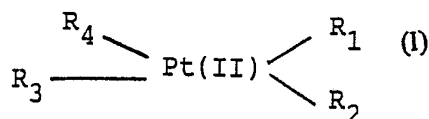




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(54) Title: HYDROPHOBIC CIS-PLATINUM COMPLEXES EFFICIENTLY INCORPORATED INTO LIPOSOMES



(57) Abstract

Platinum (II) four coordinate complex having formula (I), wherein R₁ and R₂ are carboxylato monoanions bearing a hydrophobic radical function or a single carboxylato dianion bearing a hydrophobic radical function and R₃ and R₄ are ammonia or primary amines or, when linked together represent a vicinal diaminoalkane or vicinal diaminocycloalkane. The complex is substantially soluble in methanol or chloroform and substantially insoluble in water. Said complex may be incorporated into phospholipid liposomes. Such platinum complexes encapsulated in phospholipid liposomes are useful for chemotherapy of platinum complex-sensitive tumors.

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HYDROPHOBIC CIS-PLATINUM COMPLEXES
EFFICIENTLY INCORPORATED INTO LIPOSOMES

15

This invention relates to newly synthesized platinum complexes with hydrophobic properties. The use of liposomes incorporating these new and previously synthesized complexes in anti-tumor chemotherapy is also described.

Cis-platinum (CDDP) is a highly effective drug in the treatment of several neoplastic diseases in humans (Loehrer et al (1984) Ann. Int. Med. V 100, pp 704-713).
25 However, its use is limited by severe systemic toxicity, particularly nephrotoxicity and neurotoxicity (Zwelling et al Platinum Complexes. In: Pharmacologic principles of cancer treatment (1982) Ed by B.A. Chabner, Saunders, Philadelphia, PA). In an attempt to modify the
30 therapeutic index of CDDP, new derivatives have been synthesized during the last decade. However, the development of some promising analogues has been prevented by their low hydrosolubility, which decreases their potential for clinical use (Burchenal et al (1979)
35 Cancer Treat. Rep. V 63, pp 1493-1497).

Liposomes are lipid vesicles which may form spontaneously upon addition of an aqueous solution to a dry lipid film (Mayhew et al, In: Liposomes (1983) Ed by Marc J. Ostro, Marcel Dekker, Inc., New York, N.Y.).

- 5 Liposomes may be used as drug carriers of hydrophobic or hydrophilic drugs entrapped in their hydrophobic or hydrophilic compartments respectively. Multilamellar liposomes are multilayer lipid vesicles (MLV) that are particularly suited for carrying hydrophobic drugs since
10 their hydrophobic compartment is larger than their hydrophilic compartment. When injected intravenously (iv) in animals, (Kasi et al (1984) Int. J. Nucl. Med. Biol. V 11 pp 35-37, Lopez-Berestein et al. (1)(1984) Cancer Drug Deliv. V 1, pp 199-205) and humans (Lopez-Berestein et al
15 (2)(1984), Cancer Res. V 44, pp 375-378), MLV concentrate in the liver, spleen and other organs rich in reticuloendothelial (RES) cells.

- Liposomes have been previously used in vitro to
20 deliver chemotherapeutic agents, (Mayhew et al, Liposomes (1983), Ed by Ostro, Marcel Dekker, Inc., New York, N.Y.) and immunomodulators and anti-fungal agents in vitro (Mehta et al (1984), Immunology V 51 pp 517-527, and in vivo in animals (Lopez-Berestein et al (4)(1984) Clin Exp
25 Metastasis V 2 pp 127-137 and Lopez-Berestein et al (1983), J Inf Dis V 147, pp 937-945) and in humans (Lopez-Berestein et al (1985) J. Inf. Dis. V 151 pp 704-710).

- 30 Recent studies show that liposomes can reduce certain types of drug-related toxicities such as doxorubicin cardiotoxicity (Forssen et al (1981) Proc. Natl. Acad. Sci. V 78 pp 1873-1877, Olson et al (1982), Eur. J. Cancer Clin. Oncol. V 18 pp 167-176, Gabizon et al (1982) Cancer
35 Res. V 42 pp 4734-4739, Herman et al (1983) Cancer Res. V 43 pp 5427-5432) and CDDP nephrotoxicity, (Freise et al

(1982), Arch. Int. Pharmacodynamie Therapie V 258 pp 180-192) and may increase antitumor activity as a result of a slow release mechanism (Mayhew et al (1978) Ann. N.Y. Acad. Sci. V 308, pp 371-386, Patel et al (1984) Int. J. Cancer V 34 pp 717-723) a higher drug uptake by tumor cells or due to a more selective organ distribution (Gabizon et al (1983) Cancer Res. V 43, pp 4730-4735 and Mayhew et al (1983), Cancer Drug Deliv. V 1 pp 43-58). In U.S. Patent No. 4,330,534 N⁴-acylcytosine arabinoside incorporated into liposomes, for example, was found to be therapeutically effective when administered to tumor-bearing animals. In spite of these promising results, the clinical application of antitumor agents encapsulated in liposomes has been delayed, mainly due to formulation, drug stability and large scale production problems.

CDDP has been previously encapsulated in MLV but with a very low encapsulation efficiency (7.4%) and poor stability (75% at 48 hours in 0.9% NaCl solution) (Freise et al (1982) Arch. Int. Pharmacodynamie Therapie V 258 pp 180-192).

In U.S. Patent No. 4,256,652 are described certain platinum compounds comprising resolved stereoisomers of 1,2 diaminocyclohexane (DACH). The isomers utilized were cis-DACH, trans-RR-DACH and trans-SS-DACH. The platinum compounds described therein contained, in addition to a resolved DACH isomer, two hydrophilic platinum ligands such as bromide, iodide, nitrate, bromoacetate, sulfate or glucuronate. The platinum compounds comprising the trans-RR-DACH were described as often more therapeutically effective than those bearing cis-DACH.

In European Patent Application No. 83306726.7 certain platinum compounds are described which may comprise diaminocyclohexane (non-stereochemically resolved) and do

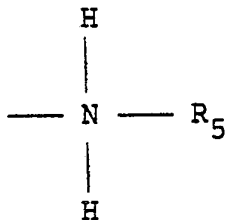
comprise phosphatidyl groups having fatty acid substituents. These compounds are described as largely insoluble in plasma and preferably employed with lipid vesicle carriers. The platinum compound-phospholipid vesicles were preferably prepared by a sonic oscillation procedure which characteristically yields unilamellar vesicles.

The present invention comprises a platinum (II) four-coordinate complex having the formula:



20 In this formula R_1 and R_2 are each carboxylates bearing a hydrophobic radical function, most preferably neodecyl, or, when linked together, are a dicarboxylato bearing a hydrophobic radical function. The R_3 and R_4 are each:

25



30

In this latter formula, R_5 is selected from the group consisting of hydrogen, an alkyl having from 2 to 6 carbon atoms such as isopropyl, aryl, aralkyl, alkenyl, a cycloalkyl such as cyclohexyl, cycloalkenyl and a

combination thereof. R_5 is preferably hydrogen, alkyl having between 1 and 20 carbon atoms, more preferably between 6 and 12 carbon atoms and most preferably between 2 and 6 carbon atoms or cycloalkyl having between 3 and 12 carbon atoms.

Additionally R_3 and R_4 may be linked together in a single function. When R_3 and R_4 are so linked together, they are preferably selected from the group consisting of cycloalkyl-1,2-diamino functions having between about 3 and 7 carbon atoms, and alkyl-1,2-diamino having between about 2 and 12 carbon atoms, preferably ethyl. A preferred cycloalkyl-1,2-diamino component is 1,2-diaminocyclohexane, preferably in the trans-R,R- or trans-S,S- form.

In the above-described complex the carboxylato of R_1 and R_2 is preferably an alkylcarboxylato having between about 5 and 20 carbon atoms, an arylcarboxylato wherein aryl is phenyl, naphthyl, or an alkylphenyl wherein the alkyl phenyl has between about 12 and 16 carbon atoms.

When the complex contains R_1 and R_2 as a dicarboxylato, the dicarboxylato is preferably an alkyl dicarboxylato having between about 5 and 20 carbon atoms, or an aryl dicarboxylato wherein aryl is naphthyl, phenyl, or alkylphenyl wherein the alkyl of the alkylphenyl has between about 6 and 12 carbon atoms. As used in the above description the term aryl is defined further as a function preferably having between about 6 and 14 carbon atoms. Likewise the term alkenyl is preferably a function having between about 5 and 20 carbon atoms.

The cycloalkyl referred to in the present descriptions is preferably a function having between about 3 and

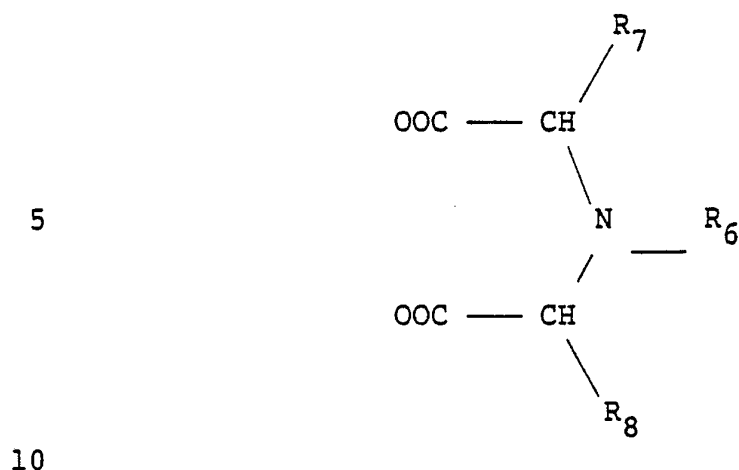
12 carbon atoms. The cycloalkenyl term as used above is preferably a function having between about 5 and 20 carbon atoms. The term aralkyl as used above is a function preferably having between about 7 and 20 carbon atoms and contains linked aryl and alkyl portions. The aryl portion herein preferably has between about 6 and 10 carbon atoms and the alkyl portion preferably has between about 1 and 10 carbon atoms. When R_3 and R_4 are taken together they are preferably a cycloalkyl-1,2-diamino having between about 3 and 20 carbon atoms. The alkyl ring of the cycloalkyl preferably has between about 3 and 12 carbon atoms.

A further aspect of the present invention concerning the platinum (II) four-coordinate complex having the formula:



25 concerns the case where R_1 and R_2 , taken together, are a dicarboxylato, preferably a cis dicarboxylato, of the formula:

30



where R_6 is alkyl having between about 10 and 20 carbon atoms, preferably pentyl, neopentyl, decyl or neodecyl and R_7 and R_8 are each hydrogen or an alkyl having between about 1 and 5 carbon atoms.

As described above R_3 and R_4 are each:



When R_3 and R_4 are taken together they are selected from the group consisting of cycloalkyl-1,2-diamino having between about 3 and 7 carbon atoms, and alkyl-1,2-diamino having between about 2 and 12 carbon atoms. The cycloalkyl-1,2-diamino is preferably 1,2-diaminocyclohexane and more preferably trans-R,R-1,2-diaminocyclohexane or trans-S,S-1,2-diaminocyclohexane. This complex is, as described above, substantially soluble in methanol or chloroform and substantially insoluble in water.

Important aspects of the present invention involve liposomes comprising fatty substances such as phospholipids, optionally cholesterol, and the four-coordinate platinum complexes described above, as well as the preparation and uses of these liposomes. Liposomes of the present invention contain the platinum complex and the phospholipid in a preferred ratio between about 1 to 10 and about 1 to 30, a more preferred ratio being 1 to 15.

Preferred phospholipids of these liposomes include phosphatidylglycerol, phosphatidylcholine, sphingomyelin, phosphatidic acid or phosphatidylserine, the more preferred phospholipids being phosphatidylglycerol, phosphatidylcholine or a combination thereof. The most preferred phosphatidylglycerol is one consisting essentially of dimyristoylphosphatidylglycerol and the most preferred phosphatidylcholine is one consisting essentially of dimyristoylphosphatidylcholine. When the liposomes of the present invention comprise dimyristoylphosphatidylglycerol and dimyristoylphosphatidylcholine they are preferably in a ratio between about 1-10 and 10-1, more preferably in a ratio of about 3 to 7.

The liposomes of the present invention may be multilamellar, unilamellar or have an undefined lamellar construction. A pharmaceutical composition comprising the liposomes of and a pharmaceutically acceptable carrier or diluent may be used for the therapy of disease conditions such as cancer.

