TRITIATED PLATINUM ANTITUMOR AGENTS CONTAINING THE TRANS-(d,l)-1,2-DIAMINOCYCLOHEXANE CARRIER LIGAND

Steven D. Wyrick and Stephen G. Chaney*
Division of Medicinal Chemistry and Natural Products, School of Pharmacy and Department of Biochemistry and Nutrition*, School of Medicine, University of North Carolina, Chapel Hill, N. C. 27514

SUMMARY

Cis-diamminedichloroplatinum (II) (cisplatin) is one of the most widely used anticancer drugs today. However, platinum compounds possessing the 1,2-diaminocyclohexane (DACH) carrier ligand offer advantages over cisplatin with regard to bioavailability, activity and decreased renal toxicity. The trans-(d,l)-1,2-diaminocyclohexane derivatives are the isomeric forms recently chosen by the NCI for large animal studies and phase I/II clinical trials. Here we report the synthesis of four trans-(d,l)-1,2-diaminocyclo-hexane platinum derivatives labeled with tritium in the cyclohexane ring by catalytic reduction of a cyclohexene precursor with carrier-free tritium gas over 10% Pd/C.

Key words: tetraplatin, trans-(d,l)-1,2-Diaminocyclohexanedichloroplatinum(II), trans-(d,l)-1,2-diaminocyclohexanenitratoplatinum(II), trans-(d,l)-1,2-diaminocyclohexanemalonatoplatinum(II), tritium, catalytic reduction

INTRODUCTION

Cis-Diamminedichloroplatinum(II) (cisplatin) is one of the most widely used anticancer drugs today.[1,2] However, because of the relatively high toxicity of cisplatin, there has been considerable effort to develop safer second generation drugs. Platinum compounds with the 1,2-diaminocyclohexane (dach) carrier ligand[3,4] are particularly interesting because (i) these compounds appear to have activity comparable to cisplatin in a wide variety of tumor screens,[5,6] (ii) they usually exhibit reduced renal toxicity[7,8] and better penetration of the blood brain barrier,[9] and (iii) they are effective against cell lines of L1210 and P388 which are resistant to cisplatin.[8] With respect to the compounds described in this manuscript, trans-(d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV) (tetraplatin) appears to have the greatest potential as a chemotherapeutic agent. It has similar therapeutic

