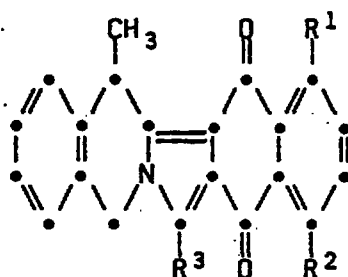


This invention relates to new isoquinoline derivatives, to processes for their preparation, to pharmaceutical compositions containing them, and to their use in medicine.

Isoquinoline compounds have been reported in, for example, EP-A-108620 and EP-A-161102 as having anti-cancer activity. We have now found a novel group of isoquinoline compounds which have good anti-cancer activity. The novel compounds of the invention also exhibit particularly advantageous physico-chemical properties.

The invention thus provides compounds of the general formula

(1)



(1)

wherein

R^1 and R^2 , which may be the same or different, each represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(OH)(OR^4)$ [where R^4 represents a hydrogen atom, or an alkyl (optionally substituted by a hydroxyl, alkoxy, cyclic ether or cycloalkyl group), alkenyl, cycloalkyl, aryl, aralkyl or aroylalkyl group], with the proviso that at least one of R^1 and R^2 represents a group $-OP(O)(OH)(OR^4)$; and R^3 is a hydrogen or halogen atom or a methyl group; and salts, especially physiologically acceptable salts, thereof.

Compounds of formula (1) may exist as stereoisomers and/or tautomers and the invention is to be understood to include all such isomers of compounds of formula (1), including mixtures thereof.

Compounds of formula (1) may form salts with bases. It will be appreciated that, for pharmaceutical use, these salts should be physiologically acceptable, but other salts may find use, for example in the preparation of compounds of formula (1) as well as physiologically acceptable salts thereof. Examples of salts of

compounds of general formula (1) include alkali metal salts such as lithium, sodium and potassium salts, amine salts, for example ammonium and mono-, di- or tri-substituted ammonium, e.g. triethylammonium salts and amino acid salts, for example arginine salts. Sodium salts are particularly useful.

References hereinafter to compounds of formula (1) and their use and preparation should, unless the context dictates otherwise, be taken to be references to the compounds and their salts, eg the physiologically acceptable salts.

In the compounds of formula (1), when the group R^4 is an alkyl group it may be for example a straight or branched C_{1-8} alkyl group, or more especially a straight or branched C_{1-6} alkyl group such as a methyl, ethyl, n-propyl, i-propyl, n-butyl or n-pentyl group. Such alkyl groups may be substituted by for example, a hydroxyl, C_{1-4} alkoxy, eg methoxy or ethoxy, 3 to 7 membered cyclic ether eg tetrahydrofuranyl, or C_{3-7} cycloalkyl eg cyclohexyl, groups.

When R^4 in the compounds of formula (1) represents an alkenyl group it may be for example a C_{3-8} alkenyl group, eg allyl.

When R^4 in the compounds of formula (1) represents a cycloalkyl group it may be for example a C_{3-7} cycloalkyl group, eg cyclohexyl.

When R^4 in compounds of formula (1) represents an aryl group it may be for example a phenyl group optionally substituted by a group R^5 where R^5 is a hydroxy, C_{1-4} alkoxy, eg methoxy, or nitro group.

When R^4 is an aralkyl group it may be for example a phenylalkyl group in which the phenyl portion is optionally substituted by a group R^5 as just defined, and may be in particular a benzyl group.

When R^4 in the compounds of formula (1) represents an aroylalkyl group it may be for example a benzoyl C_{1-3} alkyl group in which the benzoyl portion is optionally substituted in the phenyl ring by a group R^5 as defined above, and may be in particular a benzoylmethyl group.

When the group R^3 in the compounds of formula (1) is a halogen atom it may be a fluorine, chlorine, bromine or iodine atom.

According to one embodiment the invention provides compounds according to formula (1) in which;

— R^1 and R^2 , which may be the same or different, each represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(OH)(OR^4)$ [where R^4 represents a hydrogen atom or an alkyl (optionally substituted by a hydroxyl, alkoxy or cycloalkyl group) aryl or aralkyl group], with the proviso that at least one of R^1 and R^2 represents a group $-OP(O)(OH)(OR^4)$, and R^3 is a hydrogen or halogen atom or a methyl group, and salts thereof.

In the compounds of formula (1), R^3 is preferably a methyl group or, more particularly, a hydrogen atom.

Particular examples of the group R^4 include a hydrogen atom, a straight or branched C_{1-5} alkyl group, a C_{1-4} alkoxyethyl group (such as 2-methoxyethyl), a tetrahydrofuranylmethyl group (eg 2-tetrahydrofuranylmethyl), a C_{5-7} cycloalkylmethyl group (eg cyclohexylmethyl), a C_{3-6} alkenyl group (eg allyl), a C_{5-7} cycloalkyl group (eg cyclohexyl), phenyl, phenyl C_{1-3} alkyl (eg benzyl) in which the phenyl portion is optionally substituted by a nitro (eg p-nitro) group or a benzoyl C_{1-3} alkyl group (eg benzoylmethyl).

In the compounds of formula (1), R^4 is preferably a straight C_{1-5} alkyl group (eg methyl or n-propyl), a tetrahydrofuranylmethyl group, a phenyl C_{1-3} alkyl group (eg benzyl) or a benzoyl C_{1-3} alkyl group (eg benzoylmethyl).

Compounds of formula (1) in which R^1 is a hydrogen atom and R^2 is a group $-OP(O)(OH)(OR^4)$ (where R^4 is as defined above, especially benzyl or benzoylmethyl) and salts thereof are preferred.

A preferred group of compounds of formula (1) are those in which R^1 is a hydrogen atom, R^2 is a group $-OP(O)(OH)(OR^4)$ and R^3 is a hydrogen atom and salts thereof. Such compounds in which R^4 is a straight C_{1-5} alkyl group (eg methyl or n propyl), a tetrahydrofuranylmethyl group, a phenyl C_{1-3} alkyl group (eg benzyl) or a benzoyl C_{1-3} alkyl group (eg benzoylmethyl) are particularly preferred.

A particularly important compound according to the invention is phenylmethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid and the salts thereof, particularly the sodium salt.

Another important compound according to the invention is 2-oxo-2-phenylethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-

[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid and the salts thereof, particularly the sodium salt.

The compounds of formula (1) possess anticancer activity, particularly against tumours such as carcinomas, sarcomas and hepatomas.

Thus, when a compound of formula (1) is administered intraperitoneally or intravenously to mice with a subcutaneous tumour arising from an implant of S180 cells, subsequent examination has shown that tumour growth has been significantly reduced and in some cases total regression of the tumour has occurred. Activities against HT29 Human Colon Xenograft in Nude Mice and Mouse Adenocarcinoma MAC30T have also been shown.

Compounds according to the invention also have especially favourable physico-chemical properties: Thus, for example, compounds of the invention have improved water solubility at near to physiological pH [e.g. pH 6.0 to pH 8.0] over the isoquinoline compounds described in EP-A-108620 and EP-A-161102 which makes them particularly suitable for formulation for parenteral administration.

According to a further aspect of the present invention we therefore provide a compound of formula (1) or a physiologically acceptable salt thereof for use in the treatment of the human or non-human animal body to combat cancer, particularly tumours, therein.

According to a yet further aspect of the present invention we provide the use of a compound of formula (1) or a physiologically acceptable salt thereof for the manufacture of a therapeutic agent for the treatment of the human or non-human animal body to combat cancer, particularly tumours, therein.

It will be appreciated that the compounds according to the invention may advantageously be used in conjunction with one or more other therapeutic agents, such as for example anti-emetic agents,

immunosuppressive agents or different anti-cancer agents. It is to be understood that the present invention covers the use of a compound of formula (1) or a physiologically acceptable salt thereof in combination with one or more other therapeutic agents.

5 In a further aspect of the present invention we provide a pharmaceutical composition comprising as an active ingredient a compound of formula (1) or a physiologically acceptable salt thereof together with one or more pharmaceutical carriers or excipients.

10 The compounds according to the invention may be formulated for administration in any convenient way, and the invention therefore also includes within its scope pharmaceutical compositions comprising a compound of formula (1) or a physiologically acceptable salt thereof adapted for use in human or veterinary medicine. Such compositions may be presented for use in conventional manner in admixture with one
15 or more physiologically acceptable carriers or excipients. The compositions may optionally further contain one or more other therapeutic agents such as a different anti-cancer agent or an anti-emetic agent or an immunosuppressive agent.

20 Thus, the compounds according to the invention may be formulated for oral, buccal, topical, rectal or, preferably, parenteral administration (eg by bolus injection or intravenous infusion).

25 Injections are sterile products and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers, for example, with an added preservative. The compositions may take such forms as solutions, suspensions or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, bulking agents, chelating agents, antimicrobial agents,
30 solubilising agents, surfactants and/or tonicity adjusting agents. Alternatively, the active ingredient (with or without added substances) may be in a dry form for constitution with a suitable vehicle, eg sterile pyrogen-free water or dextrose, before use. The dry solid presentation may be prepared by filling a sterile powder
35 aseptically into individual sterile containers or by filling a sterile

solution aseptically into each container and freeze-drying.

The compounds according to the invention may also be formulated as compositions for oral administration. As tablets or capsules, they may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, surfactants, non-aqueous vehicles, preservatives, sugars, sweetening agents, buffers, colours, antioxidants and/or flavours. The compounds may also be formulated as suppositories, eg containing conventional suppository bases such as cocoa butter or other glycerides.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For topical administration the compounds according to the invention may be formulated as ointments, creams, lotions, powders, pessaries, sprays, aerosols or drops (eg eye or nose drops). Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Such bases may thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents and/or colouring agents. Powders may be formed with the aid of any suitable powder base, for example talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents. Aerosol sprays are conveniently delivered from pressurised packs, with the use of a suitable propellant, eg dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

The compositions may contain 0.1-100% of the active material.

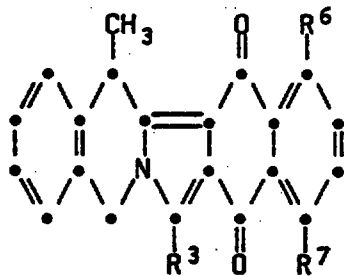
It may be possible to target a compound of the invention to a tumour by including in the composition specialised drug carrier systems such as liposomes, albumen microspheres or monoclonal antibodies.

For systemic administration the daily dosage as employed for adult human treatment will generally be within the range of from 5 mg to 5000 mg, preferably 50 mg to 2000mg, which may be administered in 1 to 5 divided daily doses, for example, depending on the route of administration and the condition of the patient. When the compositions comprise dosage units, each unit will preferably contain 10 mg to 2000 mg of active ingredient, for example 50 mg to 1000mg.