A focal point of the present invention involves a method of treating a host animal afflicted with tumor cells sensitive to the presence of a platinum (II) four-coordinate complex. This method comprises administering to the host an amount of the platinum (II) four-coordinate

complex described above or a liposome of the present invention comprising a phospholipid and a tumor cell-inhibiting effective amount of said platinum complex. The administering step is preferably parenteral and by

5 intravenous, intraarterial, intramuscular, intralymphatic, intraperitoneal, subcutaneous, intrapleural or intrathecal injection or by topical application or oral dosage. Such administration is preferably repeated on a timed schedule, for example twice daily for a period of two weeks. The

10 treatment may be maintained until tumor regression or disappearance has been achieved and may be used in conjunction with other forms of tumor therapy such as surgery or chemotherapy with different agents.

15 These antitumor methods may also be used to inhibit the metastatic spread of tumors such as reticulosarcoma. A preventative pretreatment with the platinum(II) complexes or liposomes comprising those complexes may be used to preclude metastatic spread in a vaccination-like manner.

20

Platinum (II) four-coordinate complexes were prepared utilizing racemic (unresolved) DACH, trans-RR-DACH or trans-SS-DACH. It was found that platinum complexes comprising trans-RR-DACH or trans-SS-DACH and two

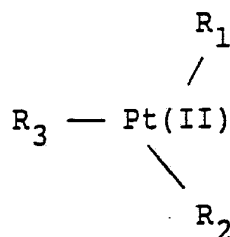
25 hydrophobic ligands such as cyclopentene-carboxylato were more efficiently incorporated into liposomes than were the analogous complexes comprising racemic DACH.

Liposomes incorporating these platinum complexes were

30 found to be stable in an aqueous milieu, non-nephrotoxic and active against murine leukemia L-1210.

In a general sense, the square-planar platinum (II) four coordinate complexes of the present invention have

35 the formula:



5

where R_1 and R_2 are preferably carboxylato monoanions bearing a hydrophobic radical function. R_1 and R_2 may also be a single carboxylato dianion where the carboxylato groups are bound to an interlinking atom which in turn is bound to a radical function. Additionally, R_3 is a vicinal diaminoalkane or vicinal diaminocycloalkane. It is contemplated that R_3 may be composed of two independent alkylamines, cycloalkylamines or ammonia. These components confer upon the complex a substantial solubility (normally greater than about 5.0 mg/ml) in methanol or chloroform at ambient temperatures and a substantial insolubility (less than about 0.5 mg/ml) in aqueous solutions at ambient temperatures.

20

The hydrophobic radical function, that function covalently pendant from the carboxyl group or from an intermediate or linking group, may be an alkyl, substituted aryl, aryl, alkenyl, cycloalkyl or cycloalkenyl group or even combinations of these functions such as alkylaryl or arylalkenyl, to name but two of the many possible hydrophobic combinations. The hydrophobic radical function characteristically has between 5 and 20 carbon atoms. When this hydrophobic radical function comprises an alkyl or alkenyl group, this group may be straight or branched. Polar functions such as hydroxyl groups, for example, substituted on the hydrophobic radicals would tend to lessen their hydrophobicity and may render them less useful for the purposes of the present invention.

35

In certain cases the R_1 and R_2 functions may be interlinked, for example when two acetate or propionate functions are bound together by a nitrogen atom. In these cases the R_1 and R_2 functions are a single carboxylato dianion. An alkyl hydrophobic function having between six and twenty carbon atoms, such as n-decane, for example, may be bound to the interlinking nitrogen and the resultant compound utilized in the production of the platinum (II) four coordinate complex of the present invention.

With certain hydrophobic radical functions (cyclopentene, for example) present on the carboxylato monoanion it was found that efficient incorporation of PT complexes into phospholipid liposomes was dependent on characteristics of the R_3 function. For example, when R_3 was 1, 2 diaminocyclohexane (DACH) the amino groups may be in several relative stereochemical configurations, cis, trans-RR and trans-SS (a mixture of these being termed "racemic"). With platinum (II) complexes containing cyclopentene radical functions (R_1 and R_2) and various stereochemical types of DACH it was found that the trans-RR-DACH and trans-SS-DACH-complexes were more efficiently incorporated into phospholipid liposomes (vesicles being a term equivalent to liposomes) than were the analogous racemic DACH complexes.

When a hydrophobic radical function was a branched alkyl containing nine carbon atoms such as that in neodecanoato, ($C_{10}H_{20}O_2$, empirical formula) the incorporation efficiency of a platinum (II) complex comprising racemic DACH or trans-RR-DACH was maximal (100%). Thus, linear or branched alkyl radical functions having from about 6 to about 12 carbon atoms confer properties of efficient phospholipid liposome incorporation upon any DACH-containing Pt (II) complex.

Liposomes containing the platinum (II) complexes described herein may be prepared from various amphipathic substances including natural or synthetic phospholipids. The phospholipids usable to produce liposomes are numerous and not exhaustively listed herein since they are generally well known in the art. These phospholipids include but are not limited to: lecithin, phosphatidylethanolamine, lysolecithin, lysophatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cardiolipin, phosphatidic acid and the cerebroside. Most preferable phospholipids for the practice of aspects of the present invention include dimyristoylphosphatidylglycerol (DMPG) and dimyristoylphosphatidylcholine (DMPC). Cholesterol in minor proportions ranging from less than 1% to about 50% may be included with phospholipids and platinum (II) complexes to produce liposomes of the present invention. A preferable but not limiting combination of DMPG and DMPC has been found to be a ratio of 3 to 7 although ratios between 1:10 and 10:1 are contemplated as satisfactory. Ratios of platinum (II) complex to phospholipid between about 1 to 10 and about 1 to 30 are contemplated as generally satisfactory although a 1 to 15 ratio was primarily used in studies thus far.

Either unilamellar or multilamellar or other platinum (II) complex - containing liposomes may be used in the practice of the present invention. Multilamellar liposomes are presently preferred since the platinum (II) complexes of the present invention are substantially water insoluble and they appear to be incorporated into the phospholipid bilayers of the liposome lamellae.

Generally, the procedure for synthesis of the platinum II compounds of the present invention may be described on one scale as follows: (1) about 10 mmole vicinal diamino cycloalkane or alkane are added to 50 ml

aqueous solution of K_2PtCl_4 (3.5 g) and stirred for six to eight hours at room temperature. The yellow solid formed comprising cis-bis-dichloro-1,2-diamine-Pt(II) may be removed by filtration and washed with fluids such as
5 water, methanol or acetone. The solid may then be suspended in about 20 ml H_2O and an aqueous solution containing about 0.75 g Ag_2SO_4 added thereto. After stirring for about 24 hours in the dark, precipitated Ag Cl may be removed by filtration. The sulfato-vicinal
10 diamine-Pt may then be dissolved in about 100 ml H_2O and added to about 2 m mol of alkali earth metal salt of a carboxylato anion prepared in situ, and stirred therewith for about 30 minutes. After removal of $BaSO_4$ by filtration, the platinum II complex of the present
15 invention may be obtained, for example by crystallization or removal of the solvent by evaporation.

The methods of preparation of particular platinum (II) complexes and chemotherapeutic treatment with
20 particular platinum (II) complexes described in the Examples contained later herein are readily adapted to the production and use of analogously described and claimed complexes by simple substitutions of appropriate vicinal diamines or hydrophobic radical-containing carboxylato
25 monoanions.

Liposomes comprising phospholipids and platinum complexes (Pt-liposomes) of the present invention are useful in inhibiting both the growth and metastatic spread
30 of tumors.

Such Pt-liposomes may be administered parenterally, topically or orally. Oral or parenteral dosages of these Pt-liposomes between about 2.5 mg/kg body weight and 25
35 mg/kg body weight are contemplated as adequate in most conditions. The particular dosages, if a tumor-bearing

human is being treated may vary in each case according to the condition of the patient, the type and extent of tumor, and particular Pt-liposome toxicity.

5 The amount of liposomal-platinum included in the pharmaceutical composition and the dosage utilized in the method of treatment of the invention will vary depending in each case upon the conditions of the patients, the nature of the tumor undergoing treatment, antitumor
10 activity of liposomal-platinum, the toxicity and solubility characteristics thereof, etc. Liposomal-platinum may also be administered in combination with other antitumor agents in a combined therapeutic regimen.

15 Parenteral administration may be intraperitoneal, subcutaneous, intrapleural, intrathecal, intraurethral, intravenous, intraarterial, intramuscular or intralymphatic. Such parenteral administration preferably involves Pt-liposome suspensions in pharmaceutically
20 acceptable solutions such as sterile isotonic aqueous solutions. These suspensions may be obtained fully prepared or may be prepared from preformed components. As known to those skilled in the art, Pt-liposomes may be prepared as pellets or powders. These pellets or powders
25 may be mixed with pharmaceutically acceptable solutions to form suspensions for parenteral administration.

 Topical administration of Pt-liposomes may involve pharmaceutical compositions such as suspensions, creams or
30 ointments which may be obtained fully prepared or prepared from Pt-liposome powders or pellets. Such topical administration may be near to sites of cancerous lesions such as the epithelium or mucosa for example.

35 Oral administrations of Pt-liposomes preferably involve encapsulation of Pt-liposome powder or pellets

whereby the Pt-liposomes are protected from much gastric and intestinal digestive activity before release from the encapsulation.

5 When desired, Pt-liposomes may be prepared to contain, for example, other therapeutic agents for treatment of tumors or anti-oxidants to aid in liposome stabilization.

10 Use of the complexes of the present invention, particularly as a component of liposomes, focuses upon the inhibition of tumor growth and prevention of the metastatic spread of tumors. For example, first a host is identified as bearing a tumor type known to generally
15 contain cells whose growth is often inhibited by platinum (II) complexes. Tumor growth in the host may be inhibited by administering to the host the PT-containing liposomes of the present invention.

20 Similarly, the metastatic spread of tumors in a host may be inhibited. A host bearing metastatic or potentially metastatic tumors of a type noted often to be sensitive to platinum (II) complexes, would first be identified. The administration of the PT-containing
25 liposomes of the present invention to that host would serve to inhibit metastatic spread.

 The following examples are presented to further illustrate preferred embodiments of the present invention;
30 they are not intended to limit the invention unless otherwise so stated in the accompanying claims.

EXAMPLE 1Materials and Analyses

5 K_2PtCl_4 was purchased from AESAR (Johnson Matthey, Inc. Seabrook, NH). Cyclopentenecarboxylic acid was purchased from Pfaltz and Bauer, Inc., Stamford, CT; 1,2-diaminocyclohexane (DACH) from Aldrich Chemical Co., Milwaukee, WI., trans-RR-DACH and trans-SS-DACH from
10 Mortol Thiokol, Inc., Danvers, MO., and neodecanoic acid from Exxon Chemical Co., Houston, Texas. Elemental analyses on the platinum complexes were performed by Integral Microanalytical Laboratories, Inc., Raleigh, NC and by Robertson Laboratory, Inc., Florham Park, N.J.
15 Infrared spectra of the complexes (as KBr pellets) were measured in the range of $600-4000\text{ cm}^{-1}$ using a Nicolet 6000 Fourier transform infrared spectrophotometer.

Chromatographically pure (thin-layer chromatography)
20 dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) used in this study were obtained from Avanti Polar Lipids (Birmingham, AL). Cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO).