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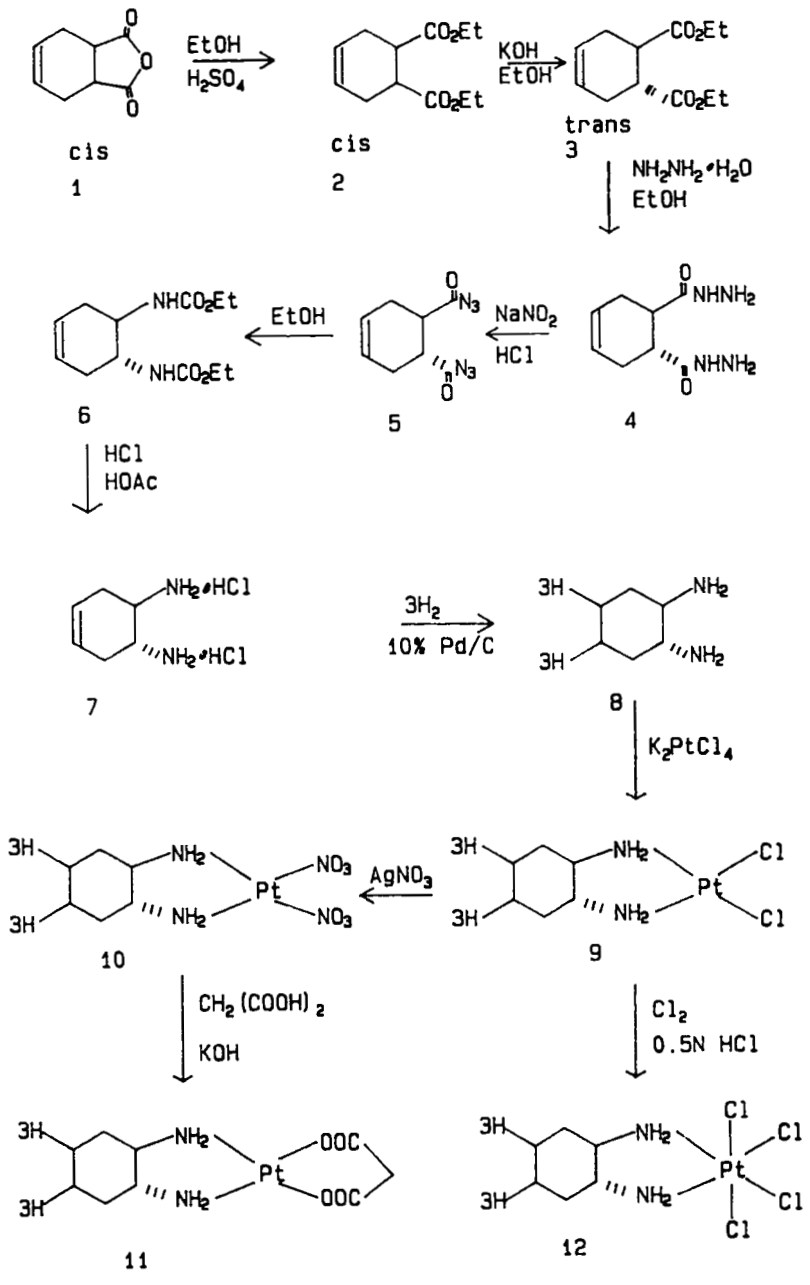
effectiveness[9,10] and reduced nephrotoxicity[11] compared to cisplatin in a variety of murine leukemia screens. In addition, its water solubility and stability make it a particularly good candidate for further drug development.[9] 1,2-Diaminocyclohexanedichloroplatinum(II) ($PtCl_2$(dach)) is of interest because it is one of the most likely major metabolites of $PtCl_4$(dach) inside the cell.[12-14] 1,2-Diaminocyclohexanemalonatoplatinum(II) (Pt(mal)(dach)) is useful primarily as a model compound to study metabolism of platinum compounds with bidentate leaving ligands.

The 1,2-diaminocyclohexane carrier ligand can exist in three different isomeric forms: cis, trans-d, and trans-l[15]. [Note: cis and trans here refer to the configuration of the 1,2-diamino substituents on the cyclohexane ring. All three isomers are complexed cis relative to the platinum atom]. Platinum compounds containing each of these isomers have slightly different effectiveness and toxicity in the various tumor cell screens[15,16] and form diasteriomerically distinct adducts with d(GpG).[17,18] The mixed trans-d,l isomers of 1,2-diaminocyclohexane are of particular interest because they are the isomeric forms of tetraplatin recently chosen by the NCI for large animal studies and phase I/II clinical trials.[9] Here, we report the synthesis of four trans-(d,l)-1,2-diaminocyclohexane platinum derivatives tritium labeled in the cyclohexane ring.

DISCUSSION

Tritium labelling was focused on the cyclohexane ring. A modification of the procedure of Kepler[19] was used to reduce trans-(d,l)-1,2-diaminocyclohex-4-ene (7) with tritium gas over 10% Pd/C in ethanol. Compound 7 was prepared by the procedure of Craven.[20] Cis-tetrahydrophthalic anhydride (1) was converted to the cis-diethyl ester 2 using ethanol and sulfuric acid. The cis diester was epimerized to the the trans isomer 3 by refluxing in ethanolic KOH. Conversion of the trans diester to the hydrazide 4 was accomplished smoothly using hydrazine monohydrate in ethanol. Conversion to the diazide 5 with $NaNO_2$/HCl followed by Curtius rearrangement to the diurethane 6 proceeded in good yield. Hydrolysis of 6 using HCl/HOAc followed by decarboxylation in the same step afforded the tritiation precursor 7. The dihydrochloride salt of 7 was converted to the diacetate to facilitate solution for tritiation and an ethanolic solution of the diacetate was reduced with 5.0 Ci of



carrier-free tritium gas over 10% Pd/C at room temperature. The crude product 8 was diluted with unlabeled trans-(d,l)-1,2-diaminocyclohexane obtained by resolution of a commercial mixture of the cis and trans isomers as the nickel complexes by the procedure of Saito.[21] The diluted [4,5-$^3H_2$(n)]-trans-(d,l)-1,2-diaminocyclohexane was then reacted with potassium tetrachloroplatinate[22] to afford [4,5-$^3H_2$(n)]-trans-

