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Synthesis of [$^3H_2$]-(*R,R*)-1,2-Diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin

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SUMMARY

A synthesis of [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, **2**, is described. *rac-trans*-4-Cyclohexene-1,2-dicarboxylic acid diethyl ester, **6**, was converted to *rac-trans*-1,2-diaminocyclohex-4-ene, **7**, by modification of known chemistry aimed at avoiding reported hazards. Resolution of the diamine, **7**, with L-(+)-tartaric acid afforded the (*R,R*)-1,2-diaminocyclohex-4-ene, **8**, which was converted to the (*R,R*)-1,2-bis(*tert*-butoxy-carbamino)cyclohex-4-ene, **10**, and tritiated to yield [$^3H_2$]-(*R,R*)-1,2-bis(*tert*-butoxycarbamino)-cyclohexane, **11**. Hydrolysis of **11** afforded [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexane, **12**, which was converted to the desired [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, **2**.

Key Words: [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexane.

INTRODUCTION

(*R,R*)-1,2-Diaminocyclohexaneoxalatoplatinum(II),[1] Oxaliplatin, **1**, is marketed in France as Eloxatin for the indication of refractory metastatic colon cancer,[2] and has activity in tumour cell lines resistant to cisplatin and carboplatin.[3] As part of a development programme, it was required to synthesise [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalato-platinum(II), [$^3H_2$]-Oxaliplatin, **2**, to facilitate metabolic and pharmacokinetic studies.

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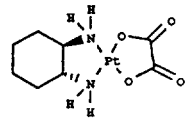
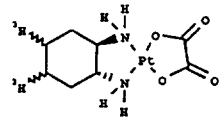
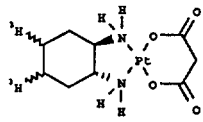
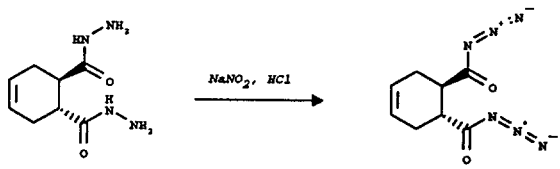
(1)   (2)

A previous synthesis of the related [$^3H_2$]-*rac-trans*-1,2-diaminocyclohexanemalonato-platinum(II), **3**, has been reported.[4]

rac-  (3)

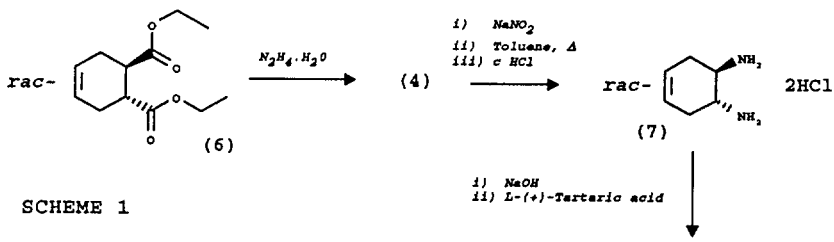
However the synthesis involved no resolution and the conversion of the bis(carbono-hydrazide), **4**, to the bis(carbonyl azide), **5**, had proved to be hazardous,[5] presumably on attempted isolation of product.