For topical administration the daily dosage as employed for adult human treatment will generally range from 0.1 mg to 1000mg, depending on the condition of the patient.

The compounds useful according to the invention may be prepared by a number of processes described in the following, wherein the various groups and symbols are as defined for formula (1) unless otherwise specified.

Thus, according to one process (A) a compound of formula (1) may be prepared by reacting a compound of formula (2)



[in which one of R^6 and R^7 represents a group $-OP(O)(X^1)(X^2)$ (where X^1 represents an atom or group convertible to a hydroxyl group and X^2 represents a hydroxyl group or an X^1 moiety) and the other represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(X^1)(X^2)$ as just defined] to convert at least one X^1 moiety to hydroxy to produce a compound of formula (1), for example by hydrolysis or ether cleavage.

Examples of X^1 as atoms capable of being converted to hydroxy include halogen atoms such as chlorine or bromine atoms.

Examples of X^1 as groups capable of being converted to hydroxy include the group OX^3 [where X^3 may be, for example, an alkyl group (optionally substituted by a hydroxyl, alkoxy, cyclic ether or cycloalkyl group) or an alkenyl, cycloalkyl, aryl, aralkyl or aroylalkyl group].

The conversion of X^1 to hydroxy may be carried out using conventional procedures. Thus, for example, a halogen atom may be displaced by a hydroxyl group by hydrolysis, eg basic hydrolysis using a suitable base such as an alkali metal hydroxide in a suitable solvent eg aqueous tetrahydrofuran.

The conversion of OX^3 to hydroxy may be carried out using a nucleophile eg a halide ion such as an iodide ion (obtained, for example, from an alkali metal halide such as sodium iodide) or a quaternary ammonium halide such as triethylamine hydroiodide in an inert solvent such as a ketone (eg acetone or butan-2-one) or a substituted amide (eg dimethylformamide), or by reaction with a trialkylsilyl iodide such as trimethylsilyl iodide in a solvent such as a halogenated hydrocarbon (eg carbon tetrachloride) followed by hydrolysis, eg basic hydrolysis using a suitable base such as an alkali metal hydroxide.

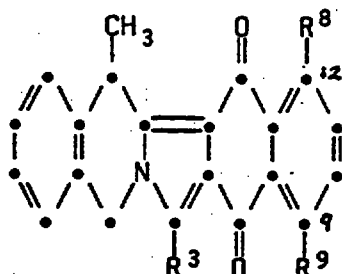
The reaction may be carried out at any suitable temperature, for example in the range -10^0 to $+100^0C$.

It will be appreciated that in accordance with conventional practice the reaction may be managed such that when $-OP(O)(X^1)(X^2)$ represents $-OP(O)(OX^3)(OX^3)$ only one of the two OX^3 groups is converted to a hydroxyl group. In these circumstances secondary phosphates of formula (1) [ie in which R^4 is an alkyl group (optionally substituted by a hydroxyl, alkoxy, cyclic ether or cycloalkyl group) or an alkenyl, cycloalkyl, aryl, aralkyl or aroylalkyl group] are prepared. Alternatively, both OX^3 groups may be converted to hydroxyl groups using trimethylsilyl iodide. In these circumstances primary phosphates of formula (1) [i.e. in which R^4 is a hydrogen atom] are prepared.

It will be further appreciated that the reaction may be managed such that when an alkali metal halide (such as lithium, sodium or

potassium iodide) is used the appropriate alkali metal salt of a compound of formula (1) is prepared.

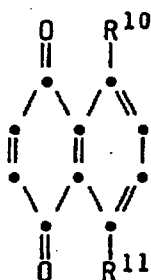
According to another process (B) a compound of formula (1) may be prepared by reacting a compound of formula (3)



(in which one of R^8 and R^9 represents a hydroxyl group and the other represents a hydrogen atom or a hydroxyl group) to convert a hydroxyl group at the 9 and/or 12 position to a group $-OP(O)(OH)(OR^4)$ where R^4 is as defined in formula (1) above.

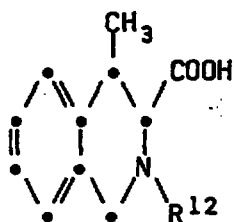
The conversion may be effected by conventional means, for example by reaction of a compound of formula (3) with a halophosphate $HalP(O)(OH)(OR^4)$ or $HalP(O)(X^1)(X^2)$ (where Hal is a halogen atom eg chlorine and X^1 and X^2 are as previously defined) or a pyrophosphate $(R^4O)(HO)(O)P-O-P(O)(OH)(OR^4)$ or $(X^1)(X^2)(O)P-O-P(O)(X^1)(X^2)$ (where X^1 and X^2 are as previously defined), followed, where necessary, by conversion of one or more X^1 moieties to hydroxy according to the procedure described in process (A) above. The reaction with the phosphorylating agent may conveniently be carried out in the presence of a base, for example an alkali metal hydride such as sodium hydride, or an alkali or alkaline earth metal carbonate eg potassium carbonate in an inert solvent such as an ether, e.g. tetrahydrofuran, an aromatic hydrocarbon eg toluene or a ketone eg acetone or a mixture of such solvents. The reaction may conveniently be carried out at a temperature in the range $0^{\circ}C$ to $100^{\circ}C$ eg $20^{\circ}C$. It will be appreciated that reaction with a halophosphate $HalP(O)(X^1)(X^2)$ or a pyrophosphate $(X^1)(X^2)(O)P-O-P(O)(X^1)(X^2)$ leads initially to the formation of a compound of formula (2) which may, if desired, be isolated.

According to another process (C) a compound of formula (1) in which R^3 is a hydrogen atom or a methyl group may be prepared by condensing a quinone of formula (4)



(4)

[in which one of R^{10} and R^{11} represents a group $-OP(O)(OH)(OR^4)$ or $-OP(O)(X^1)(X^2)$ (where X^1 and X^2 are as defined above) and the other represents a hydrogen atom, a hydroxyl group, a group $-OP(O)(OH)(OR^4)$ or a group $-OP(O)(X^1)(X^2)$ (where X^1 and X^2 are as defined above)] with a compound of formula (5)



(5)

(in which R^{12} represents a hydrogen atom or a group $-CHO$ or $-COCH_3$) and subsequently, if required, converting one or more X^1 moieties to hydroxy to form a compound of formula (1).

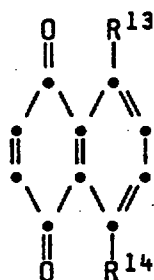
The reaction between the compounds of formulae (4) and (5) may be carried out in the presence of an alkanolic acid anhydride, such as acetic anhydride, at an elevated temperature, eg $100^\circ C$. The conversion of one or more X^1 moieties to hydroxy may be effected using conditions described in process (A) above.

The compounds of formulae (1), (2) and (3) in which R^3 is a halogen atom may be prepared by halogenating a corresponding compound in which R^3 is a hydrogen atom. Standard halogenation procedures may be used, for example, by reaction with a N-chloro-, N-bromo- or N-iodoimide, eg N-chloro, N-bromo- or N-iodosuccinimide, or perchloryl fluoride in an inert solvent such as dichloromethane at ambient temperature.

The compounds of formula (2) may be prepared from compounds of formula (3) as described above in Process (B). Alternatively, the compounds of formula (2) in which R^3 is hydrogen or methyl may be

prepared by condensing a compound of formula (4) in which one of R^{10} and R^{11} is a group $-OP(O)(X^1)(X^2)$ and the other is a hydrogen atom, a hydroxyl group or a group $-OP(O)(X^1)(X^2)$ with a compound of formula (5) according to the method of process (C) above.

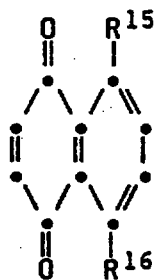
The compounds of formula (3) in which R^3 is hydrogen or methyl may be prepared by condensing a quinone of formula (6)



(6)

(in which one of R^{13} and R^{14} is a hydroxyl group or a protected hydroxyl group such as an acyloxy group eg acetoxy or halomethylcarbonyloxy and the other is a hydrogen atom, a hydroxyl group or a protected hydroxy group as just defined) with a compound of formula (5) according to the method of process (C) above, followed, where necessary, by removal of the protecting group, eg under acidic conditions using, for example, hydrochloric acid in a solvent such as tetrahydrofuran.

The compounds of formula (4) may be prepared by phosphorylation of a compound of formula (7)



(7)

(in which one of R^{15} and R^{16} is a hydroxyl group and the other is a hydrogen atom or a hydroxyl group) according to the method described in process (B) above.

The compounds of formulae (5), (6) and (7) are known compounds described in EP-A-161102 and GB-A-2175587.

The intermediates of formula (2) are novel compounds and form a further aspect of the invention. Key intermediates of formula (2) are those in which R^3 is a hydrogen atom, R^6 is a hydrogen atom and R^7 is a group $-OP(O)(X^1)(X^2)$ where X^1 and X^2 both represent OX^3 . Such intermediates in which X^3 is benzoylmethyl or, more particularly, benzyl are of special interest.

Where a salt of a compound of formula (1) is formed, the corresponding acid may be obtained by conventional means by addition of a suitable acid e.g. a mineral acid such as hydrochloric acid.

Where a compound of formula (1) is produced in the acid form, a corresponding salt may be obtained by conventional means, e.g. by addition of a base providing the required cation, for example sodium hydroxide or an alkali metal carboxylate such as a sodium carboxylate (eg sodium 2-ethylhexanoate). Thus, a salt of a compound of formula (1) may be prepared by treating a compound of formula (1) in the acid form with a suitable base in a solvent such as water, an ether eg tetrahydrofuran or an alcohol eg isopropyl alcohol or a mixture thereof. The reaction may conveniently be carried out at ambient temperature. Alternatively, a salt of a compound of formula (1) may be obtained by treating a suitable compound of formula (2) with an alkali metal halide (eg lithium iodide, sodium iodide or potassium iodide) in a suitable solvent such as water or a ketone (eg acetone or butan-2-one) or a mixture thereof, conveniently at ambient temperature. An amino acid salt of a compound of formula (1) may be obtained by treating a compound of formula (1) in the acid form with an amino acid, eg L-arginine monohydrate in water at ambient temperature.

A salt of a compound of formula (1) may be converted to a different salt, eg a physiologically acceptable salt, by exchange of ion using conventional means.