(d,l)-1,2-diaminocyclohexanedichloroplatinum(II) (9) in excellent yield (89%). Compound 9 was then stirred with an aqueous solution of $AgNO_3$ to afford the dinitrato derivative 10 in a essentially quantitative conversion. A solution of the dinitrato derivative was then treated with an aqueous solution of malonic acid (pH=5-6 with KOH) to afford an 87% yield of [4,5-$^3H_2$(n)]-trans-(d,l)-1,2-diaminocyclohexanemalonatoplatinum(II) 11. The dichloro derivative 9 was also used to prepare [4,5-$^3H_2$(n)]-trans-(d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV) (tetraplatin) (12). This conversion was accomplished in 69% yield by treatment of 9 with chlorine gas in 0.5N HCl at reflux. The specific activities of the various products are given under Experimental Procedures.

EXPERIMENTAL PROCEDURES

All chemicals were used as received from the manufacturers. Melting points were obtained on a Mel-Temp apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on either a JOEL FX 60 or Varian XL 300 spectrometer using deuterium oxide as solvent. Radiopurity was determined using a Bioscan BID-100 Image Analyser. Tritium was counted using a Packard Tricarb 4000 liquid scintillation spectrometer with Scintiverse^R counting solution. Silica gel 60 plates were used for TLC analyses. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN and are correct within 0.4% of theoretical values unless otherwise noted.

Cis-1,2-dicarbethoxycyclohex-4-ene (2). A solution of 40.0g (0.263 mol) of tetrahydrophthalic anhydride in 200 ml of absolute ethanol and 4.0 ml of conc. H_2SO_4 were stirred at reflux for 4 h. Toluene (100 ml) was added and the volume reduced by 70%. More ethanol (100 ml) was added and reflux was continued another hour. The volatiles were evaporated in vacuo and the residue partitioned between ether-H_2O. The organic layer was dried (Na_2SO_4) and evaporated in vacuo to afford 58.6 g (98%) of colorless oil.

Trans-(d,l)-1,2-dicarbethoxycyclohex-4-ene (3). Compound 2 (58.6 g, 0.259 mol) was stirred 2 h at reflux in 300 ml of 1% ethanolic KOH. The ethanol was evaporated in vacuo and the residual oil was partitioned between ether-H_2O. The ether layer was dried (Na_2SO_4) and evaporated in vacuo to afford 45.2 g of yellow oil. Distillation (155° C/30 mm Hg) afforded 40.7 g (70%) of colorless oil.

Trans-Cyclohex-4-ene-1,2-dihydrazide (4). A solution of 20.0 g (0.088 mol) of the trans diester 3 and 35.0 g (0.7 mol) of hydrazine monohydrate were stirred at reflux for 9 h. A white precipitate formed after 3 h. The suspension was cooled to 5°C and the solid filtered, washed with ethanol and then ether and dried to afford 14.5 g (83%) of pure product as a colorless solid; mp = 236-238° (lit.[20] mp = 217-218°C).

Trans-Cyclohex-4-ene-1,2-dicarbazide (5). The hydrazide 4 (14.5 g, 0.073 mol) was dissolved in 725 ml of H_2O containing 17.4 ml of conc. HCl and the solution was cooled to 7°C. A solution of 11.6 g (0.168 mol) of sodium nitrite in 58 ml of H_2O was added in portions while maintaining the temperature below 15°C. The reaction was stirred in an ice bath for 40 min. The oil which separated was extracted into ether, the extracts dried (Na_2SO_4) and evaporated *in vacuo* to afford 14.7 g (91%) of a yellow oil.

Trans-(d,l)-1,2-bis-ethoxycarbaminocyclohex-4-ene (6). The azide 5 (14.7 g, 0.067 mol) was dissolved and stirred at reflux for 12 h in 300 ml of absolute ethanol. The volume was reduced *in vacuo* to 40-50 ml and the solution stored at -10°C. The resulting precipitate was filtered, washed with cold ethanol and dried to afford 11.3 g (70%) of the diurethane as a colorless solid; mp = 135-137°C (lit.[20] mp = 136-137°C.)

