Microscale Synthesis of Nitrogen-13-Labeled Cisplatin

Bart De Spiegeleer, Guido Slegers, Carlos Vandecasteele, Walter Van den Bossche, Karel Schelstraete, Albert Claeys, and Prosper De Moerloose

Department of Pharmaceutical Chemistry and Drug Quality Control, Faculty of Pharmaceutical Sciences, State University of Ghent, Ghent, Belgium

A microscale synthesis of [^{13}N]cisplatin (cis-dichlorodiammineplatinum(II), cis-DDP) from cyclotron-produced [^{13}N]ammonia is presented. Temperature, reaction time, ratios, and concentration of reactants have been optimized for each step of the synthesis. Purification is performed by ion exchange chromatography. Radiochemical purity and optimization processes are controlled by high performance liquid chromatography and high performance thin layer chromatography—22 mCi [^{13}N]cisplatin in 10 ml of solution is produced. The entire procedure takes ~15 min and the specific activity is ~300 mCi/μmole at EOB.

J Nucl Med 27:399–403, 1986

Since the discovery of the antitumor activity of cisplatin (cis-dichlorodiammineplatinum(II), cis-DDP, cis-$Pt(NH_3)_2Cl_2$) (*1*), interest in this inorganic complex and its analogs has increased rapidly. Numerous papers have appeared concerning chemistry (*2*), analytic methods (*3*), mechanism of action (*4*), biologic properties (*5*), dose-limiting toxicity (*6*), and clinical results (*7*) of this drug. The incorporation of radionuclides of platinum (Pt), mainly the gamma-emitter ^{195m}Pt, has facilitated these studies (*8–12*). Synthesis and purification of ^{195m}Pt-labeled cisplatin has already been described. Because of the 4-day half-life and because of the relatively large quantities of starting material, insoluble intermediates can be isolated and the end product recrystallized (*13,14*).

Previous studies indicate that the biotransformation of cisplatin is extremely complex. It is now generally believed that cisplatin exercises its antitumor activity by reacting with the cellular DNA (*4*).

In vitro experiments with $Pt(H_2NC_2H_4NH_2)Cl_2$ and $Pt(CH_3NH_2)Cl_2$, both labeled with ^{195m}Pt and carbon-14, proved that the amine-ligand remains bound to platinum upon reaction with DNA (*15,16*). However, these results don't agree with in vivo experiments, where both labels do not remain together (*16*). An explanation has to be sought in the reaction with sulfur-containing compounds such as methionine, glutathione, peptides, and proteins (*17,18*). Therefore, further investigations about the fate of the amine-ligand are necessary. Nitrogen-13- (^{13}N) labeled cisplatin is an interesting compound for studying the behavior of the Pt-N bound.

Nitrogen-13 is a positron-emitter with a 10-min half-life, suitable not only for in vitro biochemical investigations (*19*), but also for in vivo imaging with positron emission tomography (PET) (*20*). Because of the short half-life, time is one of the key variables to be optimized. This paper presents in full detail the synthesis, purification, and quality control by chromatographic techniques of ^{13}N-labeled cisplatin at a sub-μmole scale.

MATERIALS AND METHODS

The entire procedure can be divided into three parts: (a) production of ^{13}N-labeled ammonia, (b) synthesis of cisplatin, and (c) purification. The completely remote-controlled setup is shown in Fig. 1. All the necessary precautions were taken to guarantee the sterility of the end product: the whole circuit was flushed with water for injection; then the ion exchanger was equilibrated with autoclaved isotonic sodium chloride solution. At the collection point, an extra Millipore filter was provided.

Received June 19, 1985; revision Nov. 11, 1985.

For reprints contact: Bart De Spiegeleer, MD, Dept. of Pharmaceutical Chemistry and Drug Quality Control, Faculty of Pharmaceutical Sciences, State University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium.