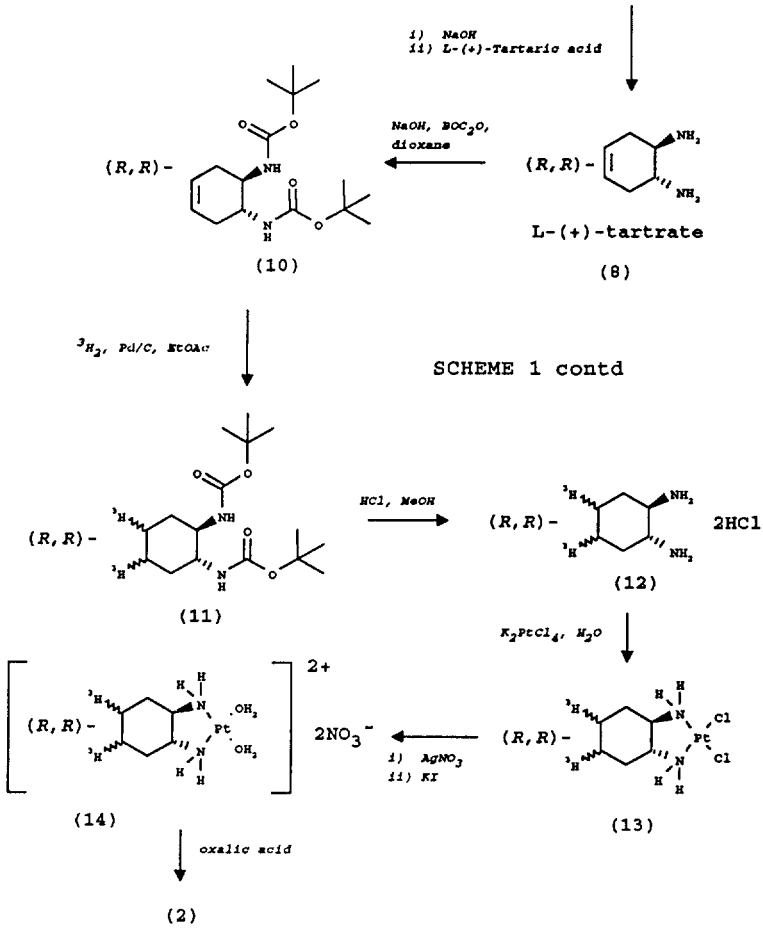
(4) (5)

RESULTS AND DISCUSSION

The synthesis of [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, **2**, was carried out as outlined in Scheme 1.



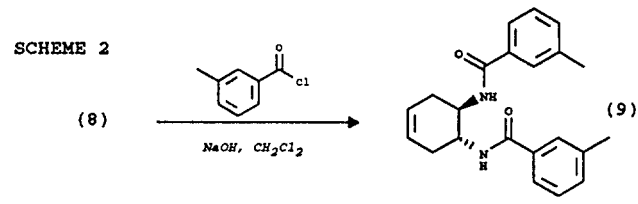
SCHEME 1



The precursor *rac-trans*-4-cyclohexene-1,2-dicarboxylic acid diethyl ester, **6**, was synthesised as reported previously,[6] and converted to the bis(carbonohydrazide), **4**, by treatment with neat hydrazine hydrate under reflux in 88% yield. The bis(carbonohydrazide), **4**, in dilute hydrochloric acid was treated with aqueous sodium nitrate at 0°C to form the bis(carbonyl azide), **5**, *in situ*.[7] The bis(carbonyl azide), **5**, was extracted from the aqueous phase in toluene and the organic phase dried before being slowly added to additional toluene under reflux. The solution was maintained at reflux for twenty minutes and then allowed to cool to room temperature before addition of concentrated hydrochloric acid to afford, after work-up, the desired racemic diamine dihydrochloride, **7**, in 71% yield.

The free diamine was liberated from the salt, **7**, by treatment with aqueous sodium hydroxide and resolved by three consecutive crystallisations with L-(+)-tartaric acid to afford the desired (*R,R*)-1,2-diaminocyclohex-4-ene tartrate, **8**, in a yield 11% of theoretical with an e.e. of >99%. The determination of e.e. was made by chiral HPLC on the bis(amide)s, **9**, formed by reaction of the diamine with *m*-toluoyl chloride (so as to provide a UV absorption for detection) as in Scheme 2.

SCHEME 2



The resolved diamine, **8**, was converted to the more soluble (*R,R*)-1,2-bis(*tert*-butoxycarbamino)cyclohex-4-ene, **10**, in 97% yield. The substrate, **10**, in ethyl acetate was then tritiated with tritium gas over palladium on charcoal to afford [$^3H_2$]-(*R,R*)-1,2-bis(*tert*-butoxycarbamino)cyclohexane, **11**, 1.47 Ci, with a radiochemical purity of >98%. ^{3}H Nmr of **11** in *d*-methanol confirmed the positions of labelling to be the methylenes of the cyclohexane ring with no incorporation at the methine. It was clear that, as anticipated, allylic incorporation of tritium had occurred. Hydrolysis of **11** afforded the desired [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexane, **12**, in 86% radiochemical yield.