Trans-(d,l)-1,2-diaminocyclohex-4-ene Dihydrochloride (7). The diurethane 6 (11.3 g, 0.047 mol) was dissolved in 113 ml of conc. HCl containing 45 ml of glacial acetic acid and the reaction was stirred at reflux for 18 h. The volatiles were removed *in vacuo* and the gummy residue was dissolved in 35 ml of conc. HCl and stored at 5°C. The resulting precipitate was filtered, washed with absolute ethanol and then ether and dried to afford 700 mg (8%) of the product as a colorless solid; mp > 330°C. ^{1}H-NMR (D_2O, H_2O as reference) δ 5.74 (t, 2H, $\underline{H}C = C\underline{H}$), 3.80 (sextet, 2H, $C\underline{H}NH_2$) and 2.48 (m, 4H, $C\underline{H}_2CHNH_2$).

Trans-(d,l)-1,2-diaminocyclohexanedichloroplatinum(II). Trans-(d,l)-1,2-diaminocyclohexane[21] (0.824 g, 0.0072 mol) was dissolved in 25 ml of H_2O containing 3.0 g (0.0072 ml) of K_2PtCl_4.[22] A precipitate began to form immediately. After 16 h at room temperature, the precipitate was filtered, washed with H_2O, methanol, then ether and dried to afford 2.6 g (95%) of product as a yellow solid. A 600 mg portion

was dissolved in 100 ml of hot DMF to give a greenish-yellow solution which was filtered and concentrated in vacuo to 35 ml. Methanol (150 ml) was added and the solution stored at -10°C. The resulting precipitate was filtered, washed with ethanol, then ether and dried to afford 418 mg (66%) of yellow solid; mp > 330°C. Anal ($C_6H_{14}Cl_2N_2Pt$) calc: C = 18.90, H = 3.68, Cl = 18.66, N = 7.37, Pt = 51.33; found: C = 19.02, H = 3.56, Cl = 18.72, N = 7.35, Pt = 51.47.

Trans-(d,l)-1,2-Diaminocyclohexanedinitratoplatinum(II). The preceeding dichloroplatinum compound (1.0 g, 2.63 mmol) was vigorously stirred for 1 h at 60°C and then overnight at room temperature in a solution of 893 mg (5.26 mmol) of $AgNO_3$ in 18 ml of H_2O. The reaction was filtered through a fine sintered glass funnel to remove the AgCl and the filtrate was divided into two equal portions. The H_2O was evaporated in vacuo from one portion to afford 520 mg (91%) of the dinitratoplatinum product as amber colored crystals. Anal ($C_6H_{14}N_4O_6Pt$) calc: C = 16.64, H = 3.23, N = 12.93, Pt = 45.04; found: C = 16.60, H = 3.32, N = 12.89, Pt = 45.02.

Trans(d,l)-1,2-diaminocyclohexanemalonatoplatinum(II). A solution of 274 mg (2.64 mmol) of malonic acid in 15 ml of H_2O (pH = 5-6 with KOH) was added to the other half of the filtrate above containing 1.32 mmol of the nitratoplatinum derivative and the reaction was stirred at 60°C for 1 h during which time a colorless precipitate formed. After stirring at room temperature for 24 h, the precipitate was filtered, washed with H_2O, methanol, then ether and dried to afford 427 mg (79%) of a colorless solid; mp = 250-252°C. Anal ($C_9H_{16}N_2Pt$) calc: C = 26.29, H = 3.89, N = 6.81, Pt = 47.45; found: C = 26.19, H = 3.91, N = 6.83, Pt = 48.84.

