (0.477 g, 2.5 mmol), para-trifluorophenyldichlorophosphate (0.700 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.950 g (89%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.49.

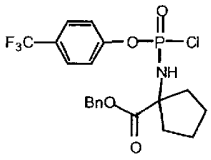
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45 (2H, m, O*Ph*), 7.2 (2H, m, O*Ph*), 5.12 (1H, bs, N*H*), 4.05 (2H, m, O*CH*$_2$$CH_3$), 2.15-2.0 (4H, m, 4H cyclopentane), 1.9-1.65 (4H, m, 4H cyclopentane), 1.2 (3H, 2t, ^{3}J=7 Hz, OCH_2*CH*$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.3 (*C*H_3CH_2), 24.2, 24.1 (2CH_2 cyclopent), 38.6, 38.5, 38.4 (2CH_2 cyclopent), 62.0 CH_3*C*H_2), 68.4 (*Cq* cyclopentane), 121.5 ('*o*', O*Ph*), 125.0 (d, J=270Hz, CF_3), 127.5 (*'m',* O*Ph*), 129.9 ('*p*', q, J=32Hz, O*Ph*), 152.8, 152.7 (*'ipso',* OPh), 174.9, 174.6 (*C*OOCH_2CH_3).

Synthesis of p-trifluorophenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

$C_{20}H_{20}ClF_3NO_4P$, MW= 461.80.

[0166]



[0167] This is synthesised according to ***Standard procedure 4,*** using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (0.700 g, 2.73 mmol), para-trifluorophenyl-dichlorophosphate (0.75 g, 2.73 mmol), and TEA (0.75 mL, 5.47 mmol) in DCM (25 mL), to yield 1.089 g (86%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.39, 8.95.

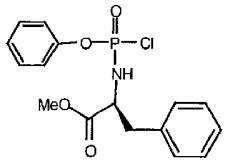
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.50 (2H, m, O*Ph*), 7.4-7.15 (7H, m, O*Ph* + CH_2*Ph*), 5.20 (2H, s, *C*H_2Ph), 4.95 (1H, bs, N*H*), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.3 ($2CH_2$ cyclopent), 38.8, 38.7, 38.6 ($2CH_2$ cyclopent), 68.1, 68.0 (*C*H_2Ph), 68.2 (*Cq* cyclopentane), 121.4, 121.3 ('*o*', O*Ph*), 125.1 (d, J=270Hz, CF_3), 126.6 (*'m', OPh*) 129.2, 128.8, 127.8 (Bn), 129.8 ('*p*', q, J=32Hz, O*Ph*), 135.7 ('*ipso*', CH_2Ph), 153.5 (*'ipso'*, OPh), 174.5, 174.4 (*C*$OOCH_2$Ph).

Synthesis of Phenyl-(methoxy-L-phenylalaninyl)-phosphorochloridate.

$C_{16}H_{17}ClNO_4P$, MW=353.74.

[0168]



[0169] This is synthesised according to ***Standard procedure 4,*** using L-phenylalanine methyl ester hydrochloride (1.08 g, 5 mmol), phenyldichlorophosphate (1.12 g, 0.75 ml, 5 mmol), and TEA (1.4ml, 10 mmol) in DCM (40 mL), to yield 1.626 g (92%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.1, 8.95.

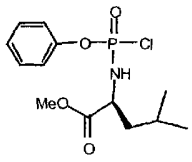
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.3-7.1 (10H, m, CH_2Ph+ O*Ph*), 5.00 (1H, bs, N*H*), 4.35 (1H, m, CHphenylala), 3.79 (3H, 2s, CH_3O), 3.00 (2H, m, CH_2Ph)

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 36.3 (CH_2phenylalanine), 53.0 (*C*H_3O), 56.6, 56.5 (CHphenylala), 121.0 ('*o*' OPh), 126.4 ('*p'*, OPh), 130.2 ('*m',* OPh), 150.2 (*'ipso',* OPh), 174.1 (*C*$OOCH_3$).

Synthesis of Phenyl-(methoxy-L-leucinyl)-phosphorochloridate

$C_{13}H_{19}ClNO_4P$, MW=319.72.

[0170]



[0171] This is synthesised according to ***Standard procedure 4,*** using L-leucine methyl ester hydrochloride (0.91 g, 5 mmol), phenyldichlorophosphate (1.12 g, 0.75 ml, 5 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.58 g (99%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.45. 9.35.