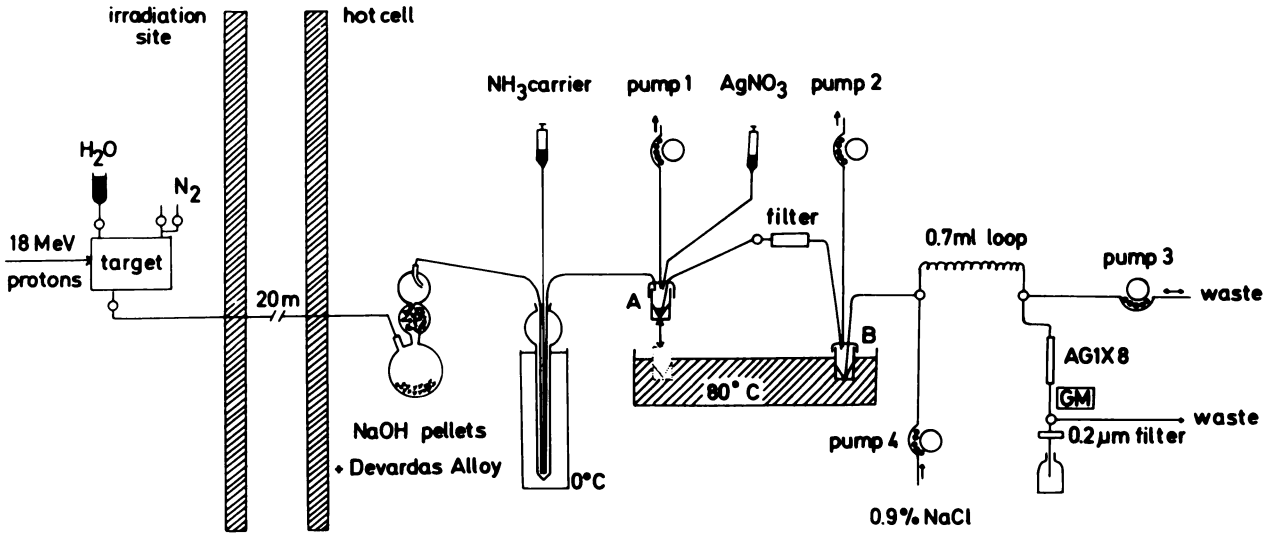

FIGURE 1
Production system

Production of ^{13}N-Labeled Ammonia

Nitrogen-13 ammonia was produced by irradiation of water as described by Slegers et al. (*21*). To prevent the NaOH solution from entering the collector, silanized glasswool was placed in the lower part of the condenser. The distilled $^{13}NH_3$ was trapped in 400 μl of water for injection, cooled to 0°. One hundred microliters of a solution containing 1 μmole of NH_3 carrier was added to the collection tube.

Synthesis

The whole synthesis was performed at a temperature of 80°C. Ten microliters of KI solution (6 μmole) and 20 μl of K_2PtCl_4 solution (1 μmole) were placed in vial A and heated for 2 min. The [^{13}N]ammonia solution was pumped from the collection tube (pump 1, Fig. 1) into the K_2PtI_4 solution. The mixture was heated again for 2 min. Subsequently, 100 μl of $AgNO_3$ solution (i 1 μmole) were added and heated for 3 min. The slurry was filtered (peristaltic pump 2, Fig. 1) through a glass tube filled with prewetted silanized glasswool into vial B, previously filled with 10 mg of finely divided NaCl. After mixing, vial B was heated for 2 min.

Purification

The resulting solution was sucked into a 700-μl loop by a peristaltic pump (pump 3, Fig. 1). By pump 4, the loop content was transferred onto an anion exchange column (AG 1X8, 100–200 Mesh; 60 × 5 mm). Elution was performed with 0.9% m/v solution of sodium chloride at a flow rate of 1 ml/min. After passage of unretained ^{13}N-labeled impurities, detected by a GM counter, a valve is switched and the flow rate increased to 3 ml/min in order to collect ^{13}N-labeled cisplatin. The entire procedure took ~15 min.

RESULTS AND DISCUSSION

Methods for the synthesis of unlabeled cisplatin were described by Kauffman (*22*), Lebedinskii (*23*), and Dhara (*24*). Because of the high yield, purity, and the absence of transderivatives, the procedure of Dhara (*24*) was followed. The reaction scheme is given in Fig. 2.

Each step was optimized by varying one factor at a time. Analysis of the end product was performed by high performance thin layer chromatography