When a specific enantiomer of formula (1) is required, this may be prepared by conventional methods of resolution known per se. Thus, for example, racemic compounds of the invention may be resolved as salts with optically active bases, eg (+)-dehydroabietylamine or (R)-(+)- or (S)-(-)-~~α~~-methylbenzylamine. Alternatively, optically active compounds of formula (1) may be prepared from optically active

intermediates, eg optically active intermediates of formula (5). Racemic compounds of formula (5) in which R¹² is hydrogen may be resolved, for example, as salts with optically active bases as above or with optically active acids, eg (L)-(+)- or (D)-(-)-tartaric acid. Compounds of formula (5) in which R¹² is CHO or COCH₃ may be similarly resolved as salts with optically active bases.

The following non-limiting Examples illustrate the invention. All temperatures are in °C. Unless otherwise stated all UV spectral data relate to solutions in ethanol of the compounds concerned.

Intermediate 1

Juglone 5-bis(phenylmethyl)phosphate

A solution of sulphuryl chloride (3.48g) in toluene (25ml) was added to a stirred solution of dibenzyl phosphite (6.08ml) in toluene (75ml) under nitrogen. The mixture was stirred for 75 min. under nitrogen, washed with 8% sodium bicarbonate solution (75ml) and the organic layer separated and dried over anhydrous sodium sulphate. After filtration the filtrate was evaporated and dried to give dibenzyl chlorophosphate (7.75g) as a colourless oil. Potassium carbonate (1.81g) was added to a solution of 5-hydroxy-1,4-naphthalenedione (2.18g) in acetonitrile (300ml) with stirring at room temperature. Freshly prepared dibenzyl chlorophosphate (7.75g, see above) in acetonitrile (50ml) was added and the mixture stirred for 20h. Potassium carbonate (907mg) was then added and a further quantity (907mg) of potassium carbonate added after another 24h. The mixture was evaporated to dryness and dried in vacuo to give an oil. The oil was subjected to column chromatography (silica, 500g, 70-230 mesh) eluting with dichloromethane:acetone (96:4). Fractions (250ml) were collected and fractions 4-12 combined and the solvent evaporated. After drying in vacuo the title compound (3.18g) was obtained as an orange solid; δ (CDCl₃) 5.28 (OCH₂), 7.38(Ph).

Intermediate 2

Bis(phenylmethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphate

Acetic anhydride (12ml) was added to a mixture of N-formyl-1,2,3,4-tetrahydroisoquinoline-4-methyl-3-carboxylic acid (625mg) and intermediate 1 (3.165g). The reaction mixture was heated at 100° for 30 min with stirring, then cooled and evaporated to dryness under reduced pressure. The resulting oil was dissolved in dichloromethane (5ml) and ether was added and the solution cooled in an acetone-drikold bath. The resulting yellow solid was collected by filtration and dried at room temperature to give the title compound (1.16g); δ (CDCl₃) 5.2-5.4 (OCH₂), 5.23+5.13 (5H) 4.95 (14H), 1.55 (14CH₃).

Intermediate 3

5,8,13,14-Tetrahydro-9-hydroxy-14-methylbenz[5,6]isoindolo[2,1-b]-isoquinolin-8,13-dione

Water (312ml) and concentrated hydrochloric acid (169ml) were added to a suspension of 5,8,13,14-tetrahydro-9-[iodoacetoxy]-14-methylbenz[5,6]isoindolo[2,1-b]isoquinolin-8,13-dione (80.0 g, Intermediate 9 in GB-A-2175587) in tetrahydrofuran (1.335L). This mixture was stirred and heated to reflux under nitrogen for 18 h and part (550ml) of the solvent was removed by distillation at atmospheric pressure. The resulting suspension was stirred and cooled to ca. 5° for 4.5 h, and the solid was collected by filtration, washed with cold tetrahydrofuran and dried in vacuo to give the title compound (48.8g); $\lambda_{\max}$ 243nm (MeOH), $E_{1\%}^{1\text{cm}}$ 1022; δ (CDCl₃) 12.98 (OH, s, 1H), 5.27 (5-H, d, 16Hz, 1H), 5.16 (5-H, d, 16Hz, 1H), 4.98 (14-H, q, 7Hz, 1H), 1.55 (14-CH₃, d, 7Hz, 3H).

Intermediate 4

Dimethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate

a) A 60%-dispersion of sodium hydride in oil (200mg) was washed free of oil with 40-60° petroleum spirit (2x10ml) under a nitrogen atmosphere. Dry tetrahydrofuran (25ml) was added to the sodium hydride, followed by addition of Intermediate 3 (1.5g) in dry tetrahydrofuran (150ml). The resulting solution was stirred at 20° under nitrogen for 15 min and then treated with a solution of freshly distilled dimethylchloro phosphate (723mg) in dry tetrahydrofuran (5ml). After 1h the reaction mixture was diluted with water (500ml) and extracted with dichloromethane (3x250ml). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green foam which was purified by column chromatography on silica (100g) eluting with dichloromethane-methanol (29:1) to give the title compound as a green foam (1.539g); $\lambda_{\max}$ 241.6nm, E_1^{254} 1052, 369.4nm, E_1^{254} 158; δ (CDCl₃) 5.26 (5-H,d,17Hz,1H), 5.14 (5-H,d,17Hz,1H), 4.96 (14-H,q,7Hz,1H), 4.02 (OCH₃,m, 6H), 1.57 (14-CH₃,d,7Hz,3H).

The following compounds were prepared in a similar manner using Intermediate 3 and the appropriate halophosphate:

b) Diethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (5.609g) from Intermediate 3 (4.0g) and diethyl chlorophosphate (2.305g); $\lambda_{\max}$ 243.5nm, E_1^{254} 1007, 372nm, E_1^{254} 155; δ (CDCl₃) 5.23 (5-H,d,16Hz,1H), 5.13 (5-H,d,16Hz,1H), 4.93 (14-H,q,7Hz,1H), 4.37 (OCH₂CH₃,m,4H), 1.55 (14-CH₃,d,7Hz,3H), 1.38 (OCH₂CH₃,m,6H).

c) Dipropyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (2.068g) from Intermediate 3 (2.0g) and dipropyl chlorophosphate (1.339g); $\lambda_{\max}$ 242.4nm, E_1^{254} 940, 369.6nm, E_1^{254} 132; δ (CDCl₃) 5.25 (5-H,d,16Hz,1H), 5.15 (5-H,d,16Hz,1H), 4.95 (14-H,q,7Hz,1H), 4.27 (OCH₂CH₂CH₃,m,4H), 1.76 (OCH₂CH₂CH₃,m,4H), 1.56 (14-CH₃,d,7Hz,3H), 0.97 (OCH₂CH₂CH₃,m,6H).

d) Bis(1-methylethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (1.563g) from Intermediate 3 (1.5g) and diisopropyl chlorophosphate (1.003g); $\lambda_{\max}$ 241.8nm, E_1^1 880, 369.6nm, E_1^1 132; $\delta(\text{CDCl}_3)$ 5.25 (5-H, d, 16Hz, 1H), 5.15 (5-H, d, 16Hz, 1H), 4.85-5.10 (14-H+OCH(CH₃)₂, m, 3H), 1.56 (14-CH₃, d, 7Hz, 3H), 1.30-1.45 (OCH(CH₃)₂, m, 12H).

e) Dibutyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (1.628g) from Intermediate 3 (1.5g) and dibutyl chlorophosphate (1.143g); $\lambda_{\max}$ 241.8nm, E_1^1 956, 369.8nm, E_1^1 129; $\delta(\text{CDCl}_3)$ 5.25 (5-H, d, 16Hz, 1H), 5.15 (5-H, d, 16Hz, 1H), 4.96 (14-H, q, 7Hz, 1H), 4.31 (OCH₂CH₂CH₂CH₃, m, 4H), 1.62-1.80 (OCH₂CH₂CH₂CH₃, m, 4H), 1.56 (14-CH₃, d, 7Hz, 3H), 1.32-1.52 (OCH₂CH₂CH₂CH₃, m, 4H), 0.92 (OCH₂CH₂CH₂CH₃, m, 6H).

f) Bis(tetrahydro-2-furanylmethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (1.00g) from Intermediate 3 (2.0g) and bis(tetrahydro-2-furanylmethyl) chlorophosphate (6.78g, freshly prepared from phosphorus trichloride and tetrahydrofurfuryl alcohol) except that purification was initially carried out by column chromatography on silica (650g) eluting with dichloromethane-acetone (4:1) and the resulting yellow foam further purified by column chromatography on silica (70g) eluting with dichloromethane-methanol (96:4); $\lambda_{\max}$ 242.2nm, E_1^1 809, 369.8nm, E_1^1 117; $\delta(\text{CDCl}_3)$, 5.24(5-H, d, 17Hz, 1H); 5.16(5-H, d, 17Hz, 1H); 4.96(14-H, q, 7Hz, 1H); 4.30(POCH₂, m, 4H), 4.21(OCH₂CHO, m, 2H); 1.56(14-CH₃, d, 7Hz, 3H)

g) Cyclohexyl methyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (1.409g) from Intermediate 3 (3.0g) and cyclohexyl methyl chlorophosphate (2.9g, freshly prepared from methyl dichlorophosphate and cyclohexanol); $\lambda_{\max}$ 242.4 nm, E_1^1 899, 369.6nm, E_1^1 150; $\delta(\text{CDCl}_3)$ 5.23 (5-H, d, 16Hz,

1H), 5.13(5-H, d, 16H, 1H), 4.95(14-H, q, 7Hz, 1H), 4.64(OCH, m, 1H), 4.01(OCH₃, m, 3H), 1.54(14-CH₃, d, 7H₃, 3H).

h) Bis(2-propenyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-
5 [5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (3.554g) from

Intermediate 3 (3.0g) and diallyl chlorophosphate (2.68g) except that purification was carried out by column chromatography on silica (300g) eluting with dichloromethane-acetone (94:4); $\lambda_{\max}$ 242.2nm, E_1^1 973, 371.8nm, E_1^1 148; δ (CDCl₃), 5.90-6.10(CH, m, 2H), 5.20-5.45 (CH₂, m, 4H), 5.25(5-H, d, 17Hz, 1H), 5.15(5-H, d, 17Hz, 1H), 4.94 (14-H, q, 7Hz, 1H) 4.75-4.90(OCH₂, m, 4H), 1.58(14-CH₃, d, 7H₃, 3H).

i) Dipentyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
15 isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (2.07g) from Intermediate