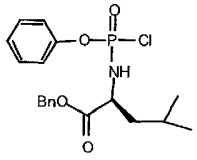
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.4-7.2 (5H, m, O*Ph*), 4.90 (1H, bs, N*H*), 3.95 (1H, m, *CH*$CH_2CH(CH_3)_2$), 3.78 (3H, s, OC*H*$_3$), 1.8 (1H, m, $CHCH_2$*CH*$(CH_3)_2$), 1.8-1.5 (2H, m, CH*C*$H_2CH(CH_3)_2$), 1.0-0.9 (6H, m, $CHCH_2CH($*C*$H_3)_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 23.2, 23.1, 22.4, 22.3 (2C, $CHCH_2CH($*C*$H_3)_2$), 24.9, 24.8 ($CHCH_2$*CH*$($*C*$H_3)_2$), 43.6 (CH*C*$H_2CH(CH_3)_2$), 53.2 (*C*H_3O), 53.7, 53.6 (*CH*CH_2*CH*$(CH_3)_2$), 120.9 ('*o*' OPh), 126.4 (*'p'*, OPh), 130.2 (*'m'*, OPh), 150.1 (*'ipso'*, OPh), 173.6 (*C*$OOCH_3$).

Synthesis of Phenyl-(benzoxy-L-leucinyl)-phosphorochloridate.

$C_{19}H_{23}ClNO_4P$, MW= 395.82.

[0172]



[0173] This is synthesised according to ***Standard procedure 4,*** using L-leucine benzyl ester hydrochloride (1.29 g, 5.0 mmol), phenyl-dichlorophosphate (1.12 g, 0.75 ml, 5.0 mmol), and TEA (1.4 mL, 10.0 mmol) in DCM (40 mL), to yield 1.88 g (95%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.93, 9.57.

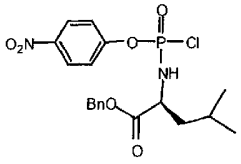
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.5-7.2 (10H, m, O*Ph*+*CH*$_2$*Ph*), 5.2 (2H, 2s, *CH*$_2$Ph), 4.95 (1H, bs, N*H*), 4.2-4.1 (1H, m, *CH*$CH_2CH(CH_3)_2$), 1.95-1.80 (1H, m, $CHCH_2$*CH*$(CH_3)_2$), 1.7 (2H, m, *CH*$CH_2CH(CH_3)_2$), 1.0-0.9 (6H, m, $CHCH_2CH($*CH*$_3)_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 23.2, 23.1, 22.4, 22.3 (2C, *CH*$CH_2CH(CH_3)_2$), 24.9 ($CHCH_2$*CH*$($*CH*$_3)_2$), 43.5 (CH*CH*$_2CH(CH_3)_2$), 53.8, 53.3 (*CH*CH_2*CH*$(CH_3)_2$), 67.8, 67.7 (*C*H_2Ph), 120.7 ('*o*' OPh), 126.4 (*'p'*, OPh), 130.2, 129,1, 128.8, 128.7 ('*m*' OPh, CH_2*Ph*), 135.8 *('ipso'*, CH_2Ph), 150.2 ('*ipso*', OPh), 174.1 (*C*$OOCH_2$Ph).

Synthesis of p-nitrophenyl-(benzoxy-L-leucinyl)-phosphorochloridate.

$C_{19}H_{22}ClN_2O_6P$, MW= 440.81.

[0174]



[0175] This is synthesised according to ***Standard procedure 4,*** using L-leucine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-nitrophenyl-dichloro phosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL), to yield 2.08g (95%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.87, 9.38.

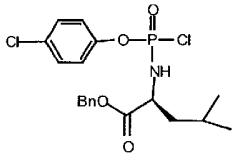
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.25-8.10 (2H, m , O*Ph*), 7.35-7.25 (7H, m, O*Ph* + *CH_2Ph*), 5.15 (2H, 2s, *CH_2*Ph), 4.95 (1H, bs, N*H*), 4.15 (1H, m, *CH*$CH_2CH(CH_3)_2$), 1.95 (1H, m, *CH*$CH_2CH(CH_3)_2$), 1.7 (2H, m, CH*CH_2*$CH(CH_3)_2$), 1.0-0.9 (6H, m, $CHCH_2CH($*CH_3*$)_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 23.2, 23.1, 22.1, 22.0 (2C, $CHCH_2CH($*CH_3*$)_2$), 24.8 ($CHCH_2$*CH*$(CH_3)_2$), 43.4, 43.3 (CH*CH_2*$CH(CH_3)_2$), 54.2, 53.9 ($CHCH_2$*CH*$(CH_3)_2$), 68.0, 67.9 (*C*H_2Ph), 121.6 ('*o*' OPh), 126.2, 126.1 ('*m*'OPh), 129.2, 129.0 (CH_2*Ph*), 135.4, 135.3 ('*ipso*', CH_2Ph), 145.8, 145.7 ('*ipso*', OPh), 154.7, 154.5 ('*p*', OPh), 173.0, 172.8 (*C*O*C*H_2Ph).

