(12) UK Patent Application (19) GB (11) 2 168 350 A

(43) Application published 18 Jun 1986

(21) Application No 8530115

(22) Date of filing 6 Dec 1985

(30) Priority data

(31) 847754 (32) 7 Dec 1984 (33) KR
856039 22 Aug 1985

(51) INT CL[4]
C07H 19/04

(52) Domestic classification (Edition H):
C2P 2E13 2E15C 2E19B 2E19C 2E19D 2E19E 2E20 2E24 2E25A 2E26B 5B 7 A1 A
U1S 1313 2410 C2P

(56) Documents cited
None

(58) Field of search
C2P
Selected US specifications from IPC sub-class C07H

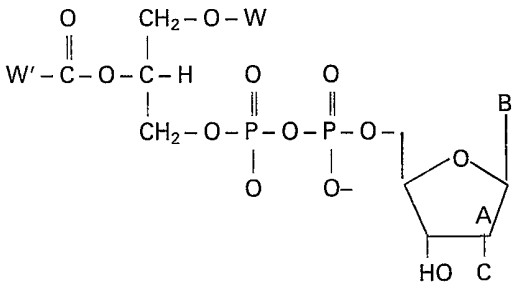
(71) Applicant
Boryung Pharmaceutical Co Ltd (South Korea),
66-21 Wonnam-Dong, Chongro-ku, Seoul, Republic of Korea

(72) Inventor
Chung Il Hong

(74) Agent and/or Address for Service
Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ

(54) Nucleoside derivatives

(57) The present invention relates to novel nucleoside derivatives of formula (I),



(I)

wherein,

B is adeninyl, cytosinyl, 5-fluorouracyl, 5-azacytosinyl, 6-mercaptopuryl or 7-deazaadeninyl;

A and C, which may be the same or different, are each hydrogen or a hydroxy group;

W is a saturated or unsaturated C_{8-20} alkyl group or a 2- or 3-alkoxyalkyl group; and

W′ is a saturated or unsaturated C_{7-19} alkyl group;

and salts thereof for use as anticancer and antiviral agents and to processes for the preparation of such novel nucleoside derivatives.

GB 2 168 350 A

Formulae in the printed specification were reproduced from drawings submitted after the date of filing, in accordance with Rule 20(14) of the Patents Rules 1982.

SPECIFICATION

Nucleoside derivatives

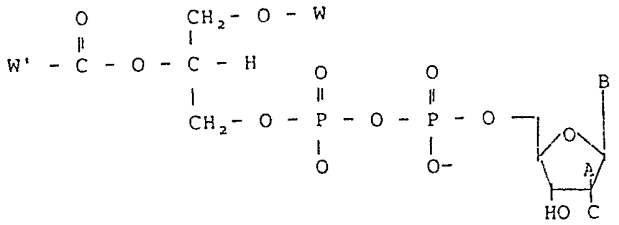
The present invention is concerned with novel nucleoside derivatives for use as anticancer and antiviral agents and with processes for the preparation of such novel nucleoside derivatives.

The preparation of 1,2-diacylglycero nucleoside conjugates is described in *Journal of Medicinal Chemistry*, 25, 1322 (1982); *Biochemical and Biophysical Research Communication*, 85, 715 (1978); and *Biochimica et Biophysica Acta*, 69,604 (1980). According to these publications, the nucleoside -5'-monophosphoromorpholidate is reacted with 1,2-diacylglycero-3-phosphates to produce 1,2-diacylglycero nucleoside conjugates.

It is one object of the present invention to provide novel neucleoside conjugates which are derived from 1-O-alkyl-2-O-acylglycero-3-phosphates instead of 1, 2-diacylglycero-3-phosphates and which are useful as anticancer and antiviral agents possessing unique molecular structures and physicochemical properties.

A further object of the present invention is to provide high yield processes for preparing the novel nucleoside conjugates of the invention.

According to one feature of the present invention, there are thus provided novel compounds of formula (I):



(I)

wherein

B is adeninyl, cytosinyl, 5-fluorouracyl, 5-azacytosinyl, 6-mercaptopuryl or 7-deazaadeninyl;

A and C, which may be the same or different , are each hydrogen or a hydroxy group;

W is a saturated or unsaturated C_{8-20} alkyl group or a 2- or 3-alkoxyalkyl group; and

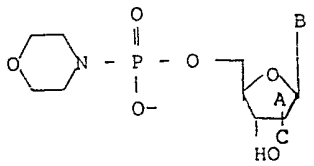
W' is a saturated or unsaturated C_{7-19} alkyl group;

and salts thereof. The compounds of formula I and non-toxic salts thereof are useful as anticancer and antiviral agents.

In formula (I), the phospholipid may be in L, D, and DL forms of optical isomers and all such forms are included. The nucleoside may for example be 9-β-D-arabinofuranosyladenine (ara-A, hereinafter), 1-β-D-arabinofuranosylcytosine (ara-C, hereinafter), 5-fluoro-2'-deoxyuridine or another nucleoside that can be used as an anticancer and antiviral agent.