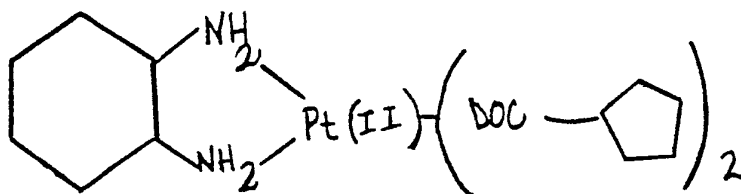
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EXAMPLE 2Synthesis of cis-bis-cyclopentenecarboxylato-1,2-DACH-platinum (II)

30

Cis-bis-cyclopentenecarboxylato-1,2-DACH-platinum (II) was one cis-platinum (CDDP) hydrophobic analogue used as a prototype to develop liposomal-platinum (L-PT) preparations. The general structure of this particular
35 complex was as follows:

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5

This prototype was synthesized using a multi-step procedure as described for racemic DACH (Khokhar et al. Inorg. Chem. Acta. Bioinorganic Section, V 108, p 63 (1985) follows: 0.96 g of DACH were added to a filtered aqueous solution of K_2PtCl_4 (3.5 g in 50 ml H_2O) and the mixture was stirred for six to eight hours at room temperature. The yellow solid containing cis-bis-dichloro-DACH-Pt (II) was removed by filtration and washed with H_2O , methanol, and finally acetone. After the final product was dried under vacuum, the yield was calculated to be 56%. Subsequently, 1.0 g of cis-bis-dichloro-DACH-Pt (II) was suspended in 20 ml H_2O and an aqueous solution of Ag_2SO_4 (0.75 g in 150 ml H_2O) was added to obtain water soluble sulfato-DACH-Pt H_2O . The reaction mixture was stirred in the dark for 24 hours and the precipitated $AgCl$ was removed by filtration. The yellow solution was evaporated to dryness at 45-50°C under reduced pressure and the yellow product was further dried over P_2O_5 in vacuum. The yield of sulfato-DACH-Pt (II) H_2O was 90%. Finally, 0.423 g (1 mmol) of sulfato-DACH-Pt (II) was dissolved in 100 ml of H_2O , and the barium cyclopentene-carboxylate was prepared in situ by the addition of 0.3 g of $Ba(OH)_2$ to 0.226 g (2 mmol) of cyclopentenecarboxylic acid in H_2O . These components were mixed and the reaction mixture was stirred for 30 minutes at room temperature. The $BaSO_4$ precipitate was filtered off, and the yellow filtrate was evaporated to dryness at 45°C under reduced pressure using a rotary evaporator. A yellow solid was

obtained, which was purified from methanol. The product was finally dried under vacuum. Yield was 70%. Analysis: C 40.26%, H 5.65% and N 5.05% $C_{18}H_{30}N_2O_4Pt$ (PT) (theoretically C 40.67%, H 5.65% and N 5.27%. The
5 infrared spectrum for the complex (as KBr pellet) $\nu_{C=O}$ 1632 cm^{-1} and ν_{C-O} 1398 cm^{-1} .

Cis-bis-cyclopentenecarboxylato-1,2-DACH-Pt (II) is highly soluble in methanol and chloroform and only
10 slightly soluble in water (less than 0.5 mg/ml). The above-described synthetic procedure was carried out with both trans-RR-DACH and trans SS-DACH to produce the trans-RR and trans-SS isomers of cis-bis-cyclopentene-carboxylato-1,2-DACH-Pt (II). The analysis of cis-bis-
15 cyclopentenecarboxylato-1,2-trans-RR-DACH-Pt (II)- $3H_2O$ was: C-37.36%, H-5.50%, N-5.12% (theoretically C-37.93%, H-5.82%, N-4.80%).

EXAMPLE 3

20

Synthesis of Cis-Bis-Neodecanoato-DACH-Platinum (II)

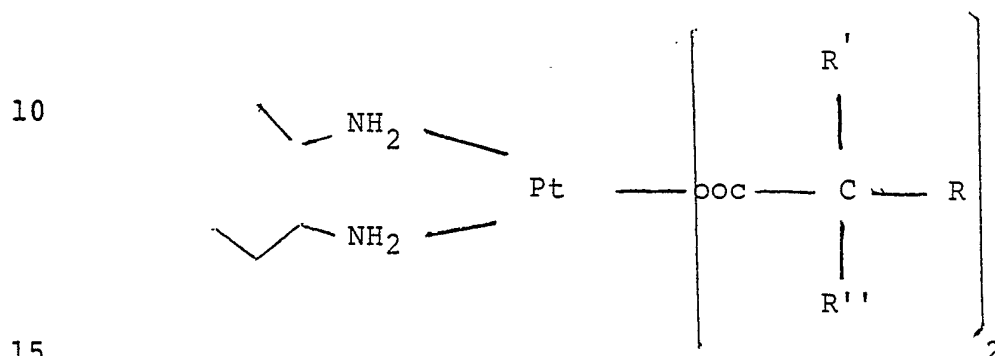
Sulfato-racemic-DACH-platinum (II) (0.423 g),
25 prepared as described in Example 2, was dissolved in 10 ml water. A potassium salt of neodecanoic acid (0.420 g) was added to this solution and the reaction mixture stirred for 30 minutes at room temperature. A gummy mass was obtained which was extracted in chloroform and the
30 chloroform extract was dried over anhydrous $MgSO_4$. The $MgSO_4$ was separated by filtration and the filtrate evaporated to dryness. An off-white solid product was obtained which was dried in vacuo and over P_2O_5 . This final product was stored at 0°C.

35

The elemental analysis of the final product was: C-48.30%; H-8.10% and N-3.92%. The calculated elemental values for a compound of the empirical formula; $C_{26}H_{52}N_2O_4Pt$. is: C-47.93%; H-8.00% and N-4.30%.

5

The structural formula of the cis-bis-neodecanoato-DACH-platinum II was as follows:



where R,R',R'' can be: CH_3 , C_2H_5 or C_3H_7 to give a carboxylato radical function $C_{10}H_{19}O_2$ (MW = 172) as empirical formula.

20

Cis,bis-neodecanoato-DACH-platinum (II) was highly soluble in methanol and chloroform and insoluble in water.

The above described procedure was carried out with
25 trans-RR-DACH to produce the trans-RR isomer of cis-bis-neodecanoato-DACH-Platinum (II). The analysis of cis-bis-neodecanoato-RR-DACH-Platinum (II) was: C-47.75%, H-8.16%, N-3.98% (theoretically C-47.93%, H-8.00%, N-4.30%).

30

EXAMPLE 4

Synthesis of Cis-Bis-n-Decyliminodiacetato-DACH-Platinum (II)

35 Sulfato-DACH-platinum (II) H_2O (0.423 g), prepared as described in Example 2, was dissolved in 10 ml of H_2O .

The sodium salt of n-decyliminodiacetic acid was prepared in situ by the addition of NaOH (0.08 g) to N-decyliminodiacetic acid (0.273 g) in 50 ml of water. This aqueous solution of sodium N-decyliminodiacetato was added to the sulfato-DACH-platinum solution and stirred for 30 minutes at room temperature. The resulting solution was evaporated to dryness on a rotary evaporator at 40°C under reduced pressure. The yellow solid thus obtained was dissolved in methyl alcohol and filtered through Celite.

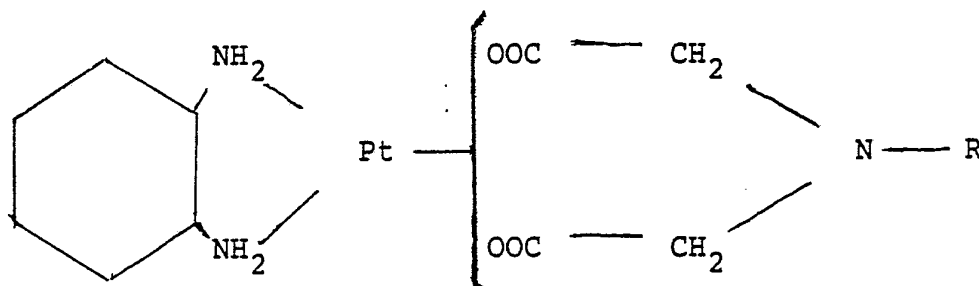
The yellow filtrate was evaporated to dryness in a rotary evaporator under reduced pressure. A yellow crystalline product was obtained, which was further purified from 1-propanol, yield 80%.

An elemental analysis of the product showed: C-39.28; H-7.12% and N-6.68%. The theoretical composition for a compound of the empirical formula, $C_{20}H_{39}N_3O_4Pt \cdot 2H_2O$, was: C-38.95%; H-7.02% and N-6.81%. The infrared spectrum for the complex (as KBr pellet) / C=O $1580cm^{-1}$ and / C-O $1410cm^{-1}$.

The structural formula of the cis-bis-n-decyliminodiaceto-DACH-platinum (II) is shown as follows:

25

30



35

where R = n-decyl or a longer straight or branched chain alkyl radical.

Cis-bis-n-decyliminodiacetato-DACH-Platinum (II) is
5 highly soluble in methanol and chloroform and insoluble in water. The above described synthetic procedure was carried out with trans-RR-DACH to produce the trans-RR isomer of cis-bis-n-decyliminodiacetato-DACH-Pt (II). The analysis of cis-bis-n-decyliminodiacetato-trans-RR-DACH-Pt
10 (II) was: C-38.60%, H-6.87%, N-6.82% (theoretically C-38.95%, H-7.02%; N-6.81%).

The above described compound represents a structure where R_1 and R_2 of the earlier described general structure
15 are one and the same and the carboxylato monoanions are bound together by a linking atom bearing an alkyl hydrophobic radical to form, in fact, a carboxylato dianion.

20 EXAMPLE 5

Platinum-Containing Liposomes (L-PT)

Multilamellar lipid vesicles (MLV) or liposomes
25 containing incorporated platinum complexes (PT) of the above descriptions were prepared as previously described for other compounds (Lopez-Berestein et al (4)(1984) Clin. Exp. Metastasis V 2 pp 127-137 and Lopez-Berestein et al (1983) J Inf Dis V 147 pp 937-945). In brief, chloroform
30 solutions of lipids (at the desired molar ratio) and PT were mixed at a lipid-PT ratio of 15:1 and the chloroform was evaporated in a rotary evaporator (Buchi, Brinkmann Instruments, Westbury, NY). The dried lipid film obtained, containing PT, was then dispersed with an
35 aqueous solution (0.9% NaCl in water) by vigorous handshaking. The suspension was subsequently centrifuged

at 30,000 x g for 45 minutes, the supernatant was discarded, and the pellet containing PT was resuspended in 0.9% NaCl solution.

5 MLV or liposomes containing platinum complexes may also be prepared from a lyophilized powder containing lipid and platinum compound. The lipid and platinum compound are dissolved in the hydrophobic solvent tertiary butanol (M.P. 26°C) at the ratios described above. The
10 solution is freeze-dried and a white powder obtained. MLV containing the platinum compound are formed upon the addition of 0.9% NaCl solution in water to the yophilized powder with mild shaking by hand.

15

EXAMPLE 6Calculation of Encapsulation
Efficiency (EE) and Stability

20 Elemental platinum (Pt) was determined in the liposome suspension and the pellet by x-ray fluorescence as previously reported (Seifert et al (1979) Proc. Amer. Ass'n. Cancer Res. V 20 p. 168) in the Department of
Analytical Chemistry, The University of Texas Medical
25 School at Houston, TX. The amount of platinum complex (PT) was determined in the supernatant by ultraviolet (UV) spectrophotometry using a wavelength of 224 nm. The EE was initially calculated with the two following formulas:

- 30 1. $EE = \frac{\text{Pt in pellet}}{\text{total Pt in the initial liposome suspension}}$
 2. $EE = \frac{\text{Total Pt initially added} - \text{Pt in supernatant}}{\text{total Pt initially added}}$

35 Since the results obtained by these two methods were highly comparable and the second method only requires PT

determination by UV spectrophotometry, most EE determinations were calculated with the second method.

The stability of the different liposome-PT (L-PT) preparations in 0.9% NaCl solution at 4°C, and 50% human (AB) serum in 0.9% NaCl solution at 37°C was determined using the following formula:

$$\text{Stability at x hours} = \frac{\% \text{ EE at x hours}}{\% \text{ EE at 0 hours}} \times 100$$

The EE values used in the stability determinations were obtained by measuring PT in the supernatants by UV spectrophotometry (0.9% NaCl solution) or x-ray fluorescence (50% human AB serum). The stability in 0.9% NaCl solution was determined up to 14 days after the initial preparation. In addition, the L-PT preparations were observed microscopically on day 14 to check the morphology of the vesicles. The stability in 50% human AB serum was determined up to 18 hours after incubation.

Different cis-platinum analogues were encapsulated in multilamellar vesicles composed of DMPC:DMPG 7:3. The encapsulation efficiency for Pt-cyclopentenecarboxylato racemic-DACH was 66%. The encapsulation efficiency was significantly increased when the pure trans RR DACH or trans-SS-DACH isomers were used (88% and 90%, respectively). The encapsulation efficiency for Pt-neodecanoato-racemic-DACH, Pt-neodecanoato trans-RR-DACH, Pt-n-decyliminodiacetato racemic DACH complexes are presented in Table I, shown below.