Synthesis of p-chlorophenyl-(benzoxy-L-leucinyl)-phosphorochloridate.

$C_{19}H_{22}Cl_2NO_4P$, MW= 430.26.

[0176]



[0177] This is synthesised according to ***Standard procedure 4,*** using L-leucine benzyl ester hydrochloride (0.644 g, 2.5 mmol), para-chlorophenyl-dichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.968 g (90%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.71, 9.55.

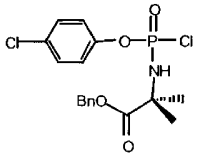
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.4-7.0 (9H, m, O*Ph* + CH_2*Ph*), 5.15 (2H, s, *CH_2*Ph), 4.5 (1H, d, ^{3}*J*=7Hz, N*H*), 4.0 (1H, m, *CH*$CH_2CH(CH_3)_2$), 1.9-1.8 (1H, m, $CHCH_2$*CH*$(CH_3)_2$), 1.7 (2H, m, CH*CH_2*$CH(CH_3)_2$), 0.85 (6H, m, $CHCH_2CH($*CH_3*$)_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 23.4, 23.3, 22.5, 22.4 (2C, $CHCH_2CH($*CH_3*$)_2$), 25.0 ($CHCH_2$*CH*$(CH_3)_2$), 43.8, 43.7 (CH*CH_2*$CH(CH_3)_2$), 54.0, 53.8 ($CHCH_2$*CH*$(CH_3)_2$), 68.2 (*C*H_2Ph), 122.5 ('*o*' OPh), 130.5, 130.4, 129.3, 129.2 ('*m*'OPh, CH_2*Ph*), 133.2 ('*p*', OPh), 135.7 ('*ipso*', CH2Ph), 149.9, 149.8 ('*ipso*', OPh), 173.4, 173.2 (*C*OO*C*H_2Ph).

Synthesis of 4-chlorophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{11}H_{14}ClNO_4P$, MW=326.11.

[0178]



[0179] This is synthesised according to ***Standard procedure 4,*** using 2-aminoisobutyrate methyl ester hydrochloride (280.0mg, 1.82 mmol), 4-chlorophenylphosphodichloride (447.4 mg, 1.82 mmol), and TEA (368.3 mg, 3.64 mmol, 507.3 µL) in DCM (20 mL), to yield 554 mg (yield 91.1 %) of crude product used without further purification.

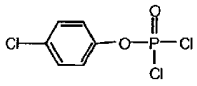
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.05 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38 (2H, d, 3 *J*=9.0 Hz, O*Ph*), 7.29-7.24 (2H, 2d, ^{3} *J*=9.0 Hz, *OPh),* 4.87-4.83 (1H, 2bs, N*H*), 3.84 (3H, s, O*CH_3*), 1.73-1.71 (6H, 2s, [*CH_3*]$_2$C). ^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.3, ([*C*H_3]$_2$C), 53.7 (O*CH_3*), 58.9 (*C*[CH_3]$_2$), 122.5 (*'o'* O*Ph*), 129.7 ('*m*', O*Ph*), 131.8 ('*p*', O*Ph*) 148.7, 148.9 ('*ipso*', *OPh),* 175.5, 175.7 (*C*OOCH_3).

Synthesis of 4-chlorophenyl-phosphodichloridate.

$C_6H_4Cl_3O_2P$, MW=245.43.

[0180]



[0181] This was synthesised according to ***Standard procedure 3,*** using phosphorus-oxychloride (1533 mg, 10.00 mmol, 932 µL), 4-chlorophenol (1.285 g, 10.00 mmol) and TEA (1.011 g, 10.00 mmol, 1394 µL) in ethylether (100 mL) to give an oil (1.897 g, 77.3 % yield).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.18.

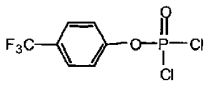
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45 (2H, d, ^{3}J=9.0 Hz, O*Ph*), 7.30 (2H, d, ^{3}J=9.0 Hz, O*Ph*).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 122.5 (*'o'*, O*Ph*), 130.6 (*'m'*, O*Ph*), 133.2 (*'p'*, O*Ph*), 148.5 (*'ipso'*, O*Ph*).

Synthesis of 4-(trifluoromethyl)-phenyl-phosphodichloridate.