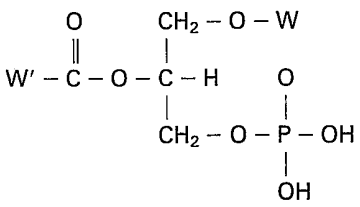
According to a further feature of the present invention, there are provided processes for the preparation of the novel compounds according to the invention as hereinbefore defined wherein:

i) a morpholidate of formula (VI)



(VI)

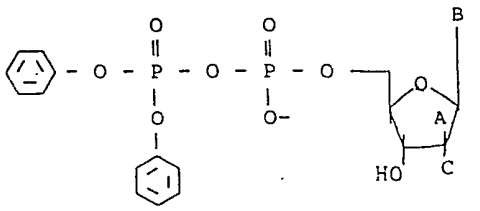
(wherein B, A and C are as hereinbefore defined) is reacted with a 1-O-alkyl-2-O-acylglycero-3-phosphate of formula (VIII)



(VIII)

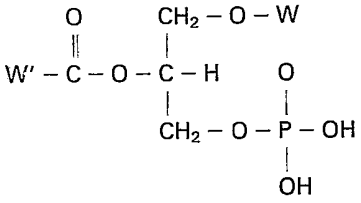
(wherein W and W' are as hereinbefore defined).

ii) a P^1-nucleoside-5'-P^2-diphenylpyrophosphate of formula (VII)



(VII)

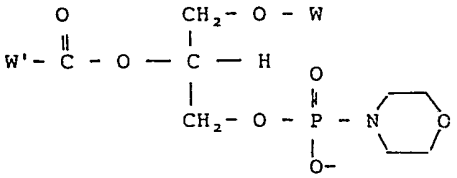
(wherein B, A and C are as hereinbefore defined) is reacted with a 1-O-alkyl-2-O-acylglycero-3-phosphate of formula (VIII)



(VIII)

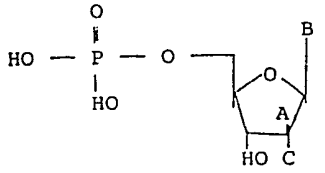
(wherein W and W' are as hereinbefore defined)

iii) a phospholipid morpholidate of formula (IX)



(IX)

(wherein W and W' are as hereinbefore defined) is reacted with a nucleoside of formula (III)

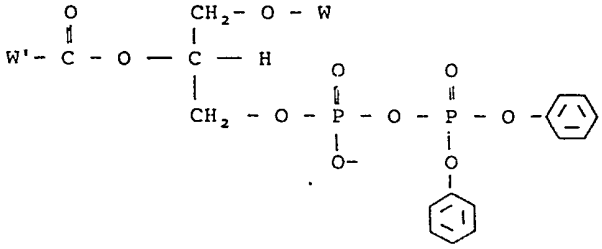


(III)

(wherein B, A and C are as hereinbefore defined);

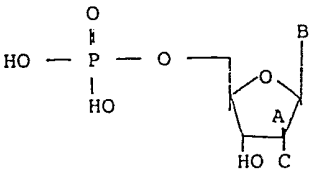
or

iv) a P^1-glycero-5'-P^2-diphenylpyrophosphate of formula (X)



(X)

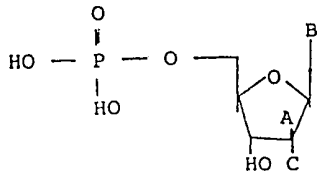
(wherein W and W' are as hereinbefore defined) is reacted with a nucleoside of formula (III)



(III)

(wherein B, A and C are as hereinbefore defined).

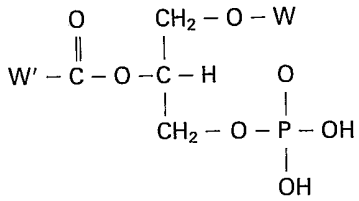
In processes (i) and (ii) the compound of formula (VI) or (VII) is conveniently first prepared by condensation of a compound of formula (III)



(III)

(wherein B, A and C are as hereinbefore defined);

In processes (iii) and (iv) the compound of formula (IX) or (X) is conveniently first prepared by condensation of a compound of formula (VIII)



(VIII)

(wherein W and W' are as hereinbefore defined).

The compounds of formulae (III), (VI), (VII) used in processes (i) to (iv) are preferably compounds in which A represents a hydroxy group and C represents hydrogen. The compounds of formula I obtained by the processes according to the invention may if desired be in the form of salts, e.g. non-toxic salts.

It is believed that the liponucleoside compounds of formula I in accordance with the invention are capable of acting as a new system for the delivery of anticancer and antiviral agents to tumor cells by providing lipid vehicles (liposomes) which penetrate cancer cell walls via the process of lysomotropism or related membrane phenomena.