3 (2.0g) and dipentyl chlorophosphate (1.715g, freshly prepared from phosphorus trichloride and pentan-1-ol) except that after evaporation the oil was dissolved in diethyl ether (50ml), stirred at 20° for 30 min. and the resulting yellow solid filtered and washed with diethyl ether; $\lambda_{\max}$ 242.4 nm, E_1^1 913, 369.4nm, E_1^1 137; δ (CDCl₃) 5.24 (5-H, d, 17Hz, 1H), 5.14(5-H, d, 17Hz, 1H), 4.96 (14-H, q, 7Hz, 1H), 4.20-4.40 (POCH₂CH₂CH₂CH₂CH₃, m, 4H), 1.65-1.85 (POCH₂CH₂CH₂CH₂CH₃, m, 4H), 1.56(14-CH₃, d, 7Hz, 3H), 1.20-1.45 (POCH₂CH₂CH₂CH₂CH₃, m, 8H), 0.88(POCH₂CH₂CH₂CH₂CH₃, m, 6H).

j) Bis(2-methoxyethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-
25 dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate (2.07g) from Intermediate 3 (2.0g) and bis (2-methoxyethyl) chlorophosphate (1.55g, freshly prepared from 2-methoxyethanol and phosphorus oxychloride) except that the column was eluted with dichloromethane-acetone (4:1); $\lambda_{\max}$ 242.2nm, E_1^1 920, 371.6nm, E_1^1 142; δ (CDCl₃), 5.23(5-H, d, 17Hz, 1H), 5.13(5-H, d, 17Hz, 1H), 4.95 (14-H, q, 7Hz, 1H), 4.48(POCH₂, m, 4H), 3.68(POCH₂CH₂, m, 4H), 3.39(OCH₃, s, 3H), 3.40(OCH₃, s, 3H), 1.57(14-CH₃, d, 7Hz, 3H).

k) Bis(cyclohexylmethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate (5.302g) from Intermediate 3 (4.0g) and bis(cyclohexylmethyl) chlorophosphate (8.22g, freshly prepared from phosphorus trichloride and cyclohexylmethanol) except that after evaporation the solid was triturated with ether (100ml) and crystallised from ethyl acetate/chloroform; $\lambda_{\max}^1$ 242.4nm, E_1^1 778, 369.6nm, E_1^1 119; δ (CDCl₃) 5.25(5-H, d, 16Hz, 1H), 5.15(5-H, d, 16Hz, 1H), 4.96(14-H, q, 7Hz, 1H), 4.09(OCH₂, m, 4H), 1.54(14-CH₃, d, 7Hz, 3H).

Intermediate 5

a) Bis(phenylmethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate

N-chlorosuccinimide (1.017g) was added to a stirred solution of dibenzyl phosphite (3.3ml) in dry benzene (30ml) and the resulting solution was stirred at 20°, the succinimide was removed by filtration and the filtrate was concentrated to a low volume (about 5-10ml). This solution was then used in the following preparation.

A 60%-dispersion of sodium hydride in oil (134mg) was washed free of oil with 40-60° petroleum spirit (2x15ml) under a nitrogen atmosphere. Dry tetrahydrofuran (25ml) was added to the sodium hydride followed by addition of Intermediate 3 (1.0g) in dry tetrahydrofuran (100ml). The resulting red solution was cooled to 10° in an ice-bath and treated with freshly prepared dibenzyl chlorophosphate in benzene (about 5-10ml, see above). After 2h at 10°, the reaction mixture was allowed to warm to 20° and maintained at this temperature for 3h. The reaction mixture was diluted with water (500ml) and extracted with dichloromethane (3x250ml). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green oil which was dissolved in dichloromethane and applied to a column of silica (300g) prepared using dichloromethane-acetone (96:4). The column was eluted with dichloromethane-acetone (96:4) and 25ml fractions were collected. The product-containing fractions were combined and evaporated to yield the title compound as a green foam (1.211g); $\lambda_{\max}^1$ 244nm, E_1^1 743, 374nm, E_1^1 112; δ (CDCl₃) 5.20-5.40

(OCH₂C₆H₅,m,4H), 5.23 (5-H,d,16Hz,1H), 5.13 (5-H,d,16Hz,1H), 4.95 (14-H,q,7Hz,1H), 1.55 (14-CH₃,d,7Hz,3H).

The following compound was prepared in a similar manner:

b) Bis[(4-nitrophenyl)methyl],[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate, (3.662g) from Intermediate 3 (2.0g) and di(p-nitrobenzyl) chlorophosphate (2.582g); $\lambda_{\max}$ 244nm, E_1^1 330; δ (d₆-DMSO) 5.48 (OCH₂,d,8Hz,4H), 5.42 (5-H,d,18Hz,1H), 5.32 (5-H,d,18Hz,1H), 4.79 (14-H,q,7Hz,1H), 1.48 (14-CH₃,d,7Hz,3H).

Intermediate 6

5,8,13,14-Tetrahydro-12-hydroxy-14-methylbenz[5,6]isoindolo[2,1-b]-isoquinolin-8,13-dione

Acetic anhydride (160ml) was added to a mixture of N-formyl-1,2,3,4- tetrahydroisoquinoline-4-methyl-3-carboxylic acid (20g) and 5-hydroxy-1,4-naphthoquinone (31.78g). The reaction mixture was heated at 100° for 30 min and then left to cool (4°) overnight. A precipitate formed which was filtered, washed with ether, dried in vacuo and purified by column chromatography on silica gel eluting with dichloromethane to yield the title compound; $\lambda_{\max}$ 243nm, E_1^1 1103, 397nm, E_1^1 381.

Intermediate 7

Bis(phenylmethyl),[7-bromo-5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate

To a solution of Intermediate 5a) (1.0g) in dichloromethane (50ml) was added N-bromosuccinimide (0.604g) in dichloromethane (50ml). After 1h at 20°, the reaction mixture was evaporated and the residue was purified by column chromatography, firstly on silica (150g) eluting with chloroform-acetone (96/4) followed by silica (40g) eluting with dichloromethane-acetone (96/4) to give the title compound as a brown foam (0.81g); $\lambda_{\max}$ 242.0nm, E_1^1 760, 378.0nm, E_1^1 108; δ

(d₆-DMSO) 5.42 (5-H, d, 17Hz, 1H), 5.34-5.17 (5-H+OCH₂, m, 5H), 4.89 (14-H, q, 7Hz, 1H), 1.49 (14-CH₃, d, 7Hz, 3H).

Intermediate 8

Bis(phenylmethyl), [7-chloro-5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate

To a solution of Intermediate 5a (1.0g) in chloroform (200ml) was added N-chlorosuccinimide (0.226g) followed by a saturated solution of hydrogen chloride in chloroform (0.5ml). After 30 min at 20⁰, the reaction mixture was evaporated to a yellow foam which was purified by column chromatography on silica (100g) eluting with dichloromethane-acetone (96/4) to give a yellow foam (0.943g). Crystallisation of this foam from acetone gave the title compound as a yellow solid (0.697g); λ_{max} 241.6nm, E₁¹ 820, 377.2nm, E₁¹ 125; δ (d₆-DMSO) 5.43 (5-H, d, 17Hz, 1H), 5.32-5.16 (5-H+OCH₂, m, 5H), 4.87 (14-H, q, 7Hz, 1H), 1.49 (14-CH₃, d, 7Hz, 3H).

Intermediate 9

Bis(2-oxo-2-phenylethyl), [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphate

A solution of dicyclohexylcarbodiimide (3.03g) in dichloromethane (125ml) was added dropwise over 3h to a stirred solution of phosphoric acid bis(2-oxo-2-phenylethyl) ester (8.93g) in dichloromethane (500ml) at 20⁰C. The reaction mixture was evaporated to low volume (ca. 100ml) and the precipitated solid was removed by filtration. The filtrate was stirred at 20⁰ for 1 h and then evaporated to give the crude phosphorylating reagent as a colourless oil.

A 60% dispersion of sodium hydride in oil (534mg) was washed free of oil with 40-60⁰ petroleum ether (2 x 25ml) under a nitrogen atmosphere. Dry tetrahydrofuran (100ml) was added to the sodium hydride followed by a solution of Intermediate 3 (4g) in dry tetrahydrofuran (400ml). The resulting solution was stirred at 20⁰ for 30 min, and then treated with the crude phosphorylating reagent. After 15 min. at 20⁰ the reaction mixture was diluted with water (2.5L) and extracted with dichloromethane (3 x 600ml). The organic

extracts were dried over anhydrous sodium sulphate and evaporated to a yellow solid (8.96g). To this solid (7.96g) was added acetone (200ml) and insoluble impurities were removed by filtration. The filtrate was evaporated and the residue was subjected to column chromatography on silica (270g) eluting with dichloromethane-methanol (96/4) to give a yellow foam. This foam was purified by further column chromatography on silica (100g) eluting with dichloromethane-acetone (96/4) to give the title compound as a yellow foam (1.14g); I.R. (CHBr₃) 1710cm⁻¹, 1659cm⁻¹; δ (CDCl₃) 5.68-7.81(OCH₂, m, H), 5.22(5-H, d, 16Hz, 1H), 5.15(5-H, d, 16Hz, 1H), 4.95(14-H, q, 7Hz, 1H), 1.55(14-CH₃, d, 7Hz, 3H).

Intermediate 10

a) Diethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-12-yl]phosphate

A 50% dispersion of sodium hydride in oil (158mg) was washed free of oil with 40-60° petroleum spirit (2x10ml) under a nitrogen atmosphere. Dry tetrahydrofuran (25ml) was added to the sodium hydride followed by addition of Intermediate 6 (1.0g) in dry tetrahydrofuran (100ml). The resulting solution was stirred at 20° under nitrogen for 15 min and then treated with a solution of freshly distilled diethyl chlorophosphate (569mg) in dry tetrahydrofuran (5ml). After 1.5h, the reaction mixture was diluted with water (500ml) and extracted with dichloromethane (3x250ml). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green solid (1.484g) which was purified by column chromatography on silica (150g) eluting with chloroform-ethanol (49/1) to give a green solid (1.254g). Crystallisation of this solid from a mixture of chloroform and ethyl acetate gave the title compound as green feathery crystals (904mg); $\lambda_{\max}$ 243nm, E₁¹ 1005, 372nm, E₁¹ 146; δ (CDCl₃) 5.25 (5-H,d,16Hz,1H), 5.15 (5-H,d,16Hz,1H), 5.01 (14-H,q,7Hz,1H) 4.39 (OCH₂CH₃,m,4H), 1.54 (14-CH₃,d,7Hz,3H), 1.42 (OCH₂CH₃,m,6H).

The following compound was prepared in a similar manner :

b) Bis(phenylmethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-12-yl]phosphate (1.5g) from
Intermediate 6 (3.0g) and dibenzyl chlorophosphate (freshly prepared
from dibenzyl phosphate (9.9ml) and N-chlorosuccinimide) in benzene
(ca. 20ml) except that elution was carried out using
dichloromethane-acetone (96:4) to give an oil which crystallised on
standing and was recrystallised from ethyl acetate/chloroform; $\lambda_{\max}$
242.6 nm, E_1^1 733, 371.4 nm, E_1^1 109; $\delta(\text{CDCl}_3)$ 5.20-5.45(OCH_2 + 5-H, m,
5H), 5.15 (5-H, d, 16Hz, 1H), 4.97(14-H, q, 7Hz, 1H), 1.53(14- CH_3 , d,
7Hz, 3H).