$C_7H_4ClF_3O_3P$, MW=278.98.

[0182]



[0183] This was synthesised according to ***Standard procedure 3***, using phosphorus-oxychloride (1.570 mg, 10.24 mmol, 954.5 µL), 4-trifluoromethylphenol (1660 g, 10.24 mmol) and TEA (1.036 g, 10.24 mmol, 1427 µL) in ethylether (100 mL) to give an oil (2.521 g, 88.2% yield).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.75.

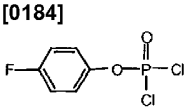
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.77 (2H, d, ^{3}J=8.4 Hz, *OPh)*, 7.49 (2H, d, ^{3}J=8.4 Hz, O*Ph*).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 121.6 (*'o'*, O*Ph*), 123.6 (*CF*$_3$, *J*=271 Hz, O*Ph*), 128.2 (*'m'*, OPh), 129.7 (*'p'*, *J*=33 Hz), 152.7 (*'ipso'*, O*Ph*).

Synthesis of 4-fluorophenyl-phosphodichloridate.

$C_6H_4Cl_2FO_2P$, MW=228.97.

[0184]



[0185] This was synthesised according to ***Standard procedure 3***, using phosphorus-oxychloride (1.395 mL, 15.00 mmol), 4-chlorophenol (1.68 g, 15.00 mmol) and TEA (2.1 mL, 15.00 mmol) in ethylether (140 mL) to give an oil (3.96 g, 96 % yield).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.52.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.15 (2H, d, ^{3} *J*=8.0 Hz, O*Ph*), 7.05 (2H, d, ^{3} *J*=8.0 Hz, O*Ph*).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 116.8 (*'o'*, O*Ph*), 122.1 (*'m'* OPh), 146.7 (*'p'*, O*Ph*), 158.7 (*'ipso'*, O*Ph*).

[0186] Experimental data are given in Table I illustrating the activity of compounds embodying the present invention, and of some comparative compounds, with respect to human breast cancer cell line MDA MB231, human colon cancer cell line HT115 and human prostrate cancer cell line PC-3. The compounds include those whose preparations are described above and compounds made by preparative methods corresponding to the methods described above.

[0187] The experimental procedures used human colon cancer cell line (HT115), human prostate cancer cell line (PC-3), human breast cancer cell line (MDA MB 231) and normal human umbilical vein endothelial cell (HUVEC). Compounds were diluted over a range of concentrations and added to cells over 1 to 3 days. The cytotoxity was determined using a MTT assay at the end of each experiment.

[0188] In the Table:

ArO refers to Ar as defined above with respect to formula I;

J refers to the moiety of the present compounds represented by, respectively, ROCOCR'R"NH-, as defined above with respect to formula I, and

B refers to the base moiety of the present compounds as defined above with respect to formula I.

[0189] BVU stands for 2-bromovinyl uridine.

[0190] GemCyt stands for Gemcitabine.

[0191] Examples A, 1 and G are comparative Examples.

[0192] Example A is 5-(2-Bromovinyl)-2'-deoxyuridine.

[0193] Example 1 is Example 1 above corresponding to compound (7) above.

[0194] Example G is gemcitabine.

[0195] Examples 51, 52 and 53 are compounds embodying formula II above.

TABLE

Ex	**ArO**	**J**	**B**	**EC50/μM**	**EC50/μM**	**EC50/μM**
				Breast	**Colon**	**Prostate**
				MDA MB231	**HT115**	**PC-3**
A	-	-	BVU	**125**	**78.7**	**120**
1	PhO	MeAlaNH	BVU	79	244.5	155
G	-	-	GemCyt	2.8	606.1	3.12
31	PhO	BnAlaNH	GemCyt	42.6	5.7	0.22
40	p-ClPhO	BnAlaNH	GemCyt	9.2	16.1	15.4
41	p-ClPhO	Bn[Me2Gly]NH	GemCyt	3.1	317.1	68.8

[0196] Gemcitabine (Example G in the Table) and compound CPF31 (Example 31 in the Table: gemcitabine-[phenyl-(benzoxy-L-alaninyl)]-phosphate) were compared in a mouse model with xenografts of human cancer (colon HT115 and prostrate PC3).

[0197] Mice were dosed daily at a range of concentrations (0.01-10μM) and tumour volume assessed versus control.

[0198] Kaplan-Meier statistics were computed regarding incident-free survival.