TABLE 1ENCAPSULATION EFFICIENCY OF
L-PT USING DIFFERENT ANALOGUES

5	Platinum Analogue	% Encapsulation Efficiency ¹
10	Pt-cyclopentenecarboxylato racemic-DACH mixture	66
	Pt-cyclopentenecarboxylato trans-RR-DACH	88
15	Pt-cyclopentenecarboxylato trans-SS-DACH	90
	Pt-neodecanoato-racemic DACH-mixture	99
20	Pt-neodecanoato-trans-RR-DACH	99
	Pt-N-decyliminodiaceto-racemic- DACH mixture	100
25	Pt-N-decyliminodiacetato-trans-RR-DACH	98

¹ Mean of at least three experiments. Liposome
composition : DMPC:DMPG 7:3

30

Analogous experiments (not shown) with DMPC alone,
DMPG alone, cholesterol alone, DMPC and DPMG combined in
other concentrations, with or without cholesterol showed
no better encapsulation efficiency and, most frequently, a
35 significantly decreased EE.

EXAMPLE 7Stability of L-PT in Aqueous Milieu

5 Preparations were suspended in 0.9% saline and
 incubated at 4°C for 14 days. The liposome compositions
 were observed by light microscopy and the amount of free
 PT in the saline determined. Liposomes containing PT and
 composed of DMPG as the only phospholipid showed
 10 significant microscopically determined loss of structure.
 Free PT was determined and stability was calculated as the
 percentage PT remaining in the liposomes. The results of
 these measurements are presented in Table 2 below.

15

TABLE 2STABILITY OF L-PT IN SALINE L-PT AT 14 DAYS¹

20	Platinum Analog	Stability %
	Pt-cyclopentenecarboxylato-racemic-DACH	89
	Pt-cyclopentenecarboxylato-trans-RR-DACH	94
25	Pt-cyclopentenecarboxylato-trans-SS-DACH	--
	Pt-neodecanoato-racemic-DACH	100
	Pt-neodecanoato-trans-RR-DACH	99
	Pt-iminodiacetato-racemic-DACH	100
	Pt-iminodiacetato-trans-RR-DACH	100

30

¹ Liposome composition : DMPC:DMPG 7:3

As may be seen in the above data, both the Pt
 cyclopentenecarboxylato trans-RR-DACH and the Pt-
 35 neodecanoato-racemic-DACH exhibit greater stability than
 the Pt cyclopentenecarboxylato-racemic-DACH.

EXAMPLE 8

Toxicity Studies of L-PT
(Cyclopentenecarboxylato-racemic-DACH)

5

Toxicology studies were carried out in 6-8 weeks old CD-1 Swiss mice weighing 22-25 gm and purchased from The University of Texas Science Park (Bastrop, TX). Free PT in suspension in hydroxypropylcellulose, DMPG, DMPG-PT, 10 DMPC:DMPG 7:3 and DMPC:DMPG 7:3-PT were administered intraperitoneally (ip) in volumes ranging between 0.1 and 0.3 ml. DMPC:DMPG 7:3-PT was also administered intravenously (iv) in one single injection or in 3 daily injections. The clinical behavior and the survival times 15 were monitored on a daily basis. The LD₁₀, LD₅₀ and LD₉₀ were calculated considering the deaths occurring up to 14 days after injection.

PT in suspension in hydroxypropylcellulose, DMPG-PT 20 and DMPC:DMPG 7:3-PT had a similar LD₅₀ dose level when given in a single intraperitoneal (ip) injection (91 mg/kg, 86 mg/kg and 75 mg/kg respectively) (Table 3). The amount of PT that would be encapsulated at the LD₅₀ dose level of empty liposomes composed of DMPG and DMPC:DMPG 25 7:3 was higher (183 mg/kg and >304 mg/kg respectively). The LD₅₀ dose for DMPC:DMPG 7:3-PT injected iv was similar for the two schedules used: single injection and daily x 3 injections (82 mg/kg vs 96 mg/kg). Most deaths in both the ip and iv toxicity studies occurred between day 5 and 30 10 after injection. The results of these studies are presented in Table 3.

TABLE 3

TOXICITY OF DIFFERENT L-PT PREPARATIONS
(CYCLOPENTENECARBOXYLATO-RACEMIC-DACH)
ADMINISTERED IP AND IV

L-PT Preparation	Route of Administration	LD ₁₀ mg/kg	LD ₅₀ mg/kg	LD ₉₀ mg/kg
Free PT	ip x 1	61	91	125
DMPG-PT	ip x 1	56	86	133
DMPC:DMPG 7:3-PT	ip x 1	54	75	94
	iv x 1	--	82	100
	iv qd x 3	63	86	107
DMPG*	ip x 1	158	183	228
DMPC:DMPG 7:3*	ip x 1	>304	>304	>304

* Results expressed in mg/kg of PT that would be encapsulated at the LD₁₀, LD₅₀ and LD₉₀ dose levels of empty liposomes.

EXAMPLE 9NEPHROTOXICITY

Blood urea nitrogen (BUN) was determined in samples obtained from the retroorbital plexus of CD₁ Swiss mice weighing 22-25 gm 96 hours after the ip injection (single dose) of CDDP, PT cyclopentenecarboxylato racemic DACH in hydroxypropylcellulose, DMPG-PT or DMPC:DMPG 7:3-PT at doses corresponding to the previously determined LD₅₀. All L-PT preparations tested for toxicity were prepared under sterile conditions on the same day of the experiments. There were no significant BUN elevations

after the ip administration of the LD₅₀ dose of PT in suspension in hydroxypropylcellulose, DMPG-PT and DMPC:DMPG 7:3-PT (BUN at 96 hours 34.4 ± 9.6 mg%, 30.0 ± 4.6 mg% and 32.0 ± 2.3 mg% respectively). The results are shown below in Table 4.

TABLE 4

ACUTE NEPHROTOXICITY OF CDDP,
FREE PT (CYCLOPENTENECARBOXYLATO-RACEMIC-DACH)
AND L-PT AT THE LD₅₀ DOSE IN CD₁ MICE

Preparation	LD ₅₀ Dose ip Single Injection (mg/kg)	BUN at 96 hours ¹ (mg%)
CDDP	17	78.3 ± 8.0
Free PT	91	34.4 ± 9.6
DMPG-PT	86	30.0 ± 4.6
DMPC:DMPG 7:3	75	32.0 ± 2.3

¹ Normal=27.2 ± mg%

EXAMPLE 10

In Vitro Antitumor Activity of
Cyclopentenecarboxylato-Racemic-
DACH-Pt Against L1210 Cells

L1210 leukemic cells were grown in a suspension culture in McCoy's 5A medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% horse serum, glutamine, streptomycin and penicillin at 37°C, 95% relative humidity in a 5% CO₂ atmosphere. Four ml of cell suspension were added to culture tubes and the appropriate concentration of free PT or L-PT was added (from 0.01 micro g/ml to 10

micro g/ml final concentration). After 96 hours, the cell concentration of control and experimental cultures were calculated with a Coulter Counter (Coulter Electronics, Hialeah, FLA) and the percent inhibition calculated. The
5 following preparations were tested: free PT, DMPG, DMPC:DMPG 7:3, DMPG-PT and DMPC:DMPG 7:3-PT. Results were expressed as the dose achieving a 50% growth inhibition (ID_{50}). Results for empty liposomes were expressed as the amount of PT that would have been encapsulated at the ID_{50}
10 concentration.

The ID_{50} for free cyclopentenecarboxylato-racemic DACH was 1.3 micro g/ml (Table 5). Of the two L-PT preparations tested, DMPG-PT was slightly more active than
15 DMPC:DMPG 7:3-PT (mean ID_{50} of three experiments; 0.7 and 1.6 micro g/ml respectively). Empty liposomes composed of DMPG had an ID_{50} of 3.7 micro g/ml while the ID_{50} for empty liposomes composed of DMPC:DMPG 7:3 was >10 micro g/ml (Table 5).

20

TABLE 5

IN VITRO ANTITUMOR ACTIVITY AGAINST L1210 CELLS OF
 FREE PT, L-PT AND EMPTY LIPOSOMES
 5 (where PT is cyclopentenecarboxylato-racemic-DACH-PT)

Preparation	ID ₅₀ (micro g/ml) ¹			
	Exp. 1	Exp. 2	Exp. 3	Mean
Free PT	1.3	--	--	--
DMPG-PT	0.7	1.0	1.4	0.7
DMPG ²	3.0	3.0	5.0	3.7
15 DMPC:DMPG 7:3-PT	1.9	2.0	0.9	1.6
DMPC:DMPG 7:3 ²	>10.0	--	--	--

¹ CDDP ID₅₀=0.1 micro g/ml.

² ID₅₀ expressed as micro g/ml of PT that would be
 20 encapsulated at the ID₅₀ concentration of empty liposomes.

EXAMPLE 11

25 In Vivo Antitumor Activity of L-PT
 Against L1210 Mouse Leukemia where
PT is cyclopentenecarboxylato-racemic-DACH

The in vivo antitumor activity of CDDP, PT in
 hydroxypropylcellulose, DMPG, DMPG-PT, DMPC:DMPG 7:3, and
 30 DMPC:DMPG 7:3-PT was tested in an L1210-BDF₁ mouse model
 (25). BDF₁ mice were purchased from Charles River
 (Wilmington, MA). Groups of 6-8 mice weighing 18-22 gm
 were inoculated ip with 1 x 10⁶ L1210 leukemia cells on
 day 0. L1210 cells were kept in DBA₂ mice were weekly
 35 passages between the different experiments. All L-PT
 preparations were injected ip in volumes of 0.1 to 0.3 ml
 24 hours after tumor inoculation. Two different schedules

of administration were used: a single injection on day 1 or an injection on days 1, 5 and 9 (multiple). The doses of CDDP used were the ones that had resulted in a maximum antitumor activity in previous experiments. The doses of PT, DMPG-PT and DMPC:DMPG 7:3-PT used ranged from 3.125 mg/kg to 50 mg/kg (approximate LD₁₀). Clinical behavior and survival times were monitored until all animals had died. Results were expressed as % T/C (median survival time of treated mice/median survival time of control mice x 100) and number of long-term survivors. Mice living more than 30 days and more than 60 days were considered to be long-term survivors for the single and multiple injection schedule respectively. All L-PT preparations tested for antitumor activity were prepared under sterile conditions on the same day of the experiment. In the first set of experiments, the effect of a single ip dose of CDDP, free PT, DMPG-PT and DMPC:DMPG 7:3-PT in the treatment of L1210 leukemia was tested. Mice treated with free PT at doses of 12.5 mg/kg and 25 mg/kg had a % T/C comparable to those treated with 10 mg/kg of CDDP (162 vs 178, means of three experiments) (Table 6). DMPG-PT at doses of 12.5 mg/kg, 6.25 mg/kg and 3.125 mg/kg had an antitumor activity comparable to free PT and CDDP (mean % T/C=175 for 12.5 mg/kg; 158 for 6.25 mg/kg; and 163 for 3.125 mg/kg) (Table 6). Doses of DMPG-PT of 25 mg/kg or more were toxic for L1210 leukemia-bearing BDF₁ mice. Mice treated with DMPC:DMPG 7:3-PT at doses of 25 mg/kg, 12.5 mg/kg and 6.25 mg/kg had a similar or slightly higher % T/C than those obtained with CDDP, free PT and DMPG-PT (mean: % T/C=215 for 25 mg/kg; 178 for 12.5 mg/kg; and 200 for 6.25 mg/kg) (Table 6). DMPC:DMPG 7:3-PT at a dose of 50 mg/kg was toxic for L1210 leukemia-bearing BDF₁ mice. Empty liposomes composed of DMPG and DMPC:DMPG 7:3, at doses equivalent to the optimal ones of loaded vesicles (L-PT) did not shown antitumor activity (% T/C=105 for DMPG and 93 for DMPC:DMPG 7:3). Long-term survivors (one

or two mice of six) were seen in the groups treated with CDDP, DMPG-PT and DMPC:DMPG 7:3-PT (Table 6).

In the experiment using a multiple dose schedule, the highest % T/C obtained was 253 for CDDP 7.5 mg/kg x 3, 284 for free PT 12.5 mg/kg x 3, 179 for DMPG-PT 6.25 mg/kg x 3 and 403 for DMPC:DMPG 7:3-PT 12.5 mg/kg x 3. Long-term survivors (1 of 6 mice) were only observed in the group treated with DMPC:DMPG 7:3-PT (Table 7).