The tritiated diamine, **12**, was converted to [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexanedichloroplatinum(II), **13**, by treatment with potassium tetrachloroplatinate(II) in 95% radiochemical yield. Dilution to approximately the desired specific activity was carried out during this step. Treatment of the dichloro- species, **13**, with aqueous silver nitrate and removal of traces of silver salts with potassium iodide afforded the dinitrate salt, **14**, which on treatment with oxalic acid gave the desired [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, **2**, in 39% radiochemical yield with a radiochemical purity of 99.6%. The specific activity of the [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II) was measured to be 127.5 μCi/mg.

EXPERIMENTAL

rac-trans-4-Cyclohexene-1,2-dicarbonohydrazide, 4: Hydrazine monohydrate (14.2 ml, 293 mmole) was added to *rac-trans*-4-cyclohexene-1,2-dicarboxylic acid diethyl ester,[6] **6**, (8.26 g, 36.5 mmole) and the reaction mixture heated under reflux for 8 hours. Water was then added to the resulting suspension. The solid was removed by filtration, washed with water, ethanol (twice) then ether (twice) before being dried under high vacuum to afford *rac-trans*-4-cyclohexene-1,2-dicarbonohydrazide,[4] **4** (4.95 g, 25 mmole, 68% yield).

rac-trans-1,2-Diaminocyclohex-4-ene dihydrochloride, 7:[7] *rac-trans*-4-Cyclohexene-1,2-dicarbonohydrazide, **4**, (4.87 g, 25 mmole) was dissolved in a mixture of water (250 ml) and concentrated hydrochloric acid (6 ml, 50 mmole). The solution was cooled to 0°C and an aqueous solution of sodium nitrite (3.75 g in 20 ml) was slowly added. A white precipitate formed during the addition. After 30 minutes, toluene (50 ml) was added to the reaction mixture. The reaction was stirred until the white precipitate had disappeared (2 hours). Toluene (50 ml) was then added and the aqueous phase decanted. The organic phase was washed sequentially with water and brine before being dried over anhydrous magnesium

sulfate. This solution was then added slowly to toluene (10 ml) under reflux under an atmosphere of nitrogen. The reaction mixture was maintained under reflux for 20 minutes after the end of the addition and then the reaction mixture was cooled to room temperature. Concentrated hydrochloric acid (5 ml, 41 mmole) was then added and the reaction mixture was stirred overnight at room temperature. The water was then azeotroped and the resulting white solid was removed by filtration and dried under high vacuum to afford the desired *rac-trans*-1,2-diaminocyclohex-4-ene dihydrochloride, **7** (3.217 g, 17.4 mmole, 71% yield).

(*R,R*)-1,2-Diaminocyclohex-4-ene tartrate, **8** *rac-trans*-1,2-Diaminocyclohex-4-ene dihydrochloride, **7** (3 g, 16.2 mmole) was suspended in dichloromethane. Sodium hydroxide (3 g) was dissolved in the minimum amount of water and added to the suspension. Diethyl ether was added and the organic phase decanted. The aqueous phase was extracted twice with a mixture of dichloromethane and diethyl ether. The combined organic phases were dried over anhydrous magnesium sulfate then the solvent removed under reduced pressure to give *rac-trans*-1,2-diaminocyclohex-4-ene (1.168 g). This diamine was dissolved in water (2 ml) and L-(+)-tartaric acid (780 mg, 5.2 mmole) added, followed by acetic acid (540 ml, 9.4 mmole). The mixture was heated to 80°C then allowed to cool to room temperature to afford a precipitate of crude (*R,R*)-1,2-diaminocyclohex-4-ene tartrate (897 mg) . The enantiomeric excess was determined to be ~80% by means of chiral HPLC analysis of the material derivatised as the bis(*m*-toluoyl amide), **9**. The crude tartrate was subjected to recrystallisation in water (twice), until the e.e. was >99% as determined by this method, to afford (*R,R*)-1,2-diaminocyclohex-4-ene tartrate, **8** (120 mg, 0.458 mmole, 11.2% of theoretical).