[0199] In the attached drawings:

Figure 1 shows for the mouse xenograft the tumour volume for prostate data at day 13 using Gemzar™ (gemcitabine available ex. Lilly);

Figure 2 shows for the mouse xenograft the tumour volume for prostate data at day 13 using CPF31;

Figure 3 shows the incident free survival functions v. day for each of CPF31 and gemcitabine; and

Figure 4 shows for the mouse xenograft the tumour volume for colon data at day 24 using, respectively, Gemzar and compound CPF31.

[0200] Referring to the drawings, CPF31 can be seen to be significantly less toxic than gemcitabine.

[0201] CPF31 was significantly effective at reducing prostate and colon tumour volume relative to control at daily dosing of 5 and 10 µM (3 and 6 µg/ml). Gemcitabine was not effective at the highest non-toxic concentration.

[0202] Gemzar is seen from Figure 1 to be toxic above 1µM. In contrast, CPF31 is seen from Figure 2 to have substantially lower toxicity.

[0203] Figure 3 shows that CPF31 has significantly lower side effects on a comparable basis: 3 animals show serious toxicity (10% body mass loss) in GMZ and in CPF31 on day 10, collectively 4 in GMZ and 1 in CPF31 on day 11 and 5 in GMZ and 1 in CPF on day 13. Using Chi square analysis by combining 5 and 10µM groups, the significance is p=0.193, 0,078 and 0.0289 on day 10, 11 and 13. It is clear that by day 13, CPF31 displayed signficantly less side effects, and the anti-cancer effects continue to exceed that of Gemzar.

[0204] Figure 3 shows the Kaplan-Meier survival curve, incidence free survival: based on the loss according to weight loss. A Cox proportion analysis shows that CPF31 is far less toxic than GMZ based on the weight-loss calculated loss (p=0.043).

[0205] CPF31 was found to be active at 5µM *in vitro,* whereas Gemzar was found to be active at 600µM, with respect to the same colon cell line. Figure 4 shows the results of testing both *in vivo* at 5µM. The greater activity of CPF31 in reducing tumour volume is shown in Figure 4.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

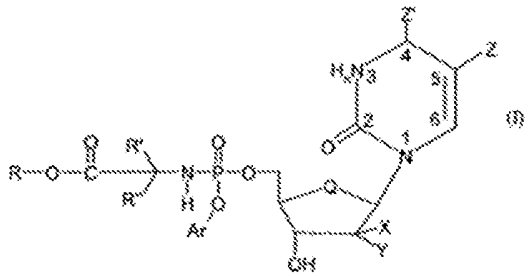
- WO0239952A [0010]
- EP1200455A [0010]
- CA2317505 [0010]
- US6339151B [0010]
- EP116797A [0010]
- AU2451601 [0010]
- US2003109697A [0012]

Non-patent literature cited in the description

- **ROSOWSKY et al.**J. Med. Chem., 1982, vol. 25, 171-8 [0003]
- **HONG et al.**J. Med. Chem., 1985, vol. 28, 171-8 [0003]
- **KODAMA et al.**Jpn. J. Cancer Res., 1989, vol. 80, 679-85 [0003]
- **JI et al.**J. Med. Chem., 1990, vol. 33, 2264-70 [0003]
- **JONES et al.**Nucleic Acids Res., 1989, vol. 17, 7195-7201 [0003]
- **HUNSTON et al.**J. Med. Chem., 1984, vol. 27, 440-4 [0003]
- **LOREY et al.**Nucleosides Nucleotides, 1997, vol. 16, 1307-10 [0003]
- **FARQUHAR et al.**J. Med. Chem., 1983, vol. 26, 1153-8 [0003]
- **SHUTO et al.**Nucleosides Nucleotides, 1992, vol. 11, 437-46 [0003]
- **LE BEC et al.**Tet. Letts., 1991, vol. 32, 6553-6 [0003]
- **PHELPS et al.**J. Med. Chem., 1980, vol. 23, 1229-32 [0003]
- **MCGUIGAN et al.**AVCC, 1998, vol. 9, 473-9 [0007]
- **MCGUIGAN et al.**Antiviral Res., 1997, vol. 35, 195-204 [0007]
- AVCC, 2000, vol. 11, 111-6 [0007]
- **SIDDIQUI et al.**J. Med. Chem., 1999, vol. 42, 393-9 [0007]
- **MCGUIGAN et al.**AVCC, 1996, vol. 7, 184-8 [0007]
- **ALLENDER et al.**Analytica Chim. Acta, 2001, vol. 435, 107-13 [0007]
- **BALZARINI et al.**BBRC, 1996, vol. 225, 363-9 [0007]
- **MCGUIGAN et al.**BioOrg. Med, Chem. Lett., 1996, vol. 6, 2369-62 [0007]
- **MCGUIGAN et al.**Bioorg. Med. Chem. Lett., 2000, vol. 10, 645-7 [0007]
- **BALZARINI et al.**PNAS, 1996, vol. 93, 7295-9 [0008]
- **MCGUIGAN et al.**J. Med. Chem., 1996, vol. 39, 1748-53 [0008]
- **LACKEY et al.**Biochem Pharmacol., 2001, vol. 61, 179-89 [0009]
- **ABRAHAM TW et al.**J. Med. Chem., 1996, vol. 39, 4569-4575 [0013]