TABLE 6

IN VIVO ANTITUMOR ACTIVITY AGAINST MOUSE L1210 LEUKEMIA
OF CDDP, FREE PT AND L-PT ADMINISTERED IP (DAY 1)
where PT is cyclopentenecarboxylato-racemic-DACH

Group Number	Preparation ⁴	% T/C (Number of Survivors on Day 30)			
		Exp.1 ¹	Exp.2 ²	Exp. 3 ³	
1	CDDP	10 mg/kg	167	--	189 (1/6)
2	Free PT	25 mg/kg	--	--	168
3		12.5 mg/kg	173	136	179
4	DMPG-PT	25 mg/kg	100	--	--
5		12.5 mg/kg	160	209 (2/6)	158
6		6.25 mg/kg	--	--	158
7		3.12 mg/kg	--	--	163 (2/6)
8	DMPC:DMPG 7:3-PT	25 mg/kg	213	200 (1/6)	232 (1/6)
9		12.5 mg/kg	167 (1/6)	--	189 (1/6)
10		6.25 mg/kg	--	--	200

¹ median survival of control = 7.5 days

² median survival of control = 11 days

³ median survival of control = 9 days

⁴ All preparations were injected ip in 0.1-0.3 ml 24 hours after the ip inoculation of 1×10^6 L1210 cells.

-34-

TABLE 7

5

IN VIVO ANTITUMOR ACTIVITY AGAINST MOUSE
L1210 LEUKEMIA OF CDDP, FREE PT AND L-PT
ADMINISTERED IP (DAYS 1, 5 and 9) WHERE
PT IS CYCLOPENTENECARBOXYLATO-RACEMIC-DACH

10

% T/C

		(Number of			
		Survivors			
Group					
Number	Preparation ¹			Dose	on Day 60)
15	1	CDDP	7.5	mg/kg x 3	253
	2	Free PT	25	mg/kg x 3	158
	3		12.5	mg/kg x 3	284
	4		6.25	mg/kg x 3	168
	5	DMPG-PT	12.5	mg/kg x 3	105
20	6		6.25	mg/kg x 3	179
	7		3.12	mg/kg x 3	168
	8		1.56	mg/kg x 3	115
	9	DMPC:DMPG 7:3-PT	12.5	mg/kg x 3	403 (1/6)
	10		6.25	mg/kg x 3	253
25	11		3.125	mg/kg x 3	210 (1/6)

¹

All preparations were injected ip in volumes of 0.1 to
0.3 ml 24 hours, 5 days and 9 days after tumor
inoculation

30

EXAMPLE 12

In Vivo Antitumor Activity of Different
L-PT Analogs Against L-1210 Leukemia

35

The antitumor activity against L-1210 murine
leukemia of the different L-PT analogs was assessed in an

L-1210-BDF, mouse model (tumor inoculation day 0, treatment day 1, ip) as described earlier herein. Compared with CDDP (% T/C=175), the L-PT analogues showed equivalent or slightly higher anti-tumor activity, with the exceptions of L-PT (cyclopentenecarboxylato trans-SS-DACH = 155 and N-decyliminodiaceto racemic-DACH = 150 and N-decyliminodiacetato trans-RR-DACH = 162). These results are shown in Table 8.

10

TABLE 8

ANTITUMOR₃ EFFECT AGAINST L1210
LEUKEMIA³ OF CIS-PLATINUM AND
DIFFERENT LIPOSOMAL-PLATINUM ANALOGUES¹

15

Platinum Analog	Dose ⁴ mg/kg	% T/C ²
PT-cyclopentenecarboxylato- racemic-DACH	25	213
25 PT-cyclopentenecarboxylato- trans-RR-DACH	25	187
PT-cyclopentenecarboxylato- trans-SS-DACH	25	155
30 PT-neodecanoato-racemic-DACH	50	187

	PT-neodecanoato-trans-RR-DACH	25	175
	PT-N-decyliminodiacetato-racemic-DACH	25	150
5	PT-N-decyliminodiacetato trans-RR-DACH	25	162
	Cis-platinum (CDDP) ⁵	10	175
	1 Liposome composition : DMPC:DMPG 7:3		
10	2 <u>Median survival of treated mice</u>		
	Median survival of control mice x 100		
	3 1×10^6 L1210 cells inoculated ip on day 0.		
15	4 Treatment : ip on day 1		
	5 Dissolved in saline, 1 mg/ml		

EXAMPLE 13

20

In Vivo Antitumor Activity of L-PT-NEODECANOATO-
RACEMIC-DACH Against Liver Metastases of Mouse
M5076 Reticulosarcoma

25 The potential antitumor effect of L-PT against liver metastases was tested using L-PT-NEODECANOATO-RACEMIC-DACH prepared as described in Example 6 and the mouse M5076 reticulosarcoma, which is a tumor that metastasizes exclusively to the liver.

30

 L-PT-NEODECANOATO-RACEMIC-DACH was more active than cis-platinum in the treatment of established liver metastases of M5076 using two different tumor inocula and schedules of administration (Table 9 and 10). L-PT-
35 NEODECANOATO-RACEMIC-DACH (25 mg/kg on days, 8, 12, 16) resulted in a more than two fold reduction in the number

of liver metastases compared with cis-platinum (7.5 mg/kg on days, 8, 12, 16) on day 30 after the intravenous inoculation of 10^5 M5076 cells (mean number of liver metastases $\pm$ SD = 39 ± 21 for L-PT vs 114 ± 38 for cis-platinum (Table 9). L-PT-NEODECANOATO-RACEMIC-DACH (25 mg/kg on days 4, 8, 12) resulted in the complete inhibition of liver metastases of M5076 on day 45 after the inoculation of 10^4 M5076 cells, while 4/6 animals treated with cis-platinum (7.5 mg/kg on days 4, 8, 12) had 175 or more liver metastases (Table 10).

L-PT-NEODECANOATO-RACEMIC-DACH was effective in the prophylaxis of liver metastases of M5076 reticulosarcoma. L-PT-NEODECANOATO-RACEMIC-DACH (37.5 mg/kg on day -1) resulted in a five-fold decrease in the number of liver metastases on day 21 after the intravenous inoculation of 10^4 M5076 cells (inoculation day 0) compared with cis-platinum (10 mg/kg on day -1) and untreated animals (mean number of liver metastases $\pm$ SD = 52 ± 20 for L-PT, 256 ± 54 for cis-platinum, and 226 ± 21 for control) (Table 11).

TABLE 9

Treatment of Liver Metastases of M5076 Reticulosarcoma
with L-PT-NEODECANOATO-RACEMIC-DACH

5				
10	Day 30	Dose	Schedule	No. Liver Metastases on
	Treatment	mg/kg	day	mean $\pm$ SD
	Cis-platinum	7.5	8,12,16	114 $\pm$ 38
15	L-PT-NEODECANOATO- 25 RACEMIC-DACH		8,12,16	39 $\pm$ 21

*Groups of 10 C57BL/6 mice were inoculated on day 0 with
10⁵ M5076 cells intravenously. Animals were treated on
20 days 8, 12, and 16 with cis-platinum or L-PT-
NEODECANOATO-RACEMIC-DACH. The median survival of
untreated animals was 19 days. Treated animals were
sacrificed on day 30, the livers dissected, placed in
Bouin's fixative, and the number of liver-tumor colonies
25 counted.

TABLE 10

Treatment of Liver Metastases of M5076 Reticulosarcoma
with L-PT-NEODECANOATO-RACEMIC-DACH

5				
	Dose	Schedule	No. Liver Metastases on	
	Day 45			
	Treatment	mg/kg	day	mean $\pm$ SD
10				
	Cis-platinum	7.5	24,8,12	0,0,175,>200,>200,>200
15	L-PT-NEODECANOATO- 25		4,8,12	0,0,0,0,0,1
	RACEMIC-DACH			

*Groups of 6 C57BL/6 mice were inoculated on day 0 with 10^4 M5076 cells intravenously. Animals were treated on days 4, 8, and 12 with cis-platinum or L-PT-NEODECANOATO-RACEMIC-DACH. The median survival of untreated animals was 30 days. Treated animals were sacrificed on day 45, the livers dissected, placed in Bouin's fixative, and the number of liver-tumor colonies counted. Two of the animals treated with cis-platinum died before day 45 versus none in the group treated with L-PT.

TABLE 11

Prophylaxis of Liver Metastases of M5076 Reticulosarcoma
with L-PT-NEODECANOATO-RACEMIC-DACH^{*}

5				
		Dose	Schedule	No. Liver Metastases on
	Day 21			
	Treatment	mg/kg	day	mean $\pm$ SD
10				
	None	--	--	226 $\pm$ 21
	Cis-platinum	10	- 1	256 $\pm$ 54
15				
	L-PT-NEODECANOATO- RACEMIC-DACH	37.5	- 1	52 $\pm$ 20

20 *Groups of 6 C57BL/6 mice were treated on day
- 1 with cis-platinum or L-PT Neodecanoato-
Racemic-DACH. Animals were inoculated on day
0 with 10^4 M5076 cells intravenously. The
median survival of untreated animals was 21
25 days. Treated animals were sacrificed on day
21, the livers dissected, placed in Bouin's
fixative, and the number of tumor colonies
counted.

EXAMPLE 14Preparation of cis-bis-neodecanoato-
cis-diamine-platinum (II).

5

Cis-diamine-diiodo-platinum (II) $(\text{NH}_3)_2 \text{Pt-I}_2$ was first prepared by the following method:

10 $\text{K}_2 \text{PtCl}_4$ (5g) was dissolved in water (20ml). An aqueous solution of KI (3g) was added and a dark brown solution was obtained. Aqueous concentrated ammonia (2ml) was added to the mixture which was stirred for 2-3 hr. at room temperature. The mixture was filtered and the solid was washed with an excess of water, ethanol and ether. The
15 product was dried over P_2O_5 under vacuum. Yield 4.5g (77%).

The compound according to the invention was then prepared as follows:

20

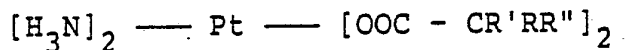
$(\text{NH}_3)_2\text{-Pt-I}_2$ (1.0g) was suspended in water (50ml), and aqueous solutions of AgNO_3 (0.68g 20ml H_2O) was added thereto. The reaction mixture was stirred overnight at room temperature in the dark. The AgI precipitate was
25 filtered and filtrate was concentrated by rotary evaporation. To the concentrated solution a solution of sodium neodecanoato prepared in situ, (neodecanoic acid 0.688g in 20ml methanol and 1N NaOH, 4ml) was added. The reaction mixture was stirred overnight at room tempera-
30 ture. The yellow reaction mixture was evaporated to dryness at 40°C under reduced pressure using a rotary evaporator. A gummy product was obtained which was extracted in chloroform and the chloroform extract was dried over anhydrous MgSO_4 . MgSO_4 was separated by
35 filtration and filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. A cream-color

solid was obtained which was dried over P_2O_5 in vacuo.
The final product was stored at 0°C.

The elemental analysis of the final product was
5 C41.58, H8.03; N 4.45%. The calculated values for
 $C_{20}H_{44}N_{24}Pt$ is C42.00; H7.7, N4.90%.

The structural formula of the cis-bis-neodecanoato-
bis-diamine-platinum (II), is as follows:

10



where R, R', R" can be CH_3 , C_2H_5 or C_3H_7 to give a
carboxylato radical function with $C_{10}H_{19}O_2$ (MW = 171) as
15 empirical formula.

Cis-bis-neodecanoato-bis-diamine-platinum (II) is highly
soluble in chloroform, methanol and other common organic
solvents, but insoluble in water.

20

EXAMPLE 15

Preparation of cis-bis-neodecanoato-bis cyclohexylamine-platinum (II) dihydrate

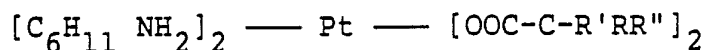
25

The method of Example 14 was followed, using the
cyclohexylamine ligand in place of ammonia. The complex
is highly soluble in chloroform, methanol and other
organic solvents, but insoluble in water.

30

Elemental Analysis; Calculated for $C_{32}H_{64}N_2O_4 \cdot 2H_2O$,
C49.71; H8.80, N3.62%, found C49.31; H8.39; N3.16%

The structural formula of the cis-bis-neodecanoato-
35 bis-cyclohexylamine-platinum (II) is as follows:



where R, R', R'' can be CH₃, C₂H₅ or C₃H₇ to give a
 carboxylato radical functions C₁₀H₁₉O₂ (MW = 171) as
 5 empirical formula.

EXMPLE 16

10 Preparation of cis-bis-neodecanoato- ethylenediamine-platinum (II) H₂O.

Cis-diiodo-ethylenediamine-platinum (II), was first
 prepared by the method of Example I, using ethylenediamine
 ligand in place of ammonia and with a 96% yield of
 15 product.

Sulfato-ethylenediamine-platinum (II). H₂O was
 prepared by the following method.