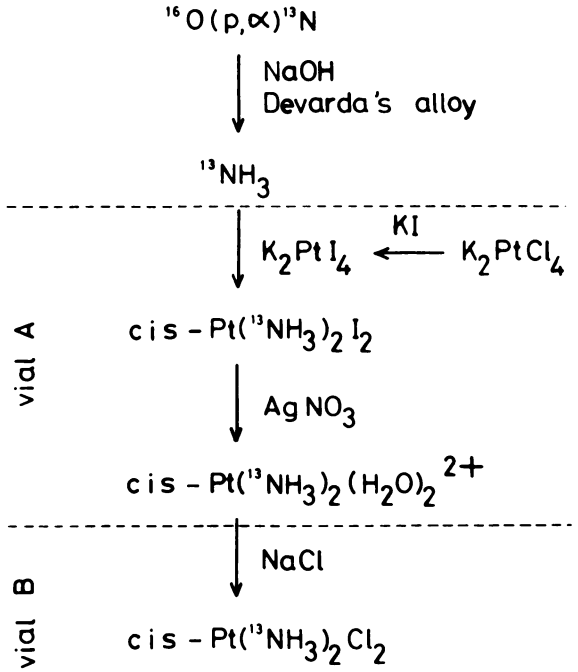

FIGURE 2
Reaction schema

TABLE 1
Influence of pH on Yield of Cisplatin

pH*	Yield (%)†
<8.02	<det. lim.
9.09	25
9.98	51
10.85	43
>11.68	<det. lim.

* pH is adjusted with NaOH or HCl.
† Yield of cisplatin from 2 μmole NH_3, determined with HPTLC.

(HPTLC) using postchromatographic derivatization with *p*-nitrosodimethylaniline (*25*) and high performance liquid chromatography (HPLC) using gamma counting and uv detection at 301 nm (*26*). Comparable results were obtained.

Both radioactive and stable NH_3 were employed in this study. Yields are higher with stable NH_3 (up to 70%), because the synthesis was done in more controlled conditions: exact amounts of NH_3 (2 μmole/500 μl) were used and exact volumes were manually transferred. In this way, losses in tubing, valves, vials and in the filter do not occur.

Step 1. Two modifications of the described $^{13}NH_3$ production (*21*) had to be made. First, in order to prevent traces of alkali from entering the collector causing a significant decrease in yield (Table 1), glass wool was placed in the lower part of the condenser. This breaks down the aerosol of NaOH, and in addition reduces the volume of distillate without significantly retaining the ammonia. The pH of the distillate without glass wool was 11.8. With glass wool, it is reduced to 9.7, i.e., the same as for a NH_3 solution in bidistilled water.

Second, in order to trap the maximum amount of ^{13}N-labeled ammonia, we used 400 μl of bidistilled water in the collection tube at 0°C. This is the minimum volume of water that results in no loss of activity (Fig. 3). At room temperature, even for higher volumes, losses of as large as 25% occur.

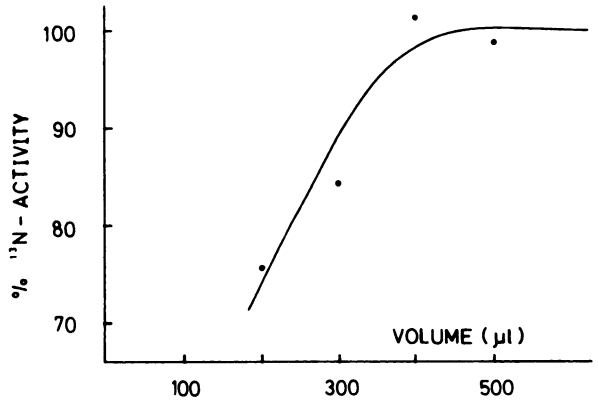

FIGURE 3
Effect of volume of water on $^{13}NH_3$ trapping efficiency. Trapping of $^{13}NH_3$ in 1 ml of 12M HCl has been taken as 100% activity

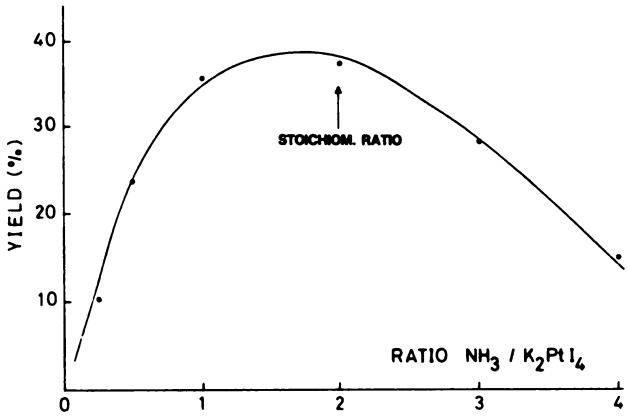

FIGURE 4
Influence of ratio of NH_3 to K_2PtI_4 on yield of cisplatin. Amount of K_2PtI_4 is 1 μmole. Reaction volume was same for all experiments

Using the Berthelot color reaction (*27*) it was shown that the amount of NH_3 in the distillate ranges from 0.46 to 0.65 μmole. Adding 1 μmole of inactive NH_3 to the distillate thus gives between 1.46 and 1.65 μmole of NH_3. Addition of carrier ammonia increases the cisplatin yield by greater than a factor of 2, so no significant decrease in specific activity occurs, but the reproducibility of the [^{13}N]cisplatin yield is enhanced. If more than the stoichiometric amount of ammonia is present, a decrease in yield and an increase in byproducts is observed (Fig. 4). If less ammonia is present, there is a slighter decrease in yield, merely as a consequence of a dilution effect, and the final product contains less impurities.