PATENTKRAV

1. Kemisk forbindelse med formlen I:



hvor:

R er udvalgt fra methyl (-CH_3), ethyl (-C_2H_5), *n*- eller *i*-propyl (-C_3H_7), *n*- eller *i*-butyl (-C_4H_9) eller benzyl (-$CH_2C_6H_5$);

R' og R" uafhængigt er udvalgt fra H, methyl (-CH_3), benzyl (-$CH_2C_6H_5$) og sekundær butyl (-$CH_2CH(CH_3)_2$), eller R' og R" sammen med det C-atom, hvortil de er bundet, tilvejebringer en C_{5-6}-ring;

Q er -O-;

X og Y begge er F;

Ar er udvalgt fra usubstitueret phenyl eller phenyl substitueret med halogen, trihalomethyl, cyan- eller nitrogrupper;

Z er H;

n er 0, Z' er -NH_2 og en dobbeltbinding eksisterer mellem position 3 og position 4;

eller et farmaceutisk acceptabelt salt, en ester eller et salt af sådan en ester fra en forbindelse med formlen I.

2. Forbindelse ifølge krav 1, hvor R er udvalgt fra methyl (-CH_3), ethyl (-C_2H_5) og benzyl (-$CH_2C_6H_5$).

3. Forbindelse ifølge krav 2, hvor R er benzyl.

4. Forbindelse ifølge et hvilket som helst af kravene 1 til 3, hvor Ar er udvalgt fra - C_6H_5, *p*$CF_3C_6H_4$-, *p*FC_6H_4-, *p*$NO_2C_6H_4$-, *p*ClC_6H_4- og *o*ClC_6H_4-.

5. Forbindelse ifølge et hvilket som helst af kravene 1 til 4, hvor R' og R" hver er methyl.

6. Forbindelse ifølge et hvilket som helst af kravene 1 til 4, hvor en af R' og R" er H, og en af R' og R" er methyl.

7. Forbindelse ifølge et hvilket som helst af kravene 1 til 4, hvor R' og R" sammen med det C-atom,

hvortil de er bundet, tilvejebringer en pentylring.

8. Forbindelse ifølge et hvilket som helst af kravene 1 til 4, hvor R' og R" svarer til sidekæderne af en naturligt forekommende aminosyre.

9. Forbindelse ifølge krav 1 udvalgt fra:
Gemcitabin-[phenyl-(benzoxy-L-alaninyl)]-phosphat (CPF 31) Gemcitabin-[para-chlorphenyl-(benzoxy-L-alaninyl)]-phosphat (CPF 40) og Gemcitabin-[para-chlorphenyl-(benzoxy-α α,-dimethylglycinyl)]-phosphat (CPF 41); og farmaceutisk acceptable salte, estere og salte af en sådan ester deraf.

10. Forbindelse ifølge krav 1, der er Gemcitabin-[phenyl-(benzoxy-L-alaninyl)]-phosphat (CPF 31) eller et farmaceutisk acceptabelt salt, en ester eller et salt af en sådan ester deraf.

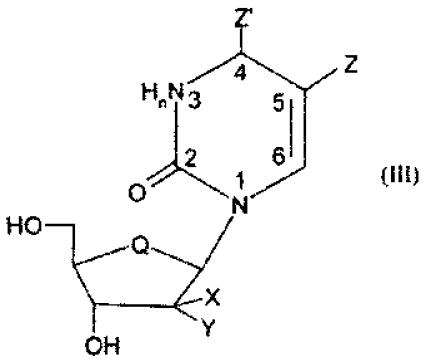
11. Forbindelse ifølge krav 1, der er Gemcitabin-[phenyl-(benzoxy-L-alaninyl)]-phosphat (CPF 31).

12. Forbindelse ifølge et hvilket som helst af de foregående krav til anvendelse i en fremgangsmåde til profylakse eller behandling af cancer.