20 Cis-bis-diiodo-ethylenediamine-platinum (II), (3.9)
 was suspended in 10ml H₂O and an aqueous solutions of
 Ag₂SO₄ (2.2g in 200 ml H₂O) was added thereto. The
 reaction mixture was stirred in the dark overnight at room
 temperature. AgI was removed by filtration and the yellow
 25 filtrate was evaporated to dryness at 40-45°C under
 reduced pressure using rotary evaporation. The final
 product was dried over P₂O₅ under vacuum. The product
 yield was 2.35g (81%).

30 The compound according to the invention was then
 prepared as follows:

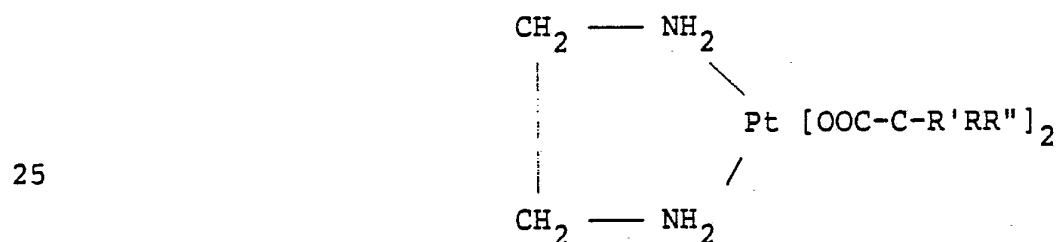
Sulfato-ethylenediamine-platinum (II), H₂O (0.369 g)
 was dissolved in H₂O (20ml) and a solution of sodium
 35 neodecanoato prepared in situ (neodecanoic acid, 0.344g in
 20 ml methanol and 1N NaOH, 2ml) was added thereto. The

reaction mixture was stirred for 2-3 hr. at room temperature. The reaction mixture was evaporated to dryness at 40-45°C under reduced pressure using rotary evaporation. A gummy product was obtained which was
 5 extracted in chloroform and the chloroform extract was dried over anhydrous MgSO_4 . The MgSO_4 was separated by filtration and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The final product was dried over P_2O_5 in vacuo. The product
 10 was stored at 0°C.

Elemental analysis; calculated for $\text{C}_{22}\text{H}_{46}\text{N}_2\text{O}_4\text{Pt} \cdot \text{H}_2\text{O}$, C42.86; H7.79; N 4.54%, found C43.07; H 7.32; N 4.71%.

15 Cis-bis-neodecanoato-ethylenediamine-platinum (II) is highly soluble in chloroform, methanol and other organic solvents, but insoluble in water.

The structural formula of the cis-bis-neodecanoato-
 20 ethylenediamine-platinum (II) is as follows:



where R, R', R" can be CH_3 , C_2H_5 or C_3H_7 to give a carboxylato radical function $\text{C}_{10}\text{H}_{19}\text{O}_2$ (MW = 171) as
 30 empirical formula.

EXAMPLE 175 Preparation of cis-bis-neodecanoato-
 bis-isopropylamine-platinum (II)

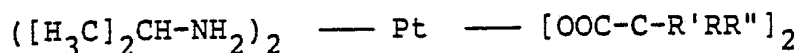
Cis-bis-neodecanoato-bis-isopropylamine-platinum (II) and sulfato-bis-isopropylamineplatinum (II) were first prepared by the method of Example 16, using isopropylamine
10 ligand in place of ethylenediamine.

The compound according to the invention was then prepared as follows:

15 Sulfato-bis-isopropylamine-platinum (II). H_2O (0.427g) was dissolved in H_2O (50ml) and a solution of sodium-neodecanoato prepared in situ (neodecanoic acid 0.344g in 20 ml of methanol and 1N NaOH, 2 ml) was added thereto. The reaction mixture was stirred for 2-3 hr. at
20 room temperature. The reaction mixture was evaporated to dryness at 40-45°C under reduced pressure using rotary evaporator. A gummy mass was obtained which was extracted in chloroform and the chloroform extract was dried over anhydrous $MgSO_4$. The $MgSO_4$ was separated by filtration
25 and filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The product was dried over P_2O_5 in vacuo. The final product was stored at 0°C.

Elemental analysis, calculated for $C_{26}H_{56}N_2O_4Pt$,
30 C47.63; H8.53; N4.26%; found C 47.74; H8.47; N 3.93%.

The structural formula of the cis-bis-neodecanoato-bis-isopropylamine-platinum (II) is as follows:



where R, R', R'' can be CH₃, C₂H₅ or C₃H₇ to give a
carboxylato radical function with C₁₀H₁₉O₂ (MW = 171) as
5 an empirical formula.

Cis-bis-neodecanoato-bis-isopropylamine-platinum
(II), is highly soluble in chloroform, methanol and other
organic solvents, but insoluble in water.

10

EXAMPLE 18

Preparation of cis-bis-decanoato-trans- R,R-1,2-diaminocyclohexane-platinum II.

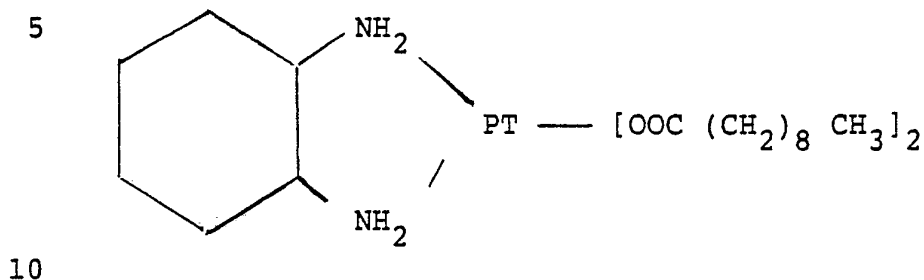
15

Sulfato-trans-R,R-1,2-diaminocyclohexane-platinum II
H₂O (0.423g) was dissolved in H₂O (20ml) and a solution of
sodium-decanoato prepared in situ (0.344g decanoic acid in
20ml methanol and 1N NaOH 2ml) was added thereto. The
20 reaction mixture was stirred for 2-3 hr. at room
temperature. The reaction mixture was evaporated to
dryness at 40-45°C under reduced pressure using a rotary
evaporator. A solid was obtained which was extracted in
chloroform and chloroform extract was dried over anhydrous
25 MgSO₄. The MgSO₄ was removed by filtration and filtrate
was evaporated to dryness under reduced pressure using
rotary evaporator. The product was dried over P₂O₅ in
vacuo. The final product was stored at 0°C.

30 Elemental analysis - calculated for C₂₆H₅₂N₂O₄Pt.H₂O
C 46.59; H 8.06; N 4.18%, found C 45.94; H 7.87 N 4.31%.

Cis-bis-decanoato-trans-R,R-1,2-diaminocyclohexane-
platinum II is highly soluble in chloroform and other
35 organic solvents, but insoluble in water.

The structural formula of the cis-bis-decanoato-trans-R,R-1,2-diaminocyclohexane-platinum II is as follows:



EXAMPLE 19

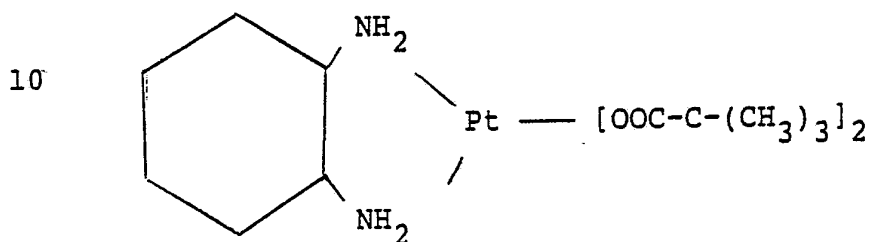
Cis-bis-neopentanoato-trans-R,R-1,2-diaminocyclohexane-platinum II.

Sulfato-trans-R,R-1,2-diaminocyclohexane-platinum II. H₂O (0.423g) was dissolved in water (20ml) and a solution of barium-neopentanoato prepared in situ (neopentanoic acid, 0.204g, in 5 ml of methanol and Ba (OH)₂·8H₂O 0.3g in 50 ml H₂O combined together) was added thereto. The reaction mixture was stirred for 2-3 hr. at room temperature. The reaction mixture was evaporated to dryness at 40-45°C under reduced pressure using a rotary evaporator. The residue was extracted with methanol, filtered and the filtrate was evaporated to dryness. A solid was obtained which was further extracted with chloroform. The chloroform extract was evaporated to dryness and a cream-color product was obtained. The product was dried over P₂O₅ under vacuum.

Elemental analysis calculated for C₁₆H₃₂N₂O₄Pt·2H₂O; C35.00; H6.57; N5.11%, found C35.16; H6.17; N5.27%.

Cis-bis-Neopentanoato-trans-R,R-1,2-diaminocyclohexane-platinum II, is highly soluble in chloroform, methanol and other common organic solvents.

- 5 The structural formula of the cis-bis-neopentanoato-trans-R,R-1,2-diaminocyclohexane-platinum II is as follows:



EXAMPLE 20

Encapsulation efficiency and antitumor activity of lipophilic cisplatin analogs

- 20 The compounds prepared in Examples 14-19 were tested for their efficiency of encapsulation in liposomes by the methods described in Example 6. These liposome-encapsulated compounds were tested to determine their optimal dose and effectiveness as inhibitors of in vivo
- 25 tumor growth as described in Example 12. The data in Table 12 reveal the resultant measurements.

Table 12

Encapsulation Efficiency and Antitumor Activity of
Lipophilic Cisplatin Analogues Incorporated in Liposomes

Compound	Encapsulation Efficiency	Optimal dose mg/kg	%T/C L1210 ip/ip single dose
Cis-bis-neodecanoato-cis-diamine-platinum (II)	95%	50	200
Cis-bis-neodecanoato-bis-cyclohexylamine-platinum (II)	89%	100	125
Cis-bis-neodecanoato-ethylenediamine-platinum (II)	82%	75	144
Cis-bis-neodecanoato-bis-isopropylamine-platinum (II)	87%	100	150
Cis-bis-decanoato-trans-R,R-1,2-diaminocyclohexane-platinum (II)	96%	50	187
Cis-bis-neopentanoato-trans-R,R-1,2-diaminocyclohexane-platinum (II)	93%	25	170

EXAMPLE 21In Vitro cytotoxicity against
human malignant cell lines

5

Cis-bis-neodecanoato-1,2-diaminocyclohexane platinum (II) was tested in the liposomal-form against three human malignant cell lines of colon carcinoma (LoVo, SW620, and
10 SW403) using a colony formation inhibition assay. The drug concentration that resulted in a 50% inhibition of colony formation (IC50) ranged from 4 to 8 uM. The IC50 of cisplatinum for these same cell lines ranged from 3 to 7 uM.

15

x x x x x x x x x x

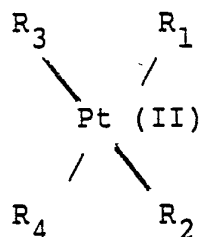
Changes may be made in the various compositions, elements, steps and procedures described herein without
20 departure from the concept and scope of the invention as defined in the following claims.

CLAIMS:

1. A platinum (II) four-coordinate complex having the formula:

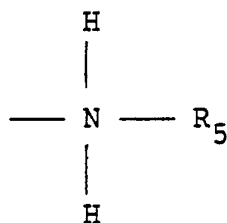
5

10



wherein R_1 and R_2 are each carboxylato bearing a
 15 hydrophobic radical function or, when linked together, are
 a dicarboxylato bearing a hydrophobic radical function,
 and wherein R_3 and R_4 are each:

20



25

wherein R_5 is selected from the group consisting of
 hydrogen, alkyl, aryl, aralkyl, alkenyl, or cycloalkyl,
 cycloalkenyl or a combination thereof; or

30

wherein R_3 and R_4 , when linked together, are selected
 from the group consisting of cycloalkyl-1,2-diamino having
 between about 3 and 7 carbon atoms, and alkyl-1,2-diamino
 having between about 2 and 12 carbon atoms; and

35

said complex is defined further as being substantially soluble in methanol or chloroform and substantially insoluble in water.

5

2. The complex of claim 1 wherein R_3 and R_4 are defined further as comprising an alkyl having between 1 and 20 carbon atoms or cycloalkyl having between 3 and 12 carbon atoms.