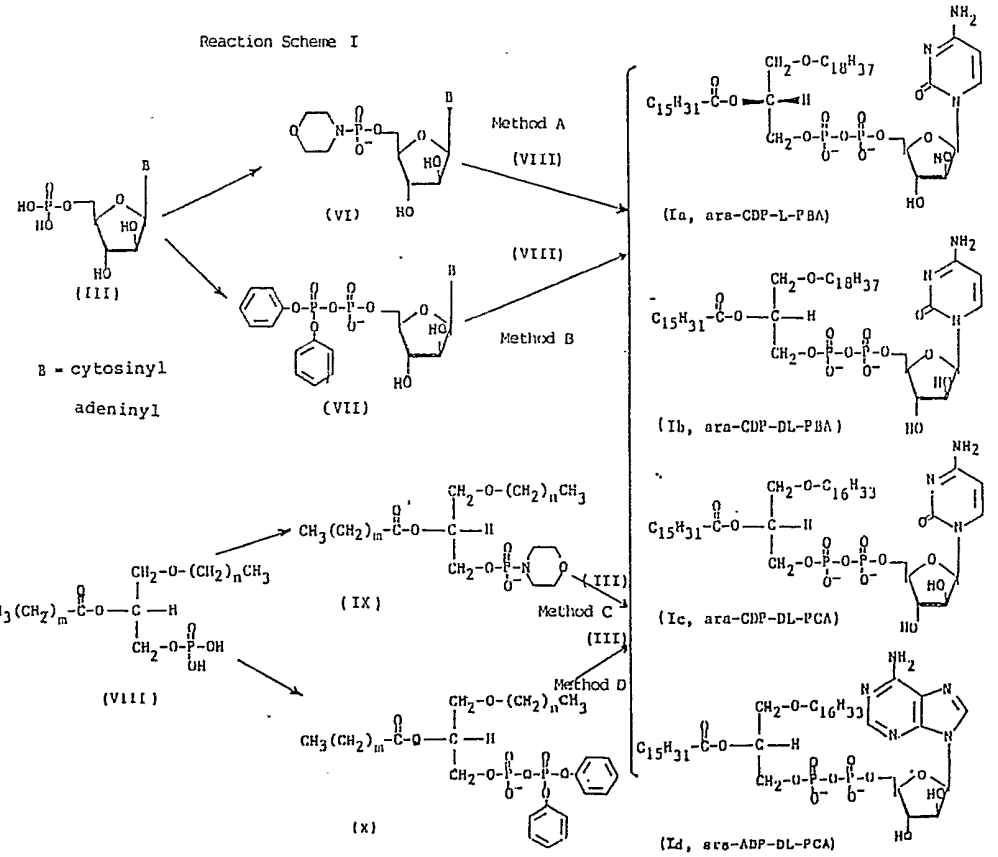
The anticancer agents according to the invention, after penetrating into the tumor cells, separate within the cells via phospholipid-enzyme specific reactions or non-specific mechanisms, into anticancer and antiviral nucleoside or nucleotide, and if the cell has a specific binding site for phospholipid, it becomes a target specific compound. Furthermore, for a nucleoside that requires phosphorylation for effective activity, a conjugate provides such a function and results in an excellent therapeutic effect for nucleoside kinase lacking resistant cells. Besides this, the 1-O-alkylphospholipid itself has pharmacological activity, especially anticancer and immunomodulating activity, and the combination of the nucleoside with the 1-O-alkylphospholipid results in an additive or synergistic effect. Among 1-O-alkylphospholipids, 1-O-alkyl-2-lysophospholipid and its derivatives, 1-O-alkyl-2-O-methyl-phosphatidyl-choline and -ethanolamine, are disclosed as having inhibitive and preventative activity against various animal cancer cells and immunomodulating activity [*Anticancer Research* 1, 135 and 345, (1981); *Seminar in Immunopathology* Vol 3, 187-203, (1979)]. As long as the conjugate form remains, the amino group of ara-C or ara-A is protected from de-amination by cytidine deaminase or adenosine deaminase. As the conjugate itself is lipophilic, it acts as a kind of sustained release prodrug and reaches the target cell. In the cell the conjugate hydrolyses to phospholipid and nucleotide. The result is the same as when two medicaments are administered and the pharmaceutical effects of both can be expected to increase the therapeutic index as an anticancer agent.

Processes according to the invention for the preparation of compounds of formula (I) are schematically shown in reaction scheme I. Nucleotide (III) is condensed to morpholidate (VI) (Method A) or P^1-nucleoside-5'-P^2-diphenyl pyrophosphate (VII) (Method B) and then reacted with 1-O-alkyl-152-O-acylglycero-phosphate (VIII) to produce the conjugates shown as compounds I_a to I_d. The other route is to condense phospholipid (VIII) to its morpholidate (IX) (Method C) or P^1-glycero-5'-P^2-diphenyl pyrophosphate (X) (Method D) and then to react it with nucleotide (III) to produce conjugates again shown as compounds I_a to I_d.

According to a still further feature of the present invention, there are provided pharmaceutical compositions comprising a compound of formula (I) or a non-toxic salt thereof together with a physiologically acceptable carrier or excipient. The compounds of the present invention and their salts can thus be employed in mixtures with conventional physiologically acceptable organic and inorganic carrier

substances. The pharmaceutical compositions according to the invention can for example be in the form of emulsions, suspensions, ampoules, powders, granules, capsules and tablets as well as in other forms. Also, they may for example include conventional fillers, preservants, stabilizers, dispersants, fumigants, buffers and colourants.