Intermediate 11

Dipropyl,[1,4-dioxonaphthalene-5,8-diyl]phosphate

5,8-Dihydroxy-1,4-naphthalenedione (1g) was suspended in
acetone (100ml) and potassium carbonate (4g) and dipropyl
chlorophosphate (5ml) were added. The reaction mixture was stirred at
40-50° for 90 min., filtered to remove excess potassium carbonate and
evaporated to dryness. The residue was dissolved in dichloromethane
and purified by column chromatography on silica using dichloromethane
and dichloromethane-methanol (99:1) as eluant to give the title
compound as a yellow oil (2.31g); $\delta(\text{CDCl}_3)$ 4.23(CH_2), 1.78(CH_2),
1.01(CH_3).

Intermediate 12

Dipropyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-[2,1-b]isoquinolin-9,12-diyl]phosphate

Acetic anhydride (10ml) was added to a mixture of N-formyl-
1,2,3,4-tetrahydroisoquinoline-4-methyl-3-carboxylic acid (440mg) and
Intermediate 11 (2.16g). The reaction mixture was kept at 10° for 30
min. and then evaporated to dryness to give a brown oil.
Chromatography on silica using petrol (60-80°)-ethyl acetate (1:1, 1:2
and 1:3) as eluant gave the title compound (984mg); $\lambda_{\max}$ 237.6nm, E_1^1
609, 371nm, E_1^1 94; $\delta(\text{CDCl}_3)$ 5.13 + 5.23 (5H), 4.93(14H), 4.20-4.35

(CH₂), 1.60-1.90(CH₂), 0.8-1.05(CH₃).

Example 1

[5,8,13,14-Tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-
5 [2,1-b]isoquinolin-9-yl]dihydrogen phosphate, disodium salt

A cooled solution (0 to 5⁰) of Intermediate 4b) (465mg) in
carbon tetrachloride (3ml) was treated under a nitrogen atmosphere
with trimethylsilyl iodide (0.32ml). After 5 min, the reaction mixture
10 was evaporated to dryness and the residual oil was treated with
methanol (5ml). The mixture was stirred at 20⁰ and after about 5 min a
clear brown solution was obtained. After 20 min the solution was
evaporated to a dark foam which was suspended in water (20ml) and the
suspension adjusted to pH 7.5 with 1M-sodium hydroxide solution
(4.00ml). The resulting mixture was extracted with chloroform (25ml)
15 and was filtered through a prewashed bed of kieselguhr using water
(50ml) to wash the product through. The resulting layers were
separated and the aqueous phase was washed with more chloroform
(10ml). The combined aqueous extracts were filtered through a
millipore filter, degassed and freeze-dried. The freeze-dried solid
20 was redissolved in water (100ml) filtered through a millipore filter
and freeze-dried to give the title compound as a bright yellow solid
(0.398g); $\lambda_{\max}$ 246nm, $E_1^{1\%}$ 667, 388nm, $E_1^{1\%}$ 165.

Example 2

25 a) Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (1.551g) was added to a solution of Intermediate
5a) (5.546g) in acetone (100ml) and the resulting solution was heated
under reflux for 1.5h. The reaction mixture was evaporated to dryness
30 and the residue was triturated with ether (100ml) to give a yellow
solid. This solid was dissolved in water (200ml) and the pH of the
solution was adjusted to pH 1 to 2 by the addition of 2M-hydrochloric
acid. The resulting mixture was diluted with water (200ml) and
extracted with dichloromethane (2x400ml). Salt solution was added
35 during the extraction procedure to disperse the emulsion which was

formed. The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green solid. This solid was suspended in water (300ml) and the suspension was adjusted to pH 7.0 by the dropwise addition of 1M-sodium hydroxide solution. The resulting solution was filtered and the filtrate was freeze-dried to a solid which was treated with acetone (400ml) and evaporated to dryness to give a solid (4.49g). Trituration of this solid with ether (200ml) gave the title compound as a yellow solid (4.232g); $\lambda_{\max}$ 243.8nm, $E_1^{1\%}$ 679, 367.2nm, $E_1^{1\%}$ 132; δ (d_6 -DMSO) 5.41 (5-H,d,16Hz,1H), 5.31 (5-H,d,16Hz,1H), 4.93 (OCH_2 ,d,6Hz,2H), 4.82 (14-H,q,7Hz,1H), 1.47 (14- CH_3 ,d,7Hz,3H).

The following compounds were prepared in a similar manner:

b) Propyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt, (1.256g) from Intermediate 4c) (1.5g) and sodium iodide (1.367g) using butan-2-one (35ml) in place of acetone; $\lambda_{\max}$ 243.6nm, $E_1^{1\%}$ 963, 360.0nm, $E_1^{1\%}$ 265; δ (d_6 -DMSO) 5.42 (5-H,d,16Hz,1H), 5.32 (5-H,d,16Hz,1H), 4.81 (14-H,m,1H), 3.78 ($OCH_2CH_2CH_3$,m,2H), 1.40-1.60 (14- CH_3 + $OCH_2CH_2CH_3$,m,3H), 0.82 ($OCH_2CH_2CH_3$,m,3H).

c) (1-Methylethyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt, (910mg) from Intermediate 4d) (1.2g) and sodium iodide (1.094g) using butan-2-one (25ml) in place of acetone; $\lambda_{\max}$ 243.4nm, $E_1^{1\%}$ 943, 366.8nm, $E_1^{1\%}$ 199; δ (d_6 -DMSO) 5.40 (5-H,d,16Hz,1H), 5.30 (5-H,d,16Hz,1H), 4.81 (14-H,q,7Hz,1H), 4.43 ($OCH(CH_3)_2$,m,1H), 1.48 (14- CH_3 ,d,7Hz,3H), 1.11 ($OCH(CH_3)_2$,d,5Hz,6H).

d) Phenylmethyl,[7-bromo-5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt, (338mg) from Intermediate 7 (705mg) and sodium iodide (174mg);

$\lambda_{\max}$ 243.6nm, E_1^1 601, 367.8nm, E_1^1 120; δ (d_6 -DMSO) 5.40
(5-H, d, 17Hz, 1H), 5.21 (5-H, d, 17Hz, 1H), 4.97-4.82 (14-H+OCH₂, m, 3H),
1.48 (14-CH₃, d, 7Hz, 3H).

5 e) Phenylmethyl, [7-chloro-5,8,13,14-tetrahydro-14-methyl-8,13-
dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium
salt, (674mg) from Intermediate 8 (1.068g) and sodium iodide (282mg);
 $\lambda_{\max}$ 243.0nm, E_1^1 688, 374.0nm, E_1^1 98; δ (d_6 -DMSO) 5.40
(5-H, d, 17Hz, 1H), 5.23 (5-H, d, 17Hz, 1H), 5.00-4.79 (14-H+OCH₂, m, 3H),
10 1.50 (14-CH₃, d, 7Hz, 3H).

f) Pentyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt (0.836g)
from Intermediate 4i) (1.207g) and sodium iodide (724mg) using
15 butan-2-one (30ml) in place of acetone; $\lambda_{\max}$ 244.0nm, E_1^1 899, 366.8nm,
 E_1^1 190; δ (d_6 -DMSO) 5.40 (5-H, d, 17Hz, 1H), 5.32 (5-H, d, 17Hz, 1H),
4.81 (14-H, q, 7Hz, 1H), 3.80 (POCH₂CH₂CH₂CH₂CH₃, 4^{et}, 7Hz, 2H),
1.35-1.55 (POCH₂CH₂CH₂CH₂CH₃ + 14-CH₃, m, 5H), 1.10-1.30
(POCH₂CH₂CH₂CH₂CH₃, m, 4H), 0.79 (POCH₂CH₂CH₂CH₂CH₃, t, 7Hz, 3H).

20 g) Cyclohexylmethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-
[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt
(1.017g) from Intermediate 4k) (1.5g) and sodium iodide (411mg) using
butan-2-one (40ml) in place of acetone; $\lambda_{\max}$ 244.0nm, E_1^1 849, 366.8nm,
25 E_1^1 198; δ (d_6 -DMSO) 5.41 (5-H, d, 16Hz, 1H), 5.31 (5-H, d, 16Hz, 1H),
4.81 (14-H, q, 7Hz, 1H), 3.62 (OCH₂, t, 6Hz, 2H), 1.48 (14-CH₃, d, 7Hz,
3H).

h) Phenylmethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-12-yl]phosphoric acid, sodium salt
30 (781mg) from Intermediate 10b) (1.2g) and sodium iodide (336mg) except
that after the pH was adjusted with hydrochloric acid the resulting
mixture was extracted with dichloromethane-methanol (3:2, 400ml and 2

x 200ml); $\lambda_{\max}$ 244.0nm, $E_1^{1\%}$ 842, 265.8nm, $E_1^{1\%}$ 295, 356.0nm, $E_1^{1\%}$ 97; δ (d_6 -DMSO) 5.41(5-H, d, 16Hz, 1H), 5.31(5-H, d, 16Hz, 1H), 4.93(OCH₂, d, 7Hz, 2H), 4.88(14-H, q, 7Hz, 1H), 1.47(14-CH₃, d, 7Hz, 3H).