10

3. The complex of claim 2 wherein R_5 is an alkyl having between 1 and 20 carbon atoms.

15

4. The complex of claim 2 wherein the alkyl has between 6 and 12 carbon atoms.

20

5. The complex of claim 2 wherein the alkyl is defined further as having between about 2 and 6 carbon atoms.

6. The complex of claim 1 wherein the carboxylato is an alkylcarboxylato having between about 5 and 20 carbon atoms, or an arylcarboxylato wherein aryl is phenyl, naphthyl, or alkylphenyl wherein the alkylphenyl has between about 12 and 16 carbon atoms.

30

7. The complex of claim 1 wherein the dicarboxylato is an alkyldicarboxylato having between about 5 and 20 carbon atoms, an aryldicarboxylato wherein aryl is naphthyl, phenyl, or alkylphenyl wherein the alkylphenyl has between about 6 and 12 carbon atoms.

35

8. The complex of claim 7 wherein the aryl is defined further as having between about 6 and 14 carbon atoms.

5 9. The complex of claim 1 wherein the alkenyl is defined further as having between about 5 and 20 carbon atoms.

10 10. The complex of claim 1 wherein the cycloalkyl is defined further as having between about 3 and 12 carbon atoms.

15 11. The complex of claim 1 wherein R_1 and R_2 are cycloalkenyl carboxylato, the cycloalkenyl having between about 5 and 20 carbon atoms, and wherein R_3 and R_4 are linked together and are trans-S,S or-R,R-1,2-diaminocyclohexane when the cycloalkenyl has 6 or less carbon atoms.

20

12. The complex of claim 1 wherein the aralkyl is defined further as containing linked aryl and alkyl portions and having between about 7 and 20 carbon atoms.

25

13. The complex of claim 12 wherein the aryl portion has between about 6 and 10 carbon atoms.

30

14. The complex of claim 12 wherein the alkyl portion has between about 1 and 10 carbon atoms.

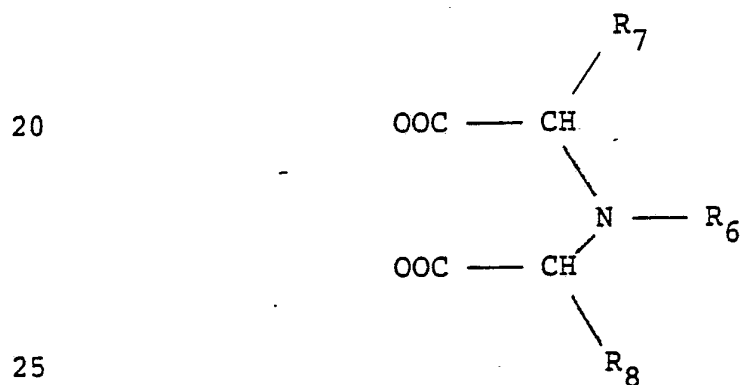
35 15. The complex of claim 1 wherein R_3 and R_4 , taken together are cycloalkyl-1,2-diamino, have between about 3

and 20 carbon atoms, and wherein the cycloalkyl ring has between about 3 and 12 carbon atoms.

5 16. The complex of claim 15 wherein the 1,2-diaminocyclohexane is trans-R,R-1,2-diaminocyclohexane, and R_1 and R_2 are each decanoato which are cis.

10 17. The complex of claim 16 wherein R_1 and R_2 are neodecanoato.

15 18. The complex of claim 1 wherein R_1 and R_2 , linked together, are a dicarboxylato of the formula:



wherein R_6 is alkyl having between about 10 and 20 carbon atoms and R_7 and R_8 are each hydrogen or an alkyl having between about 1 and 5 carbon atoms.

30

19. The complex of claim 18 wherein R_7 and R_8 are each hydrogen.

35

20. The complex of claim 18 wherein the cycloalkyl-1,2-diamino is 1,2-diaminocyclohexane.

5 21. The complex of claim 20 wherein the 1,2-diaminocyclohexane is defined further as being trans-R,R-1,2-diaminocyclohexane or trans-S,S-1,2-diaminocyclohexane.

10

22. The complex of claim 21 wherein R_6 is defined further as being decyl.

15 23. The complex of claim 21 wherein R_6 is decyl, R_7 and R_8 are hydrogen, and the dicarboxylato is cis.

20 24. The complex of claim 18 wherein R_7 and R_8 are hydrogen, and the dicarboxylato is cis.

25 25. The complex of claim 18 wherein R_7 and R_8 are hydrogen.

25

26. The complex of claim 16 wherein R_1 and R_2 are defined further as being neo-pentoato.

30

27. The complex of claim 18 wherein the dicarboxylato is cis.

35 28. The complex of claim 18 wherein the 1,2-diaminocyclohexane is in the R,R conformation.

29. The complex of claim 1 wherein R_5 is hydrogen.

30. The complex of claim 29 wherein R_1 and R_2 are each an
5 alkylcarboxylato.

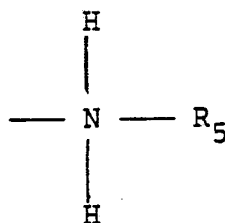
31. The complex of claim 30 wherein the alkylcarboxylato
is neo-decanoato.

10

32. The complex of claim 12 wherein R_1 and R_2 are cis.

15 33. The complex of claim 1 wherein R_3 and R_4 are unlinked
and are each:

20



25

where R_5 is cyclohexyl.

34. The complex of claim 33 wherein R_1 and R_2 are each
30 alkylcarboxylato.

35. The complex of claim 33 wherein R_1 and R_2 are each
neo-decanoato.

35

36. The complex of claim 33 wherein R_1 and R_2 are cis.

37. The complex of claim 1 wherein R_3 and R_4 are unlinked
5 and are each:



wherein R_5 is isopropyl.

15

38. The complex of claim 37 wherein R_1 and R_2 are each
neo-decanoato.

20

39. The complex of claim 37 wherein R_1 and R_2 are cis.

40. The complex of claim 1 wherein R_3 and R_4 are linked
25 and are alkyl-1,2-diamino having 2 to 12 carbon atoms.

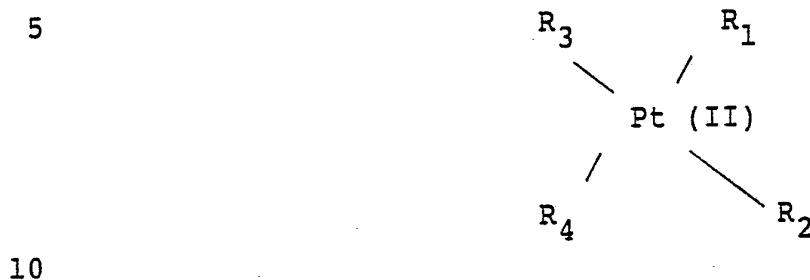
41. The complex of claim 40 wherein the alkyl is ethyl.

30

42. The complex of claim 41 wherein R_1 and R_2 are
alkylcarboxylato.

35 43. The complex of claim 42 wherein the alkylcarboxylato
is neo-decanoato.

44. A liposome comprising a four-coordinate platinum complex having the formula:



wherein R_1 and R_2 are each carboxylato bearing a hydrophobic radical function or when linked together are a dicarboxylato bearing a hydrophobic radical function, and
 15 wherein R_3 and R_4 are each:



25 and R_5 is hydrogen, alkyl having between about 1 and 20 carbon atoms, or cycloalkyl having between about 3 and 12 carbon atoms;

or wherein R_3 and R_4 when linked together are
 30 cycloalkyl-1,2-diamino having between about 3 and 7 carbon atoms, or alkenyl-1,2-diamino having between about 2 and 12 carbon atoms; and

said complex is defined further as being
 35 substantially soluble in methanol or chloroform and substantially insoluble in water, and a phospholipid.

45. A pharmaceutical composition comprising the liposome of claim 44 and a pharmaceutically acceptable carrier or diluent.

5

46. The liposome of claim 44 wherein R_5 is an alkyl having between about 6 and 12 carbon atoms.

10 47. The liposome of claim 44 wherein the carboxylato is an alkylcarboxylato having between about 5 and 20 carbon atoms, or an arylcarboxylato wherein aryl is naphthyl, phenyl, or an alkylphenyl wherein the alkylphenyl has between about 12 and 16 carbon atoms.

15

48. The liposome of claim 44 wherein the dicarboxylato is an alkylcarboxylato having between about 5 and 20 carbon atoms, or an arylcarboxylato wherein aryl is naphthyl,
20 phenyl, or alkylphenyl, wherein the alkylphenyl has between about 12 and 18 carbon atoms.

49. The liposome of claim 44 wherein each hydrophobic
25 radical function is defined further as being alkyl, aryl, aralkyl, alkenyl, or cycloalkyl, cycloalkenyl or a combination thereof.

30 50. The liposome of claim 49 wherein the complex is cis-bis-n-decyliminodiacetato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

51. The liposome of claim 44 wherein the complex is cis-bis-n-decyliminodiacetato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

5

52. The liposome of claim 44 wherein the complex is cis-bis-neodecanoato-Trans R,R-1,2-diaminocyclohexane-platinum (II).

10

53. The liposome of claim 44 wherein the complex is cis-bis-ammine-bis-neodecanoato-platinum (II).

15

54. The liposome of claim 44 wherein the complex is cis-bis-decanoato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

20

55. The liposome of claim 44 wherein the complex is cis-bis-neopentanoato-1, 2-diaminocyclohexane-platinum (II).

25

56. The liposome of claim 44 wherein the complex is cis-bis-cyclopentenecarboxylato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

30

57. The liposome of claim 44 wherein the complex is cis-bis-neodecanoato-ethylenediamine-platinum (II).

35

58. The liposome of claim 44 wherein the complex is cis-bis-neodecanoato-bis-isopropylamine-platinum (II).

59. The liposome of claim 44 wherein the complex is cis-bis-decanoato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

5

60. The liposome of claim 44 wherein the complex is cis-bis-neopentanoato-trans-R,R-1,2-diaminocyclohexane-platinum (II).

10

61. The liposome of claim 44 wherein the complex is cis-bis-neodecanoato-bis-cyclohexylamine-platinum (II).

15 62. The liposome of claim 44 wherein the phospholipid is defined further as being phosphatidylglycerol, phosphatidylcholine, sphingomyelin, phosphatidic acid or phosphatidylserine.

20

63. The liposome of claim 44 wherein the phospholipid is defined further as consisting essentially of phosphatidylglycerol, a phosphatidylcholine or a combination thereof.

25

64. The liposome of claim 44 defined further as comprising cholesterol.

30 65. The liposome of claim 63 wherein the phosphatidylglycerol is defined further as consisting essentially of dimyristoylphosphatidylglycerol and the phosphatidylcholine is defined further as consisting essentially of dimyristoylphosphatidylcholine.

35

66. The liposome of claim 65 wherein the dimyristoylphosphatidylglycerol and the dimyristoylphosphatidylcholine are defined further as being in a ratio between about 1 to 10 and 10 to 1.

5

67. The liposome of claim 65 wherein the dimyristoylphosphatidylglycerol and the dimyristoylphosphatidylcholine are defined further as being in a ratio of about 3 to 7.

10

68. The liposome of claim 44 defined further wherein the platinum complex and the phospholipid are in a ratio between about 1 to 10 and about 1 to 30.

15

69. The liposome of claim 44 defined further wherein the platinum complex and the phospholipid are in a ratio between about 1 to 15.

20

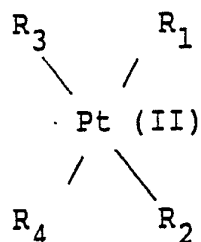
70. The liposome of claim 44 defined further as being multilamellar.

25

71. The liposome of claim 44 defined further as being unilamellar.

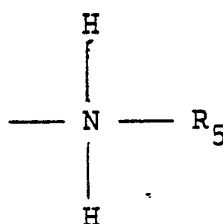
30 72. A method of treating an animal afflicted with tumor cells sensitive to a platinum (II) four-coordinate complex which comprises administering a liposome to the animal, said liposome comprising a phospholipid and a platinum (II) four-coordinate complex having the formula:

35



5

wherein R_1 and R_2 are each carboxylato bearing a hydrophobic radical function or when linked together are a dicarboxylato bearing a hydrophobic radical function, and
 10 wherein R_3 and R_4 are each:



15

20

wherein R_5 is hydrogen, alkyl having between about 1 and 20 carbon atoms, or cycloalkyl having between about 3 and 12 carbon atoms; or

25

wherein R_3 and R_4 , when taken together are cycloalkyl-1, 2-diamino having between about 3 and 7 carbon atoms, or alkenyl-1, 2-diamino having between about 2 and 12 carbon atoms; and

30

said complex is defined further as being substantially soluble in methanol or chloroform and substantially insoluble in water.