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

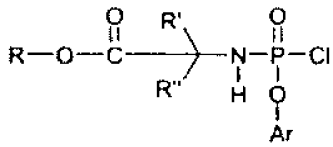
14. Fremgangsmåde til fremstilling af en farmaceutisk sammensætning, hvilken fremgangsmåde omfatter trinnet med kombinering af en forbindelse ifølge et hvilket som helst af kravene 1 til 11 med en/et farmaceutisk acceptabel/t excipiens, bærer eller fortyndingsmiddel.

15. Fremgangsmåde til fremstilling af en forbindelse med formel I ifølge krav 1, hvilken fremgangsmåde omfatter omsætning af en forbindelse med formlen (III):



(III)

med en forbindelse med formlen (IV)



(IV)

hvor Ar, n, Q, R, R', R'', X, Y, Z og Z' har betydningerne beskrevet i krav 1, og en dobbeltbinding eksisterer mellem position 3 og position 4.

DRAWINGS

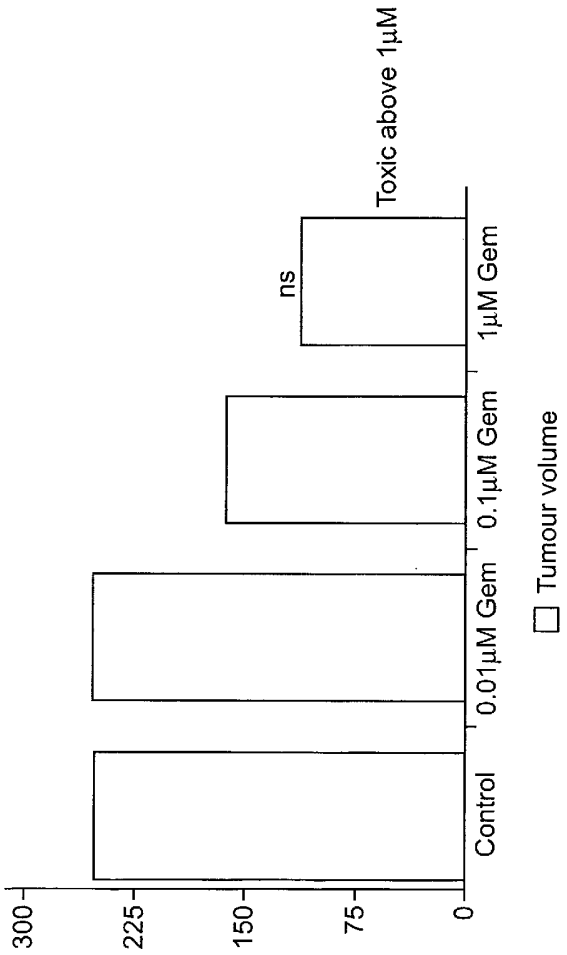


FIG. 1

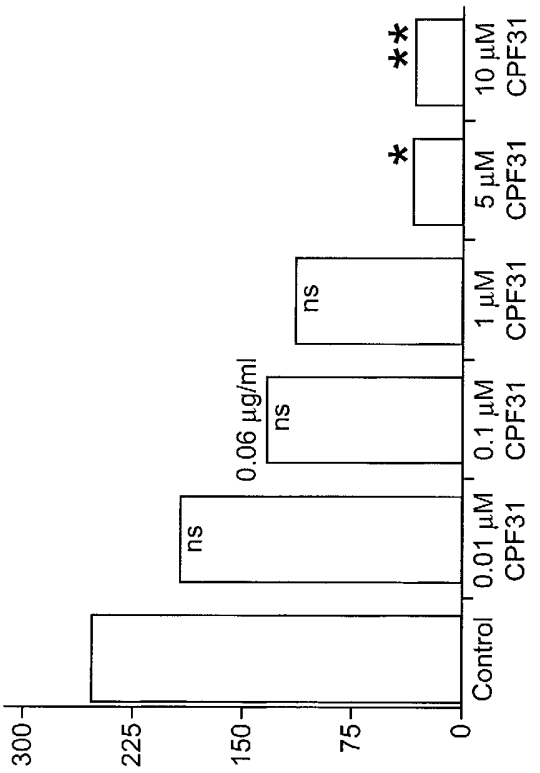

300
225
150
75
0
Control
0.01 µM CPF31
0.06 µg/ml
0.1 µM CPF31
1 µM CPF31
5 µM CPF31
10 µM CPF31
ns
ns
ns
*
**
Tumour volume
* p=0.096 vs control; ** p=0.094 vs control