Reaction Scheme I



The following examples illustrate the invention without limiting the scope thereof:

EXAMPLE 1

1-β-D-arabinofuranosylcytosine-5'-diphosphate-1-O-octadecyl-2-O-palmitoyl-S-glycerol or 1-β-D-arabino-furanosylcytosine-5'-diphosphate-β-palmitoyl-L-batyl alcohol (Ia, ara-CDP-L-PBA) (reaction Scheme Method A)

1.7 g (2.6 mmol) of 1-O-octadecyl-2-O-palmitoyl-S-glycero-3-phosphate (VIII, n=17, m=14, L-isomer) was co-evaporated with pyridine to dryness, and then mixed with 1.8 g (2.6 mmol) of ara-CMP morpholidate 4-morpholine-N,N'-dicyclohexylcarboxamidinium salt (VI, B=cytosinyl). The mixture was dissolved in 150 ml of dry pyridine and was stirred at room temperature for five days under anhydrous conditions. Pyridine was then removed under reduced pressure and a minute amount of pyridine removed by co-evaporation of toluene. The residue was dissolved in 15 ml of dry acetic acid and 150 ml of chloroform-CH_3OH-water (2:3:1) (solvent CMW) and the solution was stirred at room temperature for one hour. To the solution was added 250 ml of chloroform. The organic solvent layer was separated and concentrated under reduced pressure and a minute amount of remaining acetic acid was removed by co-evaporation with 10 ml of toluene in three steps. The residue was dissolved in 100 ml of CMW solvent and adsorbed on a DE-52 (acetate) cellulose column (2.5 × 50 cm, jacketed, 5°C) and then the column was eluated with 0-0.15 M NH_4OAc linear gradient solvent CMW (each 1500 ml). An eluate of 900-1500 ml was collected and evaporated under reduced pressure at a temperature below 30°C until white crystals formed. The white crystalline powder (ammonium salt of the desired product) was washed with water and filtered. To prepare the sodium salt, the ammonium salt was dissolved in CMW and the solution applied to an Amberlite CG-50 (Na^+) column (2.5 × 15cm). The eluate was collected and evaporated under reduced pressure. The residue was crystallised from chloroform and acetone to provide 820 mg (31.2%) of the white product.

1) Melting point : 199-202°C

2) $[\alpha]_D^{25}$ = + 33.5° (C=0.23, chloroform-methanol-water 2:3:1)

3) NMR (90MH_z) : Solvent ($CDCl_3$-CD_3OD-D_2O, 2:3:1): δ ppm 0.95 (6H, t, 2CH_3), 1.14-1.87 (58H, m, 29CH_2), 2.29 (2H, t, CH_2-C=O, 3.27-4.42 (11H, m, H2', H3', H4', H5', CH_2-O-CH_2 and CH_2-O), 515 (1H, m, glycerol CH), 5.94 (1H, d, J=7 Hz, cytosine H5, 6.18 (1H, d, J=5 Hz, HI), 7.80 (1H, d, J=7Hz, Cytosine H6)

4) Elemental analysis for $C_{46}H_{87}N_3O_{14}P_2.1.5(CH_3)_2CO.H_2O$

Elements	C	H	N	P
Calculated	56.51	9.20	3.92	5.77
Found	56.81	9.27	3.69	5.87

EXAMPLE 2

1-β-D-arabinofuranosylcytosine-5'-diphosphate-rac-1-O-octadecyl-2-O-palmitoylglycerol (Ib, ara-CDP-DL-PBA)

(1) *Method A (reaction scheme method A)*

This compound was prepared by condensation of rac-1-O-octadecyl-2-O-palmitoylglycero-3-phosphate (VIII, n=17, m=14, DL-mixture) with ara-CMP-morpholidate (VI, B=cytosinyl) and subsequently separated as described above to provide the title compound with a 35% yield. Chromatographic mobilities and NMR data were consistent with a theoretical value.

(2) *Method B (reaction scheme method B)*

The title compound can also be prepared by the following process. 323 mg (1 mmol) of ara-CMP (III, B=cytosinyl) and 371 mg (1 mmol) of tri-n-octylamine were dissolved in 7 ml of hot methanol and then the solvent was evaporated under reduced pressure. The residue was again dissolved in N,N-dimethylformamide (DMF) and evaporated under pressure to remove any trace of water remaining in the residue. The dry ara-CMP-tri-O-octylammonium salt thus obtained was dissolved in 10 ml of dioxane and 5 ml of DMF and to the solution was added 0.3 ml of diphenyl phosphochloridate and 0.45 ml of tri-n-butylamine. The mixture was reacted at room temperature for two to three hours under anhydrous conditions. The solvent was removed by evaporation under reduced pressure and then 50 ml of ether was added to precipitate P^1-(1-β-D-arabinofuranosylcytosin-5'-yl)-P^2-diphenyl pyrophosphate (VII, B=cytosinyl). The reaction mixture was maintained at 0°C for 30-60 minutes and then the ether was removed. The precipitate was dissolved in 2 ml of dioxane and any trace of water in the precipitate was removed by evaporation under reduced pressure. 663 mg (1 mmol) of rac-1-O-octadecyl-2-O-palmitoylglycero-3-phosphate (VIII, n=17, m=14, DL-mixture) was dried overnight over P_2O_5, dissolved in 1 ml of anhydrous pyridine and then reacted with a solution of the above pyrophosphate (VII) in 0.5 ml of dioxane at room temperature for a day under anhydrous conditions. After the reaction, the solvent was removed by evaporation under reduced pressure and to the residue thus obtained was added 25 ml of ether whereby the title compound was precipitated. The precipitate thus obtained was dissolved in 100 ml of CMW and adsorbed on to a DE-52 (acetate) cellulose column (2.5 × 50 cm, jacketed, 5°C) and then eluted and purified as described above to obtain the title compound with a 30% yield. The chromatographic mobilities and NMR data were consistent with theoretical values.