73. The method of claim 72 wherein the carboxylato is an alkylcarboxylato having between about 6 and 12 carbon atoms.

5

74. The method of claim 72 wherein the carboxylato is an alkylcarboxylato having between about 5 and 20 carbon atoms, or an arylcarboxylato wherein aryl is naphthyl, phenyl, or alkylphenyl and the alkylphenyl has between
10 about 7 and 16 carbon atoms.

75. The method of claim 72 wherein the dicarboxylato is an alkylcarboxylato having between about 5 and 20 carbon
15 atoms, or an arylcarboxylato wherein aryl is naphthyl, phenyl, or alkylphenyl and the alkylphenyl has between about 12 and 18 carbon atoms.

20 76. The method of claim 72 wherein each hydrophobic radical function is defined further as being alkyl, aryl, aralkyl, alkenyl, cycloalkyl or cycloalkenyl or a combination thereof having between about 5 and about 20 carbon atoms.

25

77. The method of claim 72 wherein the phospholipid is defined further as consisting essentially of phosphatidyl glycerol, phosphatidylcholine or a combination thereof.

30

78. The method of claim 72 wherein the liposome is defined further as comprising cholesterol.

35

79. The method of claim 77 wherein the phosphatidyl
glycerol is defined further as consisting essentially of
dimyristoylphosphatidylglycerol and the phosphatidyl
choline is defined further as consisting essentially of
5 dimyristoylphosphatidylcholine.

80. The method of claim 79 wherein the dimyristoylphos-
phatidylglycerol and the dimyristoylphosphatidylcholine
10 are defined further as being in a ratio between about 1 to
10 and 10 to 1.

81. The method of claim 79 wherein the dimyristoylphos-
15 phatidylglycerol and the dimyristoylphosphatidylcholine
are defined further as being in a ratio of about 3 to 7.

82. The method of claim 72 wherein the liposomes are
20 defined further as having the complex and the phospholipid
in a ratio between about 1 to 10 and about 1 to 30.

83. The method of claim 78 wherein the liposomes are
25 defined further as having the complex and the phospholipid
in a ratio of about 1 to 15.

84. The method of claim 72 wherein the liposomes are
30 defined further as being multilamellar.

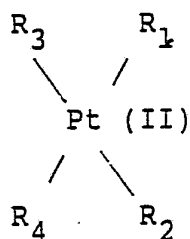
85. The method of claim 72 wherein the liposomes are
defined further as being unilamellar.

86. The method of claim 72 wherein the administering step is defined further as being intravenous, intraarterial, intramuscular, intralymphatic, intraperitoneal, subcutaneous, intrapleural or intrathecal injection or by
5 topical application or oral dosage.

87. The method of claim 72 wherein the administration is defined further as being repeated on a timed schedule.
10

88. A method for preparing a platinum (II) four-coordinate complex having the formula:
15

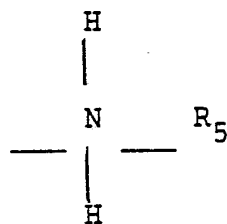
15



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wherein R_1 and R_2 are each carboxylato bearing a hydrophobic radical function or, when linked together, are
25 a dicarboxylato bearing a hydrophobic radical function, and wherein R_3 and R_4 are each:

30



35

wherein R_5 is hydrogen, alkyl having between about 1 and 20 carbon atoms, or cycloalkyl having between about 3 and 12 carbon atoms;

5 or wherein R_3 and R_4 , when linked together, are cycloalkyl-1,2-diamino having between about 3 and 7 carbon atoms, or alkenyl-1,2-diamino having between about 2 and 12 carbon atoms; and

10 said complex is defined further as being substantially soluble in methanol or chloroform and substantially insoluble in water;

the method comprising: reacting a sulfato-trans-
15 R,R-1,2-diaminoalkane-platinum (II) complex with an alkali earth metal salt of a carboxylato monoanion bearing a hydrophobic radical function to produce said platinum (II) four-coordinate complex.

20

89. The method of claim 88 wherein R_5 is alkyl having between about 1 and 20 carbon atoms.

25 90. The method of claim 88 wherein the reaction occurs in an aqueous environment.

91. The method of claim 88 wherein the sulfate salt of
30 the alkali earth metal is removed by filtration, and the platinum complex is obtained by crystallization or by removal of the solvent by evaporation.

35 92. The method of claim 88 wherein the evaporation proceeds under reduced pressure at 45-50°C.

93. The method of claim 88 wherein the platinum complex is further dried over a drying agent.

5 94. The method of claim 93 wherein the drying agent is phosphorus pentoxide.

95.. A sulfato-trans-R,R-1,2-diaminoalkane-platinum (II)
10 complex produced by the method of claim 88.

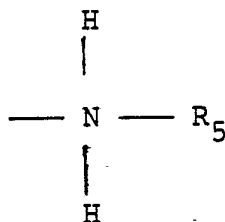
96. A method of producing a liposome comprising:

15 (a) providing platinum (II) four-coordinate complex having the formula:



25 wherein R_1 and R_2 are each carboxylato bearing a hydrophobic radical function or, when linked together, are a dicarboxylato bearing a hydrophobic radical function, and wherein R_3 and R_4 are each:

30



35

wherein R_5 is hydrogen, alkyl having between about 1 and 20 carbon atoms, or cycloalkyl having between about 3 and 12 carbon atoms; or

5 wherein R_3 and R_4 when linked together are cycloalkyl-1,2-diamino having between about 3 and 7 carbon atoms, or alkenyl-1,2-diamino having between about 2 and 12 carbon atoms;

10 said complex is defined further as being substantially soluble in methanol or chloroform and substantially insoluble in water;

(b) mixing said complex with phospholipid at a ratio
15 of about 1:15 in a hydrophobic solvent;

(c) evaporating the hydrophobic solvent to produce a film of phospholipid and complex or lyophilizing the mixture to form a powder;

20

(d) dispersing the film or powder in an aqueous solution to produce liposomes.

25 97. The method of claim 96 wherein R_5 is alkyl having between about 6 and 12 carbon atoms.

98. The method of claim 96 wherein the phospholipid is
30 defined further as consisting essentially of phosphatidylglycerol, phosphatidylcholine or a combination thereof.

99. The method of claim 96 wherein step (b) is defined
35 further as mixing said complex with phospholipid and cholesterol.

100. The method of claim 98 wherein the phosphatidyl-
glycerol is defined further as consisting essentially of
dimyristoylphosphatidylglycerol and the phosphatidylcholine
is defined further as consisting essentially of
5 dimyristoylphosphatidylcholine.

101. The method of claim 100 wherein the dimyristoylphos-
phatidylglycerol and the dimyristoylphosphatidylcholine
10 are defined further as being in a ratio between about 1-10
and 10-1.

102. The method of claim 100 wherein the dimyristoylphos-
15 phatidylglycerol and the dimyristoylphosphatidylcholine
are defined further as being in a ratio of about 3 to 7.

103. The method of claim 91 wherein the liposomes are
20 defined further as comprising the complex and the
phospholipid in a ratio between about 1 to 10 and about 1
to 30.

25 104. The method of claim 96 wherein the liposomes are
defined further as having the complex and the phospholipid
in a ratio between about 1 to 15.

30 105. The method of claim 96 wherein the liposomes are
defined further as being multilamellar.

106. The method of claim 96 wherein an additional step is
35 added of subjecting the liposomes to sonic oscillation to
produce unilamellar liposomes.

107. The method of claim 96 wherein the step (c) is defined further as comprising rotary evaporation.

5 108. A method for inhibiting the growth of tumors in a host comprising identifying a host bearing a tumor type known to generally contain cells whose growth is inhibited by platinum (II) complexes; and administering the liposomes of claim 44 to the host.

10

109. The method of claim 108 wherein the liposomes are further defined as being multilamellar.

15

110. The method of claim 108 wherein the liposomes are further defined as being unilamellar.

20 111. The method of claim 102 wherein the liposomes are administered parenterally.

112. A method for inhibiting the growth of tumors in a
25 host comprising identifying a host bearing a tumor type known to generally contain cells whose growth is inhibited by platinum (II) complexes; and administering the complex of claim 1 to the host.

30

113. A method of inhibiting the metastatic spread of tumors in a host comprising identifying a host bearing a potentially metastatic or metastatic tumor of a type known generally to be inhibited by platinum (II) complexes; and
35 administering the liposomes of claim 44 to the host.

114. The method of claim 113 wherein the liposomes are further defined as being multilamellar.

5 115. The method of claim 113 wherein the liposomes are further defined as being unilamellar.

116. The method of claim 113 wherein the liposomes are
10 administered parenterally.

117. A method for inhibiting the metastatic spread of tumors in a host comprising: identifying a host bearing a
15 potentially metastatic or metastatic tumor of a type known generally to be inhibited by platinum (II) complexes; and administering the complex of claim 1 to the host.

20 118. The method of claim 117 wherein the complex is administered parenterally.

119. A method of vaccinating a host against the metastatic
25 spread of tumors comprising administering the liposomes of claim 44 to the host.

120. The method of claim 119 wherein the liposomes are
30 unilamellar.

121. The method of claim 119 wherein the liposomes are multilamellar.

122. The method of claim 119 wherein the liposomes are administered intravenously.

5 123. The method of claim 119 wherein the metastatic spread is due to reticulosarcoma.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02181

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁴ : C 07 F 15/00; A 61 K 31/28; A 61 K 9/50						
II. FIELDS SEARCHED Minimum Documentation Searched * <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">IPC⁴</td> <td style="border: 1px solid black; padding: 5px;">C 07 F 15/00; A 61 K 31/28</td> </tr> </table> Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *			Classification System	Classification Symbols	IPC ⁴	C 07 F 15/00; A 61 K 31/28
Classification System	Classification Symbols					
IPC ⁴	C 07 F 15/00; A 61 K 31/28					
III. DOCUMENTS CONSIDERED TO BE RELEVANT *						
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³				
X	EP, A, 0130482 (RESEARCH CORPORATION) 9 January 1985, see pages 2,3; page 10-12,14; example 24; claims	1-71,88-94, 96-107				
X	EP, A, 0113508 (INCO RESEARCH & DEVELOPMENT CENTER, INC.) 18 July 1984 see examples; claims cited in the application	1-71,88-94, 96-107				
X	EP, A, 0136012 (KIDANI) 3 April 1985 see pages 1,2; examples 6,7; claims	1-71,88-94, 96-107				
X	BE, A, 898614 (FABRICA DE PRODUCTOS QUIMICOS Y FARMACEUTICOS ABELLO, S.A.) 2 May 1984, see pages 1,4,8; claims	1-71,88-94, 96-107				
X	Eur. J. Med. Chem. - Chim. Ther., volume 19, no. 4, 1984, D.G. Craciunescu et al.: "On the preparation, antitumour and cytotoxic evaluation of some new ./. "					
* Special categories of cited documents: ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Δ" document member of the same patent family						
IV. CERTIFICATION						
Date of the Actual Completion of the International Search 13th February 1987		Date of Mailing of this International Search Report 16 AVR. 1987				
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer M. VAN MOL				

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	analogues of the cis-dichloro (1,2-diamino-cyclohexane) platinum (II) complex", pages 353-357 see page 353, compounds 1a,1b,1c; page 353, tables II,III; page 356 --	1-71,88-94, 96-107
P,X	Chemical Abstracts, volume 105, no. 15, 13 October 1986, (Columbus, Ohio, US), see page 699, abstract 134160x, & JP, A, 6137794 (MITSUI PETROCHEMICAL INDUSTRIES, LTD; SUNTORY LTD) 22 February 1986. --	1-71,88-94, 96-107

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE :

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

") Claims 72-78 and 108-123

See rule 39.1.(iv) PCT

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING :

This International Searching Authority found multiple inventions in this International application as follows:

Claims 1-94, 96-123

Claim 95

Please refer to Form PCT/ISA 206 dated 27th January 1987

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-94, 96-123
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	EP, A, 0193936 (SUMITOMO PHARMACEUTICALS CO. LTD) 10 September 1986 see column 2, line 44 - column 3, line 35; examples	1-71,88-94, 96-107
X	Chemical Abstracts, volume 88, no. 2, 9 January 1978, (Columbus, Ohio, US), P. Schwartz et al.: "Preparation and antitumor evaluation of water-soluble derivatives of dichloro(1,2-diamino- cyclohexane)platinum(II)", see page 43, abstract 16014k, & Cancer Treat. Rep. 1977, 61(8), 1519-25, and Tenth Collective Index, page 40461CS	1-71,88-94, 96-107