5

Example 3

a) Methyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

10 A mixture of Intermediate 4a) (1.0g) and sodium iodide (377mg) in acetone (35ml) was heated under reflux for 5h. After this time, a second portion of sodium iodide (189mg) was added, and the reaction mixture was maintained under reflux for 1h. The reaction mixture was cooled to 20° and the precipitated product was collected by
15 filtration, washed with acetone and ether, and dried to give a green solid which was dissolved in water (50ml) the pH of the resulting solution being adjusted to pH 1 to 2 by the addition of 2M-hydrochloric acid. The resulting mixture was diluted with water (50ml) and extracted with dichloromethane (2x100ml). Salt solution was added during the extraction procedure to disperse the emulsion which
20 was formed. The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green solid which was suspended in water (70ml) the resulting suspension being adjusted to pH 7.0 by the dropwise addition of 1M-sodium hydroxide solution. The resulting solution was filtered and the filtrate was freeze-dried to a solid
25 which was suspended in acetone (100ml) and evaporated to dryness to give a solid. Trituration of this solid with ether (100ml) gave the title compound as a yellow solid (858mg); $\lambda_{\max}$ 243.4nm, $E_1^{1\%}$ 991, 365.4nm, $E_1^{1\%}$ 199; δ (d_6 -DMSO) 5.41 (5-H,d,16Hz,1H), 5.31 (5-H,d,16Hz,1H), 4.82 (14-H,q,7Hz,1H) 3.50 (OCH₃,d,11Hz,3H) 1.47 (14-CH₃,d,7Hz,3H).
30

The following compounds were prepared in a similar manner:

b) Butyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt,
 (1.041g) from Intermediate 4e) (1.255g) and sodium iodide (1.082g);
 $\lambda_{\max}$ 243.2nm, E_1^1 984, 366.6nm, E_1^1 189; δ (d_6 -DMSO) 5.41
 (5-H,d,16Hz,1H), 5.31 (5-H,d,16Hz,1H), 4.82 (14-H,q,7Hz,1H), 3.81
 (OCH₂CH₂CH₂CH₃,q,6Hz,2H), 1.15-1.60 (OCH₂CH₂CH₂CH₃+14-CH₃,m,7H), 0.83
 (OCH₂CH₂CH₂CH₃,t,6Hz,3H).

c) Cyclohexyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt, (686mg)
 from Intermediate 4g) (1.0g) except that the final trituration step
 was not necessary; $\lambda_{\max}$ 244.0 nm, E_1^1 850, 367.0 nm, E_1^1 182;
 δ (d_6 -DMSO) 5.42(5-H, d, 16Hz, 1H), 5.32(5-H, d, 16Hz, 1H), 5.48(14-H,
 q, 7Hz, 1H), 4.13(OCH, m, 1H), 1.46(14-CH₃, d, 7Hz, 1H).

Example 4

Ethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-
[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (3.0g) was added to a solution of Intermediate
 4b) (3.0g) in acetone (150ml) and the mixture was heated under reflux
 for 4h. After this time, a second portion of sodium iodide (3.0g) was
 added, and the reaction mixture was maintained under reflux for 16h.
 The reaction mixture was cooled to 20° and the precipitated product
 was collected by filtration, washed with acetone (3x30ml) and dried to
 give the title compound as a green solid (2.37g); $\lambda_{\max}$ 244.5nm, E_1^1
 1050, 368.5nm, E_1^1 222; δ (d_6 -DMSO) 5.41 (5-H,d,16Hz,1H), 5.30
 (5-H,d,16Hz,1H), 4.82 (14-H,q,7Hz,1H), 3.90 (OCH₂CH₃,m,2H), 1.49
 (14-CH₃,d,7Hz,3H), 1.14 (OCH₂CH₃,t,8Hz,3H).

Example 5

Phenyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-
[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

A 60%-dispersion of sodium hydride in oil (67mg) was washed free of oil with 40-60° petroleum spirit (2x10ml) under a nitrogen atmosphere. Dry tetrahydrofuran (10ml) was added to the sodium hydride, followed by addition of Intermediate 3 (500mg) in dry tetrahydrofuran (50ml). The resulting solution was stirred at 20° under nitrogen for 15min and then treated with phenyl dichlorophosphate (381mg). After 1h, the reaction mixture was diluted with water (250ml) and extracted with dichloromethane (3x100ml). Salt solution was added, during the extraction procedure, to disperse the emulsion which was formed. The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to an oily foam (769mg). This foam was dissolved in a mixture of tetrahydrofuran (15ml) and water (5ml) and basified with 1M-sodium hydroxide solution. After 2h at pH 9 and 2h at pH 12, the reaction mixture was warmed to 50-55° and diluted with water (5ml) and tetrahydrofuran (5ml). After 20 min, the reaction mixture was concentrated in vacuo, diluted with water (150ml) and acidified to pH 1 to 2 with 2M-hydrochloric acid. The resulting mixture was extracted with dichloromethane (3x100ml). Salt solution was added during the extraction procedure to disperse the emulsion which was formed. The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a dark green foam (705mg). This foam was suspended in water (100ml) and the suspension was adjusted to pH 7.1 by the dropwise addition of 1M-sodium hydroxide solution. The resulting mixture was filtered and the filtrate was freeze-dried to a foam which was treated with acetone (100ml) and evaporated to dryness to give a solid (440mg). Trituration of this solid with ether (100ml) gave the title compound as a dark green solid (341mg); λ_{max} 243.8nm, E_1^1 918, 369.2nm, E_1^1 174; δ (d_6 -DMSO) 5.42 (5-H,d,16Hz,1H), 5.32 (5-H,d,16Hz,1H), 4.82 (14-H,q,7Hz,1H), 1.48 (14-CH₃,d,7Hz,3H).

Example 6

(4-Nitrophenyl)methyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (330mg) was added to a suspension of Intermediate 5b) (1.359g) in butan-2-one (100ml) and the mixture was heated under reflux for 1.5h. The reaction mixture was evaporated to dryness and the residue was triturated with ether (3x100ml) to give a green solid (1.055g). The solid was recovered by filtration to give the title compound as a green solid (911mg); $\lambda_{\max}$ 243.8nm, E_1^1 837, 265.2nm, E_1^1 438, 368.8nm, E_1^1 142; δ (d_6 -DMSO) 5.39 (5-H,d,16Hz,1H), 5.29 (5-H,d,16Hz,1H), 5.09 (OCH₂,d,7Hz,2H) 4.80 (14-H,q,7Hz,1H), 1.46 (14-CH₃,d,7Hz,3H).

Example 7

[5,8,13,14-Tetrahydro-8,13-dioxo-14-methylbenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]dihydrogen phosphate, disodium salt

Intermediate 5a) (1.6g) was dissolved in dry carbon tetrachloride (5ml) and the resulting solution was cooled in an ice-bath at 0 to 5°. The mixture was allowed to warm to 20° and dry carbon tetrachloride (8ml) was added, followed by dichloromethane (5ml). The resulting solution was cooled at 0 to 5° under nitrogen and treated with trimethylsilyl iodide (0.54ml). After 5 min at 0 to 5°, the reaction mixture was evaporated to dryness and the residual oil was treated with methanol (10ml). The mixture was stirred at 20° and after about 5 min, a clear brown solution was obtained. After 10 min, the solution was evaporated to an oil which was then triturated with diethyl ether (4x25ml) to give a dark yellow solid which was suspended in sterile water (70ml). The pH was adjusted to pH 8.0 by the addition of 0.1M-sodium hydroxide solution to give a solution which was filtered through a glass-fibre filter to remove a small amount of coarse solid and then twice through a large (5cm) millipore filter. The filtrate was freeze-dried to yield the title compound as a dark yellow solid (0.738g); $\lambda_{\max}$ 246nm, E_1^1 667, 388nm, E_1^1 165.

Example 8

2-Oxo-2-phenylethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (243mg) was added to a solution of Intermediate 9 (1.12g) in acetone (25ml). The resulting solution was refluxed for 10 min. and then evaporated. The residue was triturated with ether (3 x 30ml) to give a solid. Water (140ml) was added to this solid and insoluble impurities removed by filtration. The resulting solution was acidified to pH 1.5 by the addition of 2M hydrochloric acid and extracted with dichloromethane (3 x 300ml). The organic extracts were dried over anhydrous sodium sulphate and evaporated to a solid. The solid was suspended in water (40ml) and 0.1M sodium hydroxide solution was added to pH 7.0. The resulting solution was filtered and freeze-dried to a foam. Treatment of this foam with acetone (100ml) gave a solution which was filtered and evaporated. The residue was triturated with ether (50ml) to give the title compound as a yellow solid (500mg); λ_{max} 243.6 nm, $E_1^{1\%}$ 955, 366.4nm, $E_1^{1\%}$ 147; δ (d₆-DMSO) 5.39(5-H, d, 17Hz, 1H), 5.30(5-H, d, 17Hz, 1H), 5.23(OCH₂, d, 8Hz, 2H), 4.82(14-H, q, 7Hz, 1H), 1.48(14-CH₃, d, 7Hz, 3H).

Example 9

Tetrahydro-2-furanylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (272mg) was added to a solution of Intermediate 4f) (952mg) in butan-2-one (50ml). The resulting solution was refluxed for 75min, further sodium iodide (272mg) was added and refluxing continued for 24h. The reaction mixture was evaporated to low volume (ca. 25ml) and refluxing continued for 24h. The precipitated solid was filtered and washed with ether (2 x 40ml). This solid was dissolved in water (75ml) and the solution acidified to pH 1.5 by the addition of 2M hydrochloric acid and extracted with dichloromethane (5 x 750ml). The organic extracts were dried over anhydrous sodium sulphate and evaporated to a foam. This foam was suspended in water (50ml) and 0.1M sodium hydroxide solution was added to pH 7.0. The resulting solution was filtered and freeze-dried to a foam. This foam was treated with acetone (50ml) and the resulting suspension was evaporated. The residue was triturated with ether (50ml) to give the

title compound as a yellow solid (588mg); $\lambda_{\max}$ 244.0nm, E_1^1 867, 366.2nm, E_1^1 178; δ (d_6 -DMSO) 5.40(5-H, d, 17Hz, 1H), 5.30(5-H, d, 17Hz, 1H), 4.80 (14-H, q, 7Hz, 1H), 3.50-4.00($\text{POCH}_2 + \text{OCH}_2\text{CHO} + \text{CH}_2\text{CH}_2\text{O}$ (obscured), m, 5H), 1.48(14- CH_3 , d, 7Hz, 3H).

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Example 10

(2-Propenyl),[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

10 Sodium iodide (697mg) was added to a solution of Intermediate 4h) (1.5g) in acetone (30ml) and the mixture was heated under reflux. After 3h, the reaction mixture was cooled to 20° and the precipitated product was collected by filtration, washed with acetone (10ml) and ether (10ml) and dried to a yellow solid. This solid was dissolved in water (300ml) and the pH of the solution was adjusted to pH 1.5 by the
15 addition of 2M-hydrochloric acid. The resulting mixture was extracted with dichloromethane-ethanol (4:1, 300ml) and then dichloromethane (300ml). Salt solution was added during the extraction procedure to disperse the emulsion which was formed. The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to a green
20 foam. This foam was suspended in water (150ml) and the suspension was adjusted to pH 7.0 by addition of 1M-sodium hydroxide solution. The resulting solution was filtered and the filtrate was freeze-dried to a yellow foam. Trituration of this foam with ether (100ml) gave the
25 title compound as a yellow solid (1.214g); $\lambda_{\max}$ 244.0nm, E_1^1 883, 367.4nm, E_1^1 183; δ (d_6 -DMSO), 5.85-6.00(CH, m, 1H), 4.95-5.30(CH_2 , m, 2H), 5.39(5-H, d, 17Hz, 1H), 5.30(5-H, d, 17Hz, 1H), 4.82(14-H, q, 7Hz, 1H), 4.30-4.50(OCH_2 , m, 2H), 1.48(14- CH_3 , d, 7Hz, 3H).