Trans-(d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV)[9]. A suspension of 700 mg (1.84 mmol) of trans-1,2-diaminocyclohexanedichloroplatinum(II) in 24 ml of 0.5N HCl was saturated with chlorine gas at room temperature. The solid immediately turned orange. The reaction was then stirred at reflux for 30 min during which time more chlorine was bubbled in and the solid turned from orange back to yellow. The volatiles were removed in vacuo and the yellow solid residue was dissolved in 40 ml of warm acetone and filtered. The filtrate was evaporated in vacuo and the solid redissolved in 4.0 ml of warm acetone and 5.0 ml of ether was added. After storing at -10°C, the precipitate was filtered, washed with ether and dried to afford 330 mg (40%) of product as a yellow solid; mp > 290°C. Anal ($C_6H_4N_2Cl_4Pt$) calc: C =

15.97, H = 3.10, N = 6.21, Cl = 31.44, Pt = 43.28; found: C = 15.61, H = 3.20, N = 6.09, Cl = 31.58, Pt = 42.93.

[4,5-$^3H_2$(n)]-Trans-1,2-diaminocyclohexanedichloroplatinum(II) (9). Compound 7 (46 mg, 0.25 mmol) was stirred vigorously for 1 h in 10 ml of CH_2Cl_2 to which 4 drops of 50% NaOH had been added. Excess anhydrous Na_2CO_3 was added and the suspension filtered into 1.0 ml of glacial acetic acid. The filtrate was evaporated in vacuo and the gummy residue dissolved in 2.0 ml of absolute ethanol. A 0.7 ml aliquot was stirred for 4 h under 5.0 Ci of carrier-free tritium gas over 10 mg of 10% Pd/C. The reaction mixture was filtered through a Celite pipet column, the filtrate diluted with 50 ml of absolute ethanol containing 469 mg of unlabeled trans-(d,l)-1,2-diaminocyclohexane and the volatiles removed in vacuo. The residue was again dissolved in 50 ml of absolute ethanol and the volatiles removed in vacuo. The crude labeled [4,5-$^3H_2$(n)]-Trans-(d,l)-1,2-diaminocyclohexane (8) (1.5 Ci) was dissolved in 10 ml of H_2O and added to a solution of 1.73 g (4.1 mmol) of K_2PtCl_4 in 15 ml of H_2O and the reaction was allowed to stand overnight at room temperature. The precipitate was filtered, washed with H_2O, methanol, and then ether and air dried to afford 1.39 g (89%) of product 9 as a yellow solid of > 95% radiochemical purity; mp > 250°C. The specific activity was 0.402 Ci/mmole.

[4,5-$^3H_2$(n)]-Trans-(d,l)-(1,2-diamninocyclohexanedinitratoplatinum(II) (10). Compound 9 (470 mg,1.24 mmol) was stirred vigorously in a solution of 422 mg (2.48 mmol) of $AgNO_3$ in 10 ml of H_2O at 60°C for 1 h and then overnight at room temperature. The AgCl was filtered as described above and 10% (44.7 mCi) of the filtrate (95% radiochemical purity) set aside. The remainder of the filtrate was used for the preparation of 11 below. The specific activity was 0.444 Ci/mmole.

[4,5-$^3H_2$(n)]-Trans-(d,l)-1,2-diaminocyclohexanemalonatoplatinum(II) (11). A solution of 232 mg (2.23 mmol) of malonic acid in 20 ml of H_2O (pH = 5-6 with KOH) was added to the remainder of the filtrate containing 1.12 mmol of 10 and the reaction was stirred at 60°C for 1 h and then stored at 5°C overnight. The precipitate was filtered, washed with H_2O, methanol and then ether and dried to afford 399 mg (87%) of 11 of > 95% radiochemical purity as a colorless solid; mp > 250°C. The specific activity was 0.481 Ci/mmol.

[4,5-$^3H_2$(n)]-Trans-1,2-diaminocyclohexanetetrachloroplatinum(IV) (12). Compound

9 (687 mg, 1.81 mmol) was stirred at reflux for 30 min in 25 ml of 0.5N HCl previously saturated with chlorine. Initially, the solid turned orange and then later turned yellow again. The volatiles were removed in vacuo and the yellow solid residue was dissolved in 40 ml of warm acetone and filtered. The filtrate was evaporated in vacuo and the yellow solid redissolved in 10 ml of acetone and 10 ml of ether was added. After storing at -10°C, the precipitate was filtered and washed with ether. A second crop was similarly obtained to afford a total of 559 mg (69%) of product as a yellow solid of 95% radiochemical purity; mp > 250°C. The specific activity was 0.419 Ci/mmol.

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