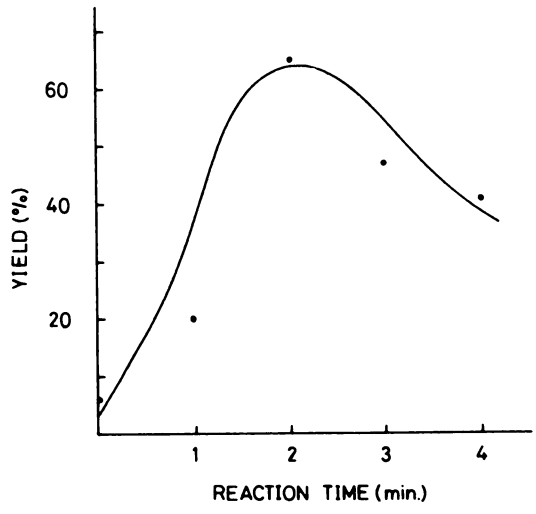

FIGURE 5
Effect on cisplatin yield of reaction time (at 80°C) of conversion of K_2PtI_4 and NH_3 to cis-$Pt(NH_3)_2I_2$.

TABLE 2
Distribution of ^{13}N Activity

Vial A	8.7%
Filter	16.9%
Vial B	9.4%
Column	5.8%
Not retained	
Waste fraction	32.1%
Cisplatin fraction	27.1%

Step 2. K_2PtCl_4 is rapidly converted to K_2PtI_4 by reacting with 1.5 times the stoichiometric amount of KI at 80°C for 1 to 3 min. A decrease in the yield of cisplatin is observed when heating is applied more than 5 min or when less KI is used. Reaction of K_2PtI_4 with ammonia is one of the most critical steps; not only the pH and the NH_3 to K_2PtI_4 ratio, but also the reaction time is critical. As can be seen in Fig. 5, the optimal reaction time is 2 min.

No cisplatin is formed when the ammonia is trapped directly in a diluted solution of K_2PtI_4. Instead, a black-green precipitate, most probably a mixture of metallic platinum and tetramineplatinum(II) complexes like Magnus' green salt (*28,29*), is seen in the collection tube.

Step 3. Addition of a slight excess (10%) of $AgNO_3$ and 3 min reaction at 80°C is sufficient. Adding more $AgNO_3$ or prolonged heating does not improve the yield.

Step 4. After the formation of the aquoderivatives, on-line filtration of the insoluble silver halogenides is performed. Conversion to cisplatin by reaction with 10 mg NaCl takes 2 min at 80°C. A greater excess of NaCl and prolonged heating gives a decrease in yield.

Purification

Cisplatin, a neutral molecule with a dipole moment, can be separated from transplatin, which has no dipole moment, from positively charged impurities, e.g., the aquo compounds and $(NH_4)^+$, from negatively charged impurities, e.g., the starting materials $(PtCl_4)^{2-}$ and $(PtI_4)^{2-}$, by ion exchange chromatography. Elution on AG 1X8 (100–200 mesh) anion exchanger with 0.9% m/v NaCl solution gives no retention of the positive impurities and of transplatin, which are eluted as the "solvent" peak. Cisplatin shows some retention and is eluted after the solvent peak. The negative impurities are strongly retained and remain on the column. For the elution experiments, the platinum compounds were detected by the color reaction with $SnCl_2$ (*30*).

Elution with 0.9% m/v NaCl solution has the advantage of the product being obtained in a solution suitable for i.v. injection.

Yield and Specific Activity

Table 2 shows the distribution of the ^{13}N activity. For a 20-min irradiation with 18 MeV protons at a 20-μA intensity and a synthesis time of 15 min, 22 mCi of ^{13}N-labeled cisplatin in 10 ml of solution are produced, which represents a sufficient amount for PET. The radiochemical purity of the ^{13}N-labeled cisplatin fraction was determined by HPLC analysis (Fig. 6), and found to be pure. The carrier amount, determined by HPLC, was at most 0.2 μmole, which is far below the dose given in chemotherapy. The collected ^{13}N-labeled cisplatin fraction could not be analyzed with HPTLC because of the large dilution (20X) as a result of the ion exchange chromatography, and of the very low application volumes in HPTLC (200 nl) in contrast to the 100-μl injection volume in HPLC.