EXAMPLE 3

1-β-D-arabinofuranosylcytosine-5'-diphosphate-rac-1-O-hexadecyl-2-O-palmitoyl glycerol or 1-β-D-arabino-furanosylcytosine-5'-diphosphate-β-palmitoyl-DL-chimyl alcohol (Ic, ara-CDP-DL-PCA)

(1) *Method A (reaction scheme method A)*

This compound is prepared by the same process as described in Example 1. 4.19 g (6.6 mmol) of rac-1-O-hexadecyl-2-O-palmitoylglycero-3-phosphate (VIII, n=15, m=14 DL-mixture) which had been co-evaporated with pyridine was reacted with 3.43 g (5 mmol) of ara-CMP morpholidate (VI, B=cytosinyl) in 300 ml of anhydrous pyridine at room temperature for five days under anhydrous conditions. Pyridine was evaporated under reduced pressure and the residue was treated as described in Example 1 and adsorbed on to DE-52 (acetate) cellulose column (2.5 × 50 cm, jacketed 5°C) and then eluted with CMW solvent containing 0-0.15 M NH_4OAc linear gradient (each 1500 ml). The portion that contained the title compound was collected and concentrated at a temperature below 30°C under reduced pressure until white crystals were formed. The crystals thus obtained were converted into the sodium salt by passing through an Amberlite CG-50 (Na+) column to obtain the title compound in 30% yield.

1) Melting Point : 202-205°C (decomposition)

2) NMR (90MHz) : Solvent ($CDCl_3$-CD_3OD-D_2O, 2:3:1): ppm 0.87 (6H, t, $2CH_3$), 1.07-1.78 (54H, m, 27 CH_2), 2.35 (2H, t, CH_2-C=O), 3.27-4.35 (11H, m, H_2^1, H_3^1, H_4^1, H_5^1, CH_2-O-CH_2,CH_2-O), 5.15 (1H, m, glycerol CH), 5.92 (1H, d, J=7 Hz, cytosine H_5), 6.14 (1H, d, J=5 Hz, $H1'$), 7.88 (1H, d, J=7 Hz, cytosine H_6).

3) Elemental analysis for $C_{44}H_{81}N_3O_{14}P_2Na_2.0.5H_2O$

Elements	C	H	N	P
Calculated	53.21	8.43	4.23	6.24
Found	53.69	9.27	3.92	5.72

(2) *Method B (reaction scheme method C)*

To a reflux mixture comprising 3.17 g (5 mmol) of rac-1-O-hexadecyl-2-O-palmitoylglycero-3-phosphate (VIII, m=15, n=14, DL-mixture), 1.7 ml (20 mmol) of morpholine, and 50 ml of t-butyl alcohol was added (dropwise) a solution of 4.12 g (20 mmol) of N,N'-dicyclohexylcarbodiimide (DCC) and 75 ml of t-butyl alcohol. The reaction mixture was refluxed for two hours, stirred at room temperature overnight and then 20 ml of water was added with stirring at room temperature for two hours in order to decompose the remaining DCC. The white crystals thus formed were removed by filtration and the filtrate was concentrated by evaporation under reduced pressure and extracted with ether. The extract was evaporated under reduced pressure and the resulting residue (IX, n=15, m=14, DLC-mixture) was twice co-evaporated with toluene. 2.13 g (6.6 mmol) of ara-CMP (III, B=cytosinyl) and 4.67 g (13.2 mmol) of tri-n-octylamine were then added and the mixture was dried by co-evaporation with pyridine in three steps and then dissolved in 200 ml of pyridine. The reaction mixture thus obtained was stirred and reacted at room temperature for seven days under anhydrous conditions. Pyridine was removed by evaporation under reduced pressure and any trace of pyridine still remaining was completely removed by co-evaporation with a small amount of toluene. The residue was dissolved in 30 ml of acetic acid and 300 ml of CMW solvent and the solution stirred at room temperature for one hour followed by the addition of 500 ml of chloroform. The organic layer was separated and concentrated under reduced pressure and any trace of acetic acid remaining was completely removed by co-evaporation with 10 ml of toluene in three steps. The residue was dissolved in 100 ml of CMW solvent, adsorbed on a DE-52 (acetate) cellulose column (2.5 × 50 cm, jacketed 5°C) and then eluted with CMW solvent containing 0-0.15M NH_4OAc linear gradient, as in the method described in Example 1, to obtain the title product in 30% yield. The chromatographic mobilities and NMR data were consistent with the theoretical values.

EXAMPLE 4

9-β-D-arabinofuranosyladenine-5'-diphosphate-rac-1-O-hexadecyl-2-O-palmitoylglycerol or 9-β-D-arabinofuranosyladenine-5'-diphosphate-β-palmitoyl-DL-chimyl alcohol (Id, ara-ADP-DL-PCA) (Reaction Scheme Method A)

1.90 g (3 mmol) of rac-1-O-hexadecyl-2-O-palmitoylglycero-3-phosphate (VIII, n=15, m=14, DL-mixture) were dried by co-evaporation with pyridine in three steps and 2.13 g (3 mmol) of ara-AMP morpholidate-4-morpholine-N, N'-dicyclohexyl-carboxamidinium salt (VI, B=adeninyl) were dissolved in 200 ml of dry pyridine and the solution was stirred at room temperature for seven days under anhydrous conditions. Pyridine was removed by evaporation under reduced pressure and the residue was treated using the same process as described in Example 1, adsorbed on to a DE-52 (acetate) cellulose column (2.5 × 50 cm, jacketed 5°C) eluted with CMW solvent containing 0-0.15M NH_4OAc linear gradient and then passed through an Amberlite CG-50 (Na+) column to obtain 851 mg (29%) of the sodium salt of the title compound.