(*R,R*)-1,2-Bis(*tert*-butoxycarbamino)cyclohex-4-ene, **10:** (*R,R*)-1,2-Diaminocyclohex-4-ene tartrate, **8** (120 mg, 0.458 mmole) was dissolved in aqueous sodium hydroxide 4.5 M (250 ml, 1.12 mmole). A solution of *tert*-butyl pyrocarbonate (300 mg, 1.376 mmole) in dioxane (1 ml) was then added and the reaction stirred overnight at room temperature. The reaction mixture was dissolved in ethyl acetate, dried over anhydrous magnesium sulfate before the solvents were removed under reduced pressure. The crude product was chromatographed on silica gel eluting with hexane : ethyl acetate (4:1) to give (*R,R*)-1,2-bis(*tert*-butoxycarbamino)-cyclohex-4-ene, **10** (155 mg, 0.445 mmole, 97% yield).

[$^3H_2$]-(*R,R*)-1,2-Bis(*tert*-butoxycarbamino)cyclohexane, **11:** (*R,R*)-1,2-Bis(*tert*-butoxy-carbamino)cyclohex-4-ene, **10** (8 mg, 25.6 mmole) was dissolved in ethyl acetate (1 ml) to which was added 10% palladium on charcoal (8 mg). The reaction mixture was deoxygenated by a freeze/vacuum process (4 times). Tritium gas (2.5 ml, 6.25 Ci) was then added (pressure = 0.5 atm) and the reaction mixture stirred vigorously for 1 hour at room temperature. The catalyst was then removed by filtration and washed with ethanol. Labile tritium was removed by distillation of the ethanol under reduced pressure (3 times) to afford the desired [$^3H_2$]-(*R,R*)-1,2-bis(*tert*-butoxycarbamino)cyclohexane, **11** (1.47 Ci). TLC on silica gel eluting with hexane : ethyl acetate (4:1) indicated a radiochemical purity >98%.

[$^3H_2$]-(*R,R*)-1,2-Diaminocyclohexane, 12: A solution of hydrogen chloride in methanol was prepared from methanol (50 ml) and acetyl chloride (5 ml). This solution was added to [$^3H_2$]-(*R,R*)-1,2-bis(*tert*-butoxycarbamino)cyclohexane, **11** (1.46 Ci) and the resulting mixture stirred for 20 hours at room temperature. The solvent was then removed under reduced pressure and the residue dissolved in ethanol. The residual hydrogen chloride was removed by distillation of the ethanol under reduced pressure (3 times), to afford [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexane, **12** (1.26 Ci, 86% radiochemical yield).

[$^3H_2$]-(*R,R*)-1,2-Diaminocyclohexanedichloroplatinum(II), 13: A solution of potassium tetrachloroplatinate (10 g, 24.1 mmole) was dissolved in milliQ water (50 ml) and added to [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexane, **12** (1.26 Ci) ABA-6003-107. Unlabelled (*R,R*)-1,2-diaminocyclohexane (2.75 g, 24.1 mmole) was dissolved in milliQ water (5 ml) and added to the above solution. After 1 hour the resulting yellow precipitate was removed by filtration. On being left to stand overnight, a second crop was obtained from the mother liquor. The solids were combined to give [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexanedichloroplatinum(II), **13** (8.942 g, 1.21 Ci, 95% radiochemical yield).

[$^3H_2$]-(*R,R*)-1,2-Diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, 2: To a suspension of [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexanedichloroplatinum(II), **13** (8.942 g, 1.21 Ci) in milliQ water (400 ml) was added a solution of silver nitrate (7.15 g, 42 mmole) in milliQ water (100 ml). After 20 hours the insolubles were removed by filtration and the solution concentrated under reduced pressure at 25°C to approximately 125 ml. Potassium iodide (1 g) was then added. The resulting solution was stored for 2 days at 7°C and then the resulting precipitate was removed by filtration to leave a slightly yellow solution (785 mCi). Oxalic acid (1.43 g, 15.9 mmole) was added to this solution and after 6 hours the resulting precipitate was removed by filtration, washed with milliQ water (2 x 5 ml) then dried under high vacuum to give the desired [$^3H_2$]-(*R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II), [$^3H_2$]-Oxaliplatin, **2** (3.722 g, 468 mCi, 39% radiochemical yield).

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7. ***** WARNING ***** The authors want to make it quite clear that the synthesis of azides such as **5** inevitably involves some risk. We consider that the methodology described here involves a reduced risk over that published previously, but must stress that this must still not be considered negligible.