Example 11

30 Ethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-[2,1-b]isoquinolin-12-yl]phosphoric acid, sodium salt

Sodium iodide (2.0g) was added to a solution of Intermediate 10a) (2.0g) in acetone (100ml) and the mixture was heated under reflux for 5h. After this time, a second portion of sodium iodide (2.0g) was

35

added and the reaction mixture was maintained under reflux for 16h. The reaction mixture was cooled to 20° and the precipitated product was collected by filtration, washed with acetone (2x20ml) and dried to give a green solid (1.75g). Crystallisation of this solid (1.75g) from a mixture of water (20ml) and acetone (800ml) gave the title compound as a green solid (1.125g); $\lambda_{\max}^1$ 243.6nm, E_1^1 973, 367.2nm, E_1^1 173; δ (d_6 -DMSO) 5.41 (5-H, d, 16Hz, 1H), 5.31 (5-H, d, 16Hz, 1H), 4.87 (14-H, q, 7Hz, 1H), 3.88 (OCH_2CH_3 , m, 2H), 1.45 (14- CH_3 , d, 7Hz, 3H), 1.10 (OCH_2CH_3 , t, 6Hz, 3H).

Example 12

2-Methoxyethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz-[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

Sodium iodide (0.637g) was added to a solution of Intermediate 4j) (2.03g) in acetone (40ml). The resulting solution was refluxed for 3h and more sodium iodide (1.274g) was added. Refluxing was continued for 20h. The reaction mixture was evaporated and the residue was redissolved in 2-butanone (40ml). The resulting solution was refluxed for 2h and then evaporated. The residue was triturated with ether (3 x 25ml) to give a solid. Water (200ml) was added and the resulting suspension was acidified to pH 1.5 with 2M hydrochloric acid and extracted with dichloromethane (3 x 300ml). Salt solution was added during the extraction to disperse an emulsion. The organic extracts were dried over anhydrous sodium sulphate and evaporated to a solid. This solid was suspended in water (200ml) and 0.1M sodium hydroxide solution was added to pH 7.0. The mixture was filtered and the filtrate was freeze-dried to a foam. Treatment of the foam with acetone (120ml) gave a solution which was filtered and evaporated. The residue was triturated with ether (40ml) to give the title compound as a yellow solid (1.34g); $\lambda_{\max}^1$ 244.0nm, E_1^1 1034, 366.8nm, E_1^1 208; δ (d_6 -DMSO) 5.40 (5-H, d, 17Hz, 1H), 5.30 (5-H, d, 17Hz, 1H), 4.80 (14-H, q, 7Hz, 1H), 3.96 ($POCH_2$, m, 2H), 3.44 ($POCH_2CH_2$, q, 5Hz, 2H), 3.22 (OCH_3 , s, 3H), 1.47 (14- CH_3 , d, 7Hz, 3H).

Example 13

Propyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo-
[2,1-b]isoquinolin-9,12-diyl]phosphoric acid, sodium salt

Sodium iodide (2.5g) was added to a solution of Intermediate 12 in butan-2-one (50ml) and the reaction mixture was stirred under reflux for 4h and evaporated to dryness. The residue was dissolved in methanol and chromatographed on Sephadex LH 20 in methanol twice to give title compound as a dark yellow solid (595mg), λ_{max} (water) 244.8nm, $E_1^{1\%}$ 637, 356.4nm, $E_1^{1\%}$ 140; δ (DMSO) 5.28 + 5.38 (5H), 4.82 (14H), 3.65-3.85 (CH_2), 1.35-1.6 (14 CH_3 + CH_2), 0.70-0.90 CH_3 . (Sephadex is a registered Trade Mark).

Example 14

4-Nitrophenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz
[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, triethylamine
salt

Triethylamine hydroiodide (278mg) was added to a solution of Intermediate 5b) (750mg) in acetone (75ml). The resulting solution was refluxed for 4h. Further triethylamine hydroiodide (278mg) was added and refluxing continued for a further 17h. The reaction mixture was evaporated and the residual oil was washed with ether. The oil was dissolved in water (100ml) and extracted with dichloromethane (2 x 100ml) then with dichloromethane-methanol (1:1, 100ml) then with dichloromethane (100ml). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated. Water (250ml) was added to the residue and the resulting solution was filtered and freeze-dried to a foam. This foam was dissolved in water (50ml) and the solution filtered. The filtrate was acidified to pH 1.5 with 2M hydrochloric acid and extracted with dichloromethane-methanol (2:1, 450ml) then with dichloromethane (100ml). The combined organic extracts were washed with water and dried over anhydrous sodium sulphate. Triethylamine (0.1ml, 0.7g) was added and the solution was evaporated. The residual oil was dissolved in water (30ml), the solution was filtered and the filtrate was freeze-dried to give the title compound as a yellow foam (256mg); λ_{max} 244.2nm, $E_1^{1\%}$ 739, 365.8nm, $E_1^{1\%}$ 129; δ (d_6 -DMSO) 5.39(5-H, d, 17Hz, 1H), 5.29(5-H, d, 17Hz,

1H), 5.10(POCH₂, d, 8Hz, 2H), 4.80(14-H, q, 7Hz, 1H),
2.98-3.13(N⁺-CH₂CH₃, m, 6H), 1.47(14-CH₃, d, 7Hz, 3H),
1.18(N⁺-CH₂CH₃, t, 7Hz, 9H).

5 Example 15

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl] phosphoric acid

10 Sodium iodide (285mg) was added to a solution of Intermediate
5a) (1.019g) in acetone (25ml) and the mixture was heated under
reflux. After 1.5h, the reaction mixture was evaporated to dryness
and the residue was diluted with water (100ml), acidified to pH 2 to 3
with 2M-hydrochloric acid and extracted with dichloromethane (3 x
100ml). Salt solution was added during the extraction procedure to
15 disperse the emulsion which was formed. The combined organic extracts
were dried over anhydrous sodium sulphate and evaporated to a green
oil which solidified. The solid was stirred in ether (50ml) for 10
min. and then collected by filtration to give a green solid.
Crystallisation of the solid from a mixture of chloroform (containing
a trace of ethanol) and ether gave the title compound as green
20 crystals (427mg); $\lambda_{\max}$ 245.0nm, E_1^{1979} , 369.0nm, E_1^{193} ; δ (d₆-DMSO) 5.44
(5-H, d, 16Hz, 1H), 5.36(5-H, d, 16Hz, 1H), 5.23(POCH₂, d, 8Hz, 2H),
4.86 (14-H, q, 7Hz, 1H), 1.51(14-CH₃, d, 7Hz, 3H).

25 Example 16

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

30 A solution of sodium 2-ethylhexanoate (1.55g) in
tetrahydrofuran (14ml) was added over 2 min to a stirred suspension of
the product of Example 15 (4g) under nitrogen. Water (0.4ml) was
added to the resulting clear solution which was then diluted with
propan-2-ol (215ml) over 40 min. The resulting suspension was stirred
for 20 min and the solid was collected by filtration, washed with
propan-2-ol (3x30ml), sucked dry under nitrogen and dried in vacuo to
give the title compound as a yellow-green amorphous solid (3.19g);
35 $\lambda_{\max}$ 244nm (MeOH), E_1^{1843} , having a similar ¹H N.m.r. spectrum to the

sample obtained in Example 2a).

Example 17

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, potassium salt

Potassium iodide (1.83g) was added to a suspension of Intermediate 5a) (5.90g) in a mixture of acetone (118ml) and water (2.93ml). This mixture was stirred and heated to reflux under nitrogen for 2.5 h, a crystalline precipitate forming after 40 min. The suspension was cooled to 21° and stirred for 1 h and the solid was collected by filtration, washed with acetone (2x25ml) and dried in vacuo to give the title compound (4.32g); $\lambda_{\max}$ 244nm (MeOH), $E_1^{1\%}$ 813; δ (d_6 -DMSO) 5.36 (5-H,d,16Hz,1H), 5.24 (5-H,d,16Hz,1H), 4.96 (OCH₂,d,6Hz,2H), 4.80 (14-H,q,7Hz,1H), 1.48 (14-CH₃,d,7Hz,3H).

Example 18

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, lithium salt

Lithium iodide trihydrate (1.88g) was added to a suspension of Intermediate 5a) (5.90g) in a mixture of acetone (118ml) and water (2.3ml). This mixture was stirred and heated to reflux under nitrogen for 2.7 h, a crystalline precipitate forming after 20 min. The suspension was cooled to ca. 20° and stirred for 2 h and the solid was collected by filtration, washed with acetone (3x50ml) and dried in vacuo at 21° to give the title compound (4.72g); $\lambda_{\max}$ 244nm (MeOH), $E_1^{1\%}$ 847; δ (d_6 -DMSO) 5.43 (5-H,d,16Hz,1H), 5.31 (5-H,d,16Hz,1H), 5.04 (OCH₂,d,6Hz,2H), 4.84 (14-H,q,7Hz,1H), 1.49 (14-CH₃,d,7Hz,3H)

Example 19

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-
isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, L-arginine salt

A suspension of the product of Example 15 (2g) in water (80ml) was stirred under nitrogen and a solution of L-arginine monohydrate (0.70g) in water (10ml) was added dropwise over 15 min to adjust the pH to 6.1. The pH was raised to 7.0 by the addition of part (2ml) of

a solution of L-arginine (0.1g) in water (5ml), and the resulting clear solution was filtered. The filtrate was freeze-dried to give the title compound as a yellow-green solid (2.61g); $\lambda_{\max}$ 244 nm (MeOH), $E_1^{1\%}$ 648; δ (d_6 -DMSO) 5.41 (5-H,d,16Hz,1H), 5.29 (5-H,d,16Hz,1H), 5.02 (OCH₂,d,6Hz,2H), 4.83 (14-H,q,7Hz,1H), 3.14 (NCH₂,t,6Hz,2H), 1.50 (14-CH₃,d,7Hz,3H).