1) NMR (90 MHz) : Solvent ($CDCl_3$-CD_3OD-D_2, 2:3:1):
ppm 0.92 (6H, t, $2CH_3$), 1.07-1.78 (54H, m, $27CH_2$), 2.35 (2H, t, CH_2-C=O), 3.27-4.35 (11H, m, H^1_2, H^1_3, H^1_4, H^1_5, CH_2-O-CH_2; CH_2-O), 5.07 (1H, m, glycerol CH). 6.33 (IH, d, J=4.5H_2, H_1), 8.17 (1H, s, adenine H_2), 8.40 (1H, s, adeninyl H_8).

The following experiments demonstrate the pharmacological properties of compounds according to the invention:

Experiment 1

Antitumour activity against i.p. inoculated L 1210 lymphoid leukemic mice:

DBA/2J mice (average weight 25 g, male) received i.p. inoculation of 1×10^6 or 1×10^5 L 1210 lymphoid leukemic cells and after 24 hours, the drug was dissolved in 0.9% NaCl, the solution being injected into the mice; the survival rate after 45 days was recorded.

The test was conducted in accordance with United States National Cancer Institute Protocols (Cancer Chemotherapy Reports 3, 1-103, 1972). The treatment schedules were qd 1, qd 1,5,9 and qd 1-5. The optimal dosage in Table 1 is the amount which produces maximum activity. A broad range of dosage levels were tested. Activity was measured by comparing the increase in life span. This was done by comparing the average life spans of the test and control group mice. Table 1 shows the therapeutic results of ara-C and its conjugate ara-CDP-DL-PBA (I_b) and ara-CDP-DL-PCA (I_c). The first part shows results of ara-C sensitive L 1210 lymphoid leukemic mice. The non-treated control mice died seven or eight days after the inoculation of tumour cells. when ara-C was injected with an optimal single dosage of 400 mg (1644 umol/kg), the increase in life span (% ILS) was 14%, and injecting 200 mg (822 umol) once a day for five days resulted in a 129% ILS. But conjugate ara-CDL-DP-PBA (I_b) and ara-CDP-DL-PCA (I_c) at an optimal single dosage of 400 mg (395 and 407 umol/kg) showed excellent ILS values of 257% and 293%, respectively. When the optimal dosage was injected five times it still resulted in excellent ILS values of 229% and 264%, respectively. The comparison of the single dose for ara-C and its conjugate is out of the range. In the five dosage schedules, the conjugate shows twice the effectiveness at a dosage of 1/8 or 1/10 of the ara-C molar dose, and the toxicities are much less. Secondly, results of therapeutic treatments of ara-C resistant L 1210 lymphoid leukemic mice, which is due to deoxycytidine kinase existing in small quantities, show non-treated control mice died eight to elevent days after the inoculation of tumour cells. Single dose treatments with ara-C exhibited hardly any effects (ILS, 6%), and five day treatments resulted in 65% ILS. These results indicate that ara-C containing

deoxycytidine kinase but in small amounts. But, the conjugate of ara-C, that is, ara-CDP-DL-PBA (I_b) and ara-CDP-DL-PCA (I_c) produces a remarkable therapeutic result and promises to be a great hope in combating cancer. At the optimal dosage of 400 mg/kg whether used in a single dose or in amounts of 80 mg/kg/day for 5 days, the result is an ILS of 259-356%, and among the six mice, one to three mice survived more than 45 days and were completely cured. Moreover, at a dosage of 167 mg/kg/day for the first, fifth, and nineth day (three doses) schedule, ara-CDP-PBA-(I_b) showed more than 290% ILS, and ara-CDP-DL-PCA-(I_c) showed more than 374% ILS and more than three mice, (in the case of ara-CDP-DL-PCA six mice) out of six mice survived more than 45 days and were cured. Thus, one third (1/3 of the molar dose of ara-C produced a five fold increase in effectiveness. The fact that the conjugate is effective on deoxycytidine kinase shortage ara-C resistant L 1210 lymphoid leukemic mice shows that the conjugate is introduced into cells by endocytosis or some other mechanism and is hydrolysed by an enzyme to phospholipid and ara-CMP. Thus liberated ara-CMP is continuously phosphorylated to ara-CTP. Consequently, as the ara-CMP is liberated from the conjugate, deoxycytidine kinase normally needed for phosphorylation of ara-C is not required.