FIG. 2

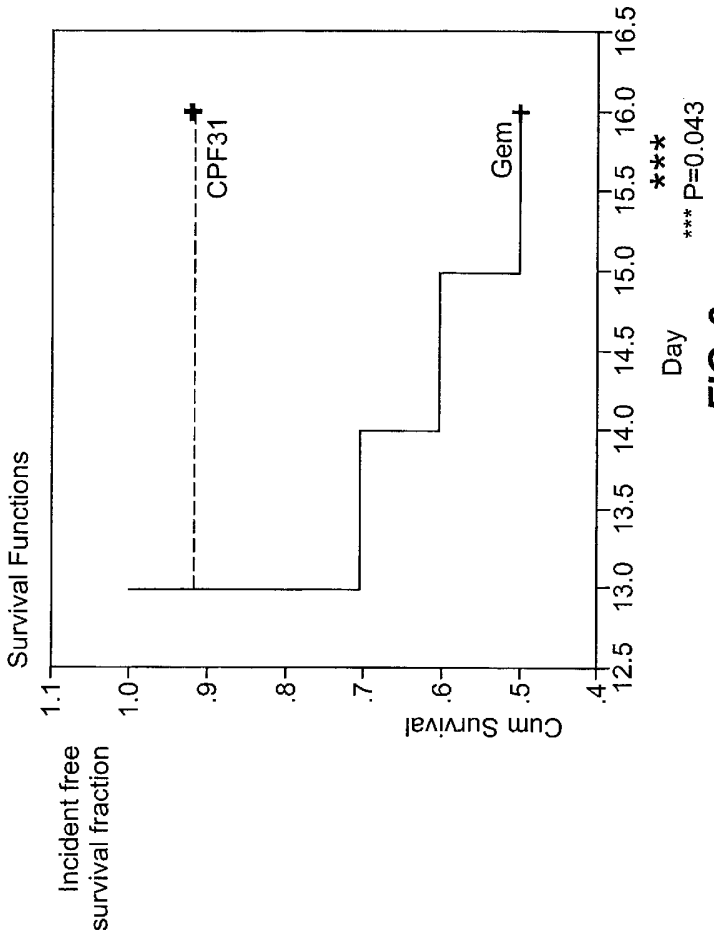

Survival Functions
Incident free survival fraction
1.1
1.0
.9
.8
.7
.6
.5
.4
Cum Survival
12.5
13.0
13.5
14.0
14.5
15.0
15.5
16.0
16.5
Day
CPF31
Gem
***
*** P=0.043

FIG. 3

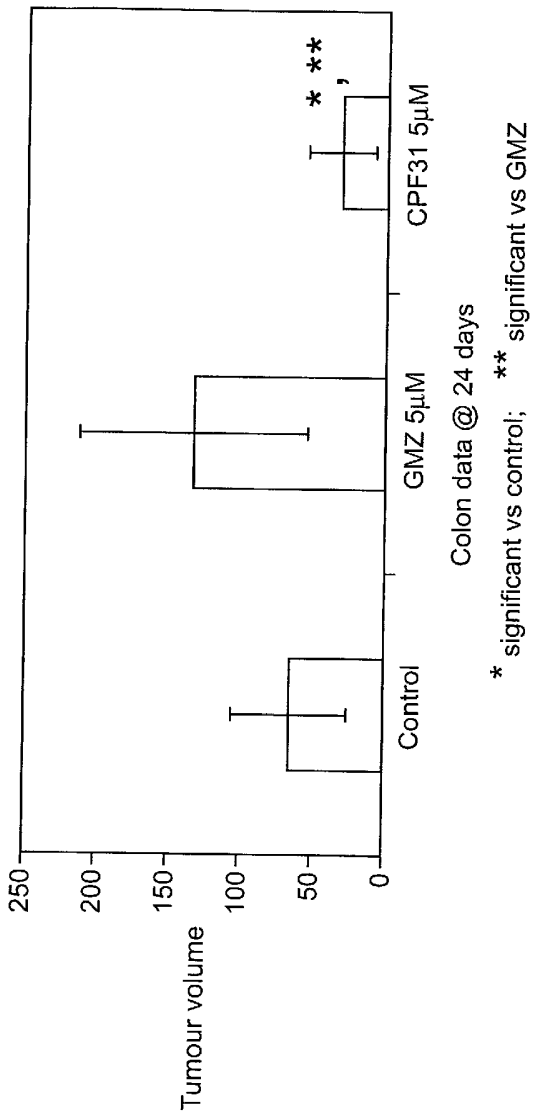

250
200
150
100
50
0
Tumour volume
Control
GMZ 5µM
CPF31 5µM
* **
Colon data @ 24 days
* significant vs control; ** significant vs GMZ

FIG. 4