Example 20

Phenylmethyl,[5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, sodium salt

a) Sodium iodide (5.94g) was added to a suspension of Intermediate 5a (21.2g) in acetone (420ml). This mixture was stirred and heated to reflux under nitrogen for 1.5h, and the resulting solution was cooled to ca. 35° and filtered. The filtrate was concentrated in vacuo to ca. 50 ml, and the residue was stirred while diisopropyl ether (350ml) was added. The resulting suspension was stirred for 45 min, and the green solid was collected by filtration, washed with diisopropyl ether (2x100ml) and dried in vacuo at 30°. Part (2.0g) of this solid was suspended in refluxing acetone (20g) and water (2.0ml) was added. The resulting solution was seeded and stirred under reflux for 5 min while crystallisation occurred. The mixture was cooled to ca 20° over 30 min and refrigerated overnight, and the solid was collected by filtration, washed with chilled acetone (2x5ml) and dried in vacuo to give the title compound as crystals (1.77g); $\lambda_{\max}$ 244nm (MeOH), $E_1^{1\%}$ 857; $\nu_{\max}$ (Nujol mull) 1662 (C=O), 1250 and 1110cm⁻¹ (phosphate); δ (d_6 -DMSO) 5.41 (5-H,d,16Hz,1H), 5.29 (5-H,d,16Hz,1H), 4.96 (OCH₂,d,6Hz,2H), 4.82 (14-H,q,7Hz,1H), 1.49 (14-CH₃,d,7Hz,3H). (Nujol is a registered Trade Mark).

b) Sodium iodide (1.65g) was added to a suspension of Intermediate 5a (5.90g) in a mixture of acetone (118ml) and water (2.93ml). This mixture was stirred and heated to reflux under nitrogen for 2 h, a pale yellow crystalline precipitate forming after 0.8 h. The suspension was cooled to 21° and stirred for 1 h, and the solid was collected by filtration, washed with acetone (2x25ml) and dried in vacuo to give the title compound as crystals (4.96g); $\lambda_{\max}$ 244nm (MeOH) $E_1^{1\%}$ 842, having infrared and ¹H N.m.r. spectra similar to the sample obtained in Example 20a).

The following are examples of pharmaceutical compositions according to the invention. The term 'Active Ingredient' as used hereinafter means a compound of the invention and may be for example the compounds of Examples 2a) or 8.

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Example A-Dry Powder for Injection

	<u>mg/vial</u>
Active ingredient	equivalent to 100mg acid
10 Trisodium citrate	8.8
Citric acid	0.2

Method 1

Blend the sterile ingredients until homogeneous. Fill
15 aseptically into glass vials. Purge the headspace with nitrogen and close the vials using rubber closures and metal overseals.

Method 2

Dissolve the ingredients in water for injections B.P. Sterilise
20 the solution by membrane filtration. Aseptically fill into freeze-drying vials and place suitable rubber freeze-drying closures in position. Freeze-dry, filling the vials with nitrogen at the end of the cycle. Fully insert the closures and apply metal overseals.

25 Constitution

Constitute with a suitable sterile vehicle, e.g. using water for injections or using a 5% w/v dextrose solution, as an injection (e.g. in a 10ml volume) or an infusion (e.g. in a 100ml volume).

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Example B - Oral Tablet

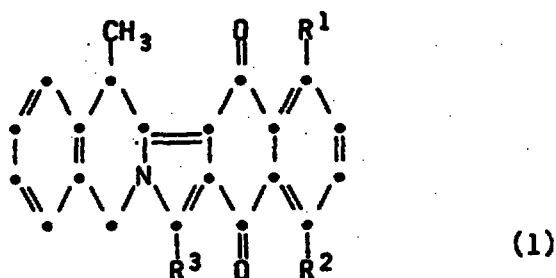
		<u>mg/tablet</u>
	Active Ingredient	equivalent to 250mg acid
5	Microcrystalline cellulose	231
	Sodium starch glycolate	6
	Magnesium stearate	2

10 Sieve the ingredients and blend until homogeneous. Compress
with appropriate punches. The tablets may be covered with a thin
polymer coat applied by the usual film coating techniques. A pigment
may be included in the film coat.



CLAIMS:

1. Compounds of the general formula (1)



(wherein R^1 and R^2 , which may be the same or different, each represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(OH)(OR^4)$ [where R^4 represents a hydrogen atom or an alkyl (optionally substituted by a hydroxyl, alkoxy, cyclic ether or cycloalkyl group), alkenyl, cycloalkyl, aryl, aralkyl or aroylalkyl group], with the proviso that at least one of R^1 and R^2 represents a group $-OP(O)(OH)(OR^4)$; and R^3 represents a hydrogen or halogen atom or a methyl group), and salts thereof.

2. Compounds of formula (1) as claimed in claim 1 wherein R^4 represents a hydrogen atom or a straight-chain or branched C_{1-8} alkyl (optionally substituted by a hydroxyl, C_{1-4} alkoxy, 3-7 membered cyclic ether, or C_{3-7} cycloalkyl group), C_{3-8} alkenyl, C_{3-7} cycloalkyl, phenyl, phen C_{1-3} alkyl or benzoyl C_{1-3} alkyl group, wherein any phenyl moiety may optionally be substituted by a hydroxyl, C_{1-4} alkoxy or nitro group, and salts thereof.

3. Compounds of formula (1) as claimed in either of claims 1 and 2 wherein R^4 represents a straight



chained C₁₋₅ alkyl group, a tetrahydrofuranylmethyl group, a phen C₁₋₃ alkyl group or a benzoyl C₁₋₃ alkyl group, and salts thereof.

4. Compounds of formula (1) as claimed in any one of claims 1 to 3 wherein R³ represents a hydrogen atom, and salts thereof.

5. Compounds of formula (1) as claimed in any one of claims 1 to 4 wherein R¹ represents a hydrogen atom, and salts thereof.

6. Compounds of formula (1) as claimed in claim 1 wherein R¹ and R², which may be the same or different, each represents a hydrogen atom, a hydroxyl group or a group -OP(O)(OH)(OR⁴) [where R⁴ represents a hydrogen atom or an alkyl (optionally substituted by a hydroxyl, alkoxy or cycloalkyl group), aryl or aralkyl group], with the proviso that at least one of R¹ and R² represents a group -OP(O)(OH)(OR⁴), and R³ is a hydrogen or halogen atom or a methyl group, and salts thereof.

7. A compound as claimed in claim 1 being phenylmethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, or 2-oxo-2-phenylethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid, or a salt thereof.

8. A compound as claimed in claim 1 being the sodium salt of phenylmethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]-isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid.

9. A compound as claimed in claim 1 being the sodium salt of 2-oxo-2-phenylethyl, [5,8,13,14-tetrahydro-14-methyl-8,13-dioxobenz[5,6]isoindolo[2,1-b]isoquinolin-9-yl]phosphoric acid.



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10. A compound of formula (1) as claimed in any one of the preceding claims, or a physiologically acceptable salt thereof, for use in the treatment of the human or non-human animal body to combat cancer therein.

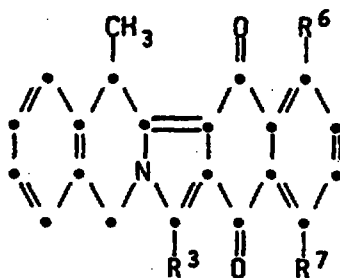
11. Isoquinoline compounds substantially as herein disclosed in any one of Examples 1 to 20.

12. A pharmaceutical composition comprising as an active ingredient a compound of formula (1) (as defined in claim 1), or a physiologically acceptable salt thereof, together with one or more pharmaceutical carriers or excipients.

13. Pharmaceutical compositions as claimed in claim 12 adapted for parenteral administration.

14. A process for the preparation of compounds of formula (1) (as defined in claim 1) and salts thereof, which process comprises at least one of the following steps:

(A) reacting a compound of formula (2)



(2)

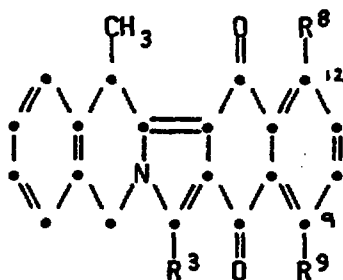
[wherein R^3 is as defined in claim 1; and one of R^6 and R^7 represents a group $-OP(O)(X^1)(X^2)$ (where X^1 represents an atom or group convertible to a hydroxyl group and X^2 represents a hydroxyl group or an X^1 moiety) and the other represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(X^1)(X^2)$]



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to convert at least one X^1 moiety to hydroxyl to produce a compound of formula (1);

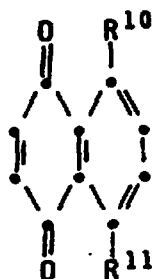
(B) reacting a compound of formula (3)



(3)

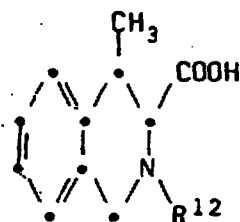
[wherein R^3 is as defined in claim 1; and one of R^8 and R^9 represents a hydroxyl group and the other represents a hydrogen atom or a hydroxyl group] to convert a hydroxyl group at the 9 and/or 12 position to a $-OP(O)(OH)(OR^4)$ group (wherein R^4 is as defined in claim 1);

(C) condensing a quinone of formula (4)



(4)

[wherein one of R^{10} and R^{11} represents a group $-OP(O)(OH)(OR^4)$ or $-OP(O)(X^1)(X^2)$ (where X^1 and X^2 are as hereinbefore defined) and the other represents a hydrogen atom, a hydroxyl group or a group $-OP(O)(OH)(OR^4)$ or $-OP(O)(X^1)(X^2)$] with a compound of formula (5)



(5)



[wherein R^{12} represents a hydrogen atom or a -CHO or -COCH₃ group] and subsequently, if required, converting one or more X^1 moieties to hydroxyl;

(D) halogenating a compound of formula (1) in which R^3 represents a hydrogen atom; and

(E) converting a free acid of formula (1) into a salt thereof or a salt of a compound of formula (1) into the free acid.

15. A process as claimed in claim 14 wherein process step (A) is effected by hydrolysis or ether cleavage.

16. A process as claimed in claim 14 wherein process step (B) is effected by reaction of the compound of formula (3) with a halophosphate or pyrophosphate of formula $\text{HalP}(\text{O})(\text{OH})(\text{OR}^4)$, $\text{HalP}(\text{O})(\text{X}^1)(\text{X}^2)$, $\text{O}(\text{P}(\text{O})(\text{OH})(\text{OR}^4))_2$ or $\text{O}(\text{P}(\text{O})(\text{X}^1)(\text{X}^2))_2$ (where R^4 is as defined in claim 1 and X^1 and X^2 are as defined in claim 14), followed where required by conversion of one or more X^1 moieties to hydroxyl.

17. A process as claimed in claim 14 wherein process step (C) is effected in the presence of an alkanolic acid anhydride and at elevated temperature.

18. The use of a compound of formula (1) as defined in claim 1 or a physiologically acceptable salt thereof for the manufacture of a therapeutic agent for the treatment of the human or non-human animal body to combat cancer therein.

19. Compounds of formula (2) (as defined in claim 14) and salts thereof.