Advantages

As shown in the above tests, the conjugates according to the invention demonstrate a greater activity than the parent drug ara-C, even at lower dosages, and this in turn may contribute to the improvement in the therapeutic index of the parent drug. Moreover, ara-C has a short half-life, and must be administered continuously to be effective, but the conjugate has a greater and superior activity even in a single dosage. Therefore, the conjugate can be used as a sustained release drug. The fact that the conjugate is effective on ara-C resistant L 1210 lymphoid leukemic mice means that it hydrolyses and liberates ara-CMP and phospholipid in cancerous cells and exerts great effect upon deoxycytidine kinase lacking resistant cells. The phospholipid is 1-O-alkyl-2-O-acylglycero-3-phosphate and after biochemical reaction it is converted into 1-O-alkyl-2-lysophosphatidyl-choline or -ethanolamine, and as these compounds themselves also possess growth and transfer retarding activities against cancer cells and immunomodulating activity, they can be expected to display additive and synergistic effects. The conjugate compounds are not simply a prodrug of ara-C, but are considered to be a new drug. Furthermore, compared with other lipophilic prodrugs, the present conjugates have the advantage of forming a transparent suspension in water when ultrasonically irradiated. It has been reported in the literature that in treating leukemic patients with ara-C the generation of resistance to the drug is related to the cell's relative content of deoxycytidine kinase and cytidine deaminase [Annals of New York Academy of Science 255, 247, (1975)]. However, the ara-C conjugate has a resistance to cytidine deaminase and does not hydrolyse the amino group. It is reasonable to believe that it will be greatly effective in treating ara-C resistant leukemia patients.

TABLE 1

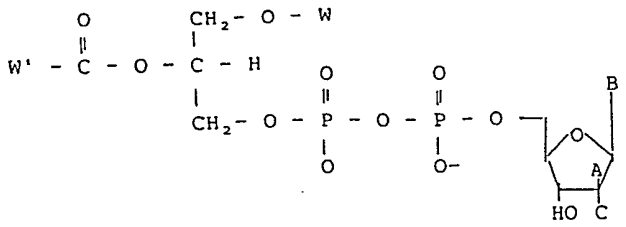
Antitumor activity against i.p. inoculated 1,1210 lymphoid leukemic mice[a].

compound	*treatment schedule (qd)*	*optimal dose[b] mg(μmole)/kg/day*	*survival days range*	*median (T/C)*	*% ILS[c]*	*45-day survivors*
against L1210/0[d]						
ara-C	1	400(1644)	8-10	8.0/7.0	14	0
	1-5	200(822)	7-18	16.0/7.0	129	0
ara-CDP-DL-PBA (Ib)	1	400(395)	15-29	25.0/7.0	257	0
	1-5	100(99)	18-32	23.0/7.0	229	1
ara-CDP-DL-PCA (Ic)	1	400(407)	20-33	27.5/7.0	293	0
	1-5	80(81)	21-30	25.5/7.0	264	0
against L1210/ara-C[e]						
ara-C	1	300(1233)	9-10	9.5/9.0	6	0
	1-5	60(247)	14	14.0/9.0	65	0
ara-CDP-DL-PBA (Ib)	1	400(395)	25→45	36.0/9.0	300	2
	1-5	80(79)	30→45	>41.0/9.0	>356[f]	3
	1,5,9	167(165)	24→45	>39.0/10.0	>290[f]	3
ara-CDP-DL-PCA (Ic)	1	400(407)	11→45	>38.5/9.0	>328[f]	3
	1-5	80(81)	26→45	30.5/8.5	259	1
	1,5,9	167(169)	-	>45.0/9.5	>374[f]	6
ara-CMP and DL-PCA-P[g]	1	131 and 258 (407 each)	3-10	10.0/9.0	11	0
	1-5	26.2 and 51.4 (81 each)	4-15	14.0/9.0	56	0

a: Each group of six DBA/2J mice (av wt 25 g, male) received i.p. inoculation of 1×10^6 L 1210/0 cells or 1×10^5 L 1210/ara-C cells on day O.
b: Producing maximum activity.
c: Percentage increase in life span: (T/C-1) × 100.
d: ara-C sensitive L 1210
e: ara-C resistant L 1210 due to the shortage of deoxycytidine
f: 45 days
g: ara-CMp (III, B=cytosinyl) and rac-1-O-hexadecyl-2-O-palmitoylglycerol-3-phosphate (VIII, n=15, m=14, DL-mixture).

CLAIMS

1. Compounds of formula (I):



(I)

wherein,
B is adeninyl, cytosinyl, 5-fluorouracyl, 5-azacytosinyl, 6-mercaptopuryl or 7-deazaadeninyl;
A and C, which may be the same or different, are each hydrogen or a hydroxy group;
W is a saturated or unsaturated C_{8-20} alkyl group or a 2- or 3- alkoxyalkyl group; and
W' is a saturated or unsaturated C_{7-19} alkyl group;
and salts thereof.

2. Compounds as claimed in claim 1 wherein the nucleoside portion of the conjugate is provided by 9-β-D-arabinofuranosyladenine, 1-β-D-arabinofuranosylcytosine or 5-fluoro-2'-deoxyuridine.

3. 1-β-D-Arabinofuranosylcytosine-5'-diphosphate-1-O-octadecyl-2-O-palmitoyl-S-glycerol and salts thereof.

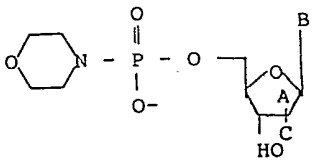
4. 1-β-D-Arabinofuranssylcytosine-5'-diphosphate-rac-1-O-octadecyl-2--O-palmitoylglycerol and salts thereof.

5. 1-β-D-Arabinofuranosylcytosine-5'-diphosphate-rac-1-O-hexadecyl-2-O-palmitoylglycerol and salts thereof.

6. 9-β-D-Arabinofuranosyladenine-5'-diphosphate-rac-1-O-hexadecyl-2-O-palmitoylglycerol and salts thereof.

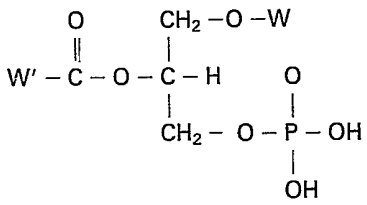
7. Compounds as claimed in any of the preceding claims in the form of non-toxic salts thereof.

8. A process for the preparation of compounds as claimed in any of the preceding claims wherein a morpholidate of formula (VI)



(VI)

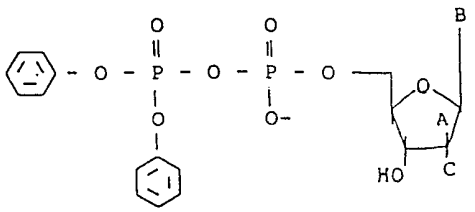
(wherein B, A and C are as defined in claim 1) is reacted with a 1-O-alkyl-2-O-acylglycero-3-phosphate of formula (VIII)



(VIII)

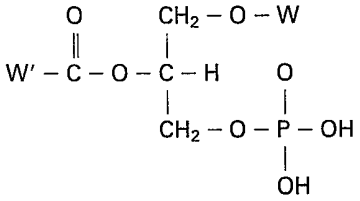
(wherein W and W' are as defined in claim 1).

9. A process for the preparation of compounds as claimed in any of claims 1 to 7 wherein a P^1 nucleoside-5'-P^2-diphenylpyrophosphate of formula (VII)



(VII)

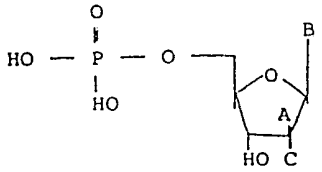
(wherein B, A and C are as defined in claim 1) is reacted with a 1-O-alkyl-2-O-acylglycero-3-phosphate of formula (VIII)



(VIII)

(wherein W and W′ are as defined in claim 1).

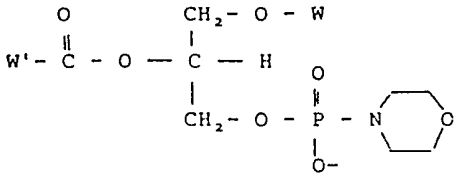
10. A process according to either of claims 8 and 9 wherein the compound of formula (VI) or (VII) is first prepared by condensation of a compound of formula (III)



(III)

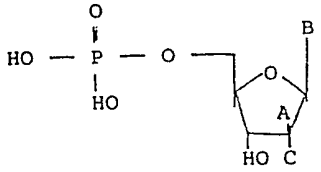
(wherein B, A and C are as defined in claim 1).

11. A process for the preparation of compounds as claimed in any of claims 1 to 7 wherein a phospholipid morpholidate of formula (IX)



(IX)

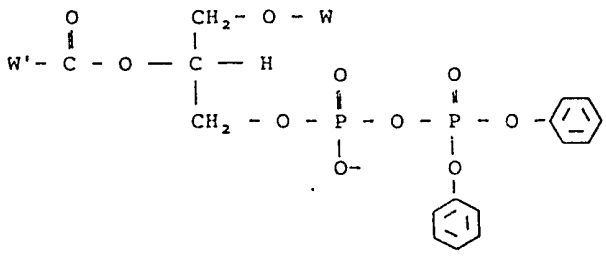
(wherein W and W′ are as defined in claim 1) is reacted with a nucleoside of formula (III)



(III)

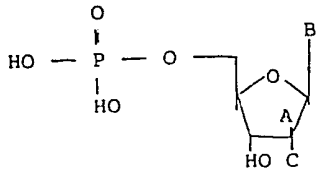
(wherein B, A and C are as defined in claim 1).

12. A process for the preparation of compounds as claimed in any of claims 1 to 7 wherein a P^1-glycero-5′-P^2-diphenylpyrophosphate of formula (X)



(X)

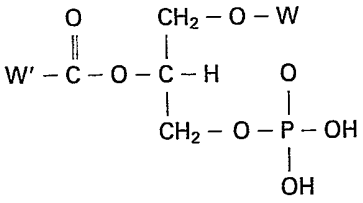
(wherein W and W' are as defined in claim 1) is reacted with a nucleoside of formula (III)



(III)

(wherein B, A and C are as defined in claim 1).

13. A process according to either of claims 11 and 12 wherein the compound of formula (IX) or (X) is first prepared by condensation of a compound of formula (VIII)



(VIII)

(wherein W and W' are as defined in claim 1).

14. A process according to any of claims 8 to 13 wherein compounds of formulae (VI), (VII) and (III) are used in which A represents a hydroxy group and C represents hydrogen.

15. A process according to any of claims 8 to 14 wherein the compound of formula (I) is obtained in the form of a salt thereof.

16. Pharmaceutical compositions comprising a compound of formula (I) as defined in claim 1 or a non-toxic salt thereof, together with a physiologically acceptable carrier or excipient.

Printed in the UK for HMSO, D8818935, 4/86, 7102.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.