The specific activity was ~100 mCi/μmole, corresponding to ~300 mCi/μmole at EOB.

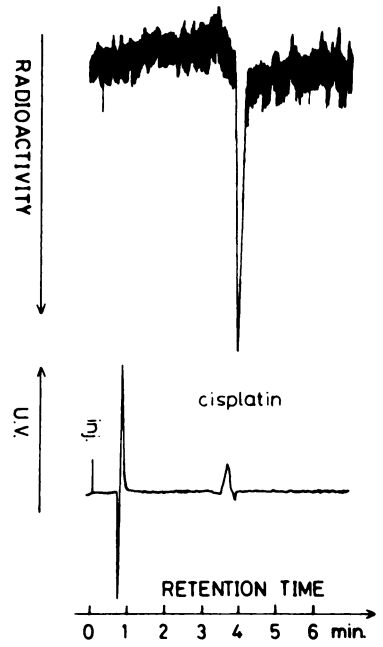

FIGURE 6
HPLC chromatogram of cisplatin fraction. Stationary phase: Lichrosorb 10 RP 18 (250 × 4.6 mm) preloaded with hexadecyltrimethylammoniumbromide (0.5% m/v HTAB in water); mobile phase: $10^{-4}M$ HTAB in $10^{-2}M$ citrate buffer at pH 7

REFERENCES

1. Rosenberg B, Van Camp L, Trosko JE, et al: Platinum compounds: A new class of potent antitumor agents. *Nature* 222:385–386, 1969
2. Lippard SJ: New chemistry of an old molecule: cis-$[Pt(NH_3)_2Cl_2]$. *Science* 218:1075–1082, 1982
3. Riley CM, Sternson LA: Recent advances in the clinical analysis of cisplatin. *Pharm Int* 5:15–19, 1984
4. Roberts JJ, Thomson AJ: The mechanism of action of antitumor platinum compounds. *Prog Nucl Acid Res Mol Biol* 22:71–133, 1979
5. Drobnik J, Urbankova M, Krekulova A: The effect of cis-dichlorodiammineplatinum(II) on *Escherichia coli*

B. Mutat Res 17:13-20, 1973
6. Hardaker WT, Stone RA, McCoy R: Platinum nephrotoxicity. *Cancer* 34:1030-1032, 1974
7. Prestayko AW, Crooke ST, Carter SK, eds: *Cisplatin, Current Status, and New Developments*. New York, Academic Press, 1980
8. Wolf W, Manaka R, Leh F: The use of radionuclides of platinum. *J Clin Hematol Oncol* 7: 741-755, 1977
9. Lange RC, Spencer RP, Harder HC: Synthesis and distribution of a radiolabeled antitumor agent: cis-diamminedichloroplatinum(II). *J Nucl Med* 13:328-330, 1972
10. Lange RC, Spencer RP, Harder HC: The antitumor agent cis-$Pt(NH_3)_2Cl_2$: distribution studies and dose calculations for ^{193m}Pt and ^{195m}Pt. *J Nucl Med* 14:191-195, 1973
11. Smith PHS, Taylor DM: Distribution and retention of the antitumor agent ^{195m}Pt-cis-dichlorodiammineplatinum(II) in man. *J Nucl Med* 15:349-351, 1974
12. Wolf W, Manaka R: Synthesis and distribution of ^{195m}Pt cis-dichlorodiammineplatinum(II). *J Clin Hematol Oncol* 7:79-94, 1977
13. Hoeschele JD, Butler TA, Roberts JA: Microscale synthesis and biodistribution of Pt-195m-labeled cis-dichlorodiammineplatinum(II), cis-DDP. *Proc Int Symp Radiopharm* 2:173-182, 1979
14. Hoeschele JD, Butler TA, Roberts JA, et al: Analysis and refinement of the microscale synthesis of the ^{195m}Pt-labeled antitumor drug, cis-dichlorodiammineplatinum(II), cis-DDP. *Radiochim Acta* 31:27-36, 1982
15. Robins AB: The reaction of ^{14}C-labelled platinum ethylenediamine dichloride with nucleic acid constituents. *Chem Biol Interact* 6: 35-45, 1973
16. Tullius TD, Ushay HM, Merkel CM, et al: Structural Chemistry of Platinum-DNA adducts. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*, Lippart SJ, ed. Washington, American Chemical Society, 1983, pp 51-74
17. Odenheimer B, Wolf W: Reactions of cisplatin with sulfur-containing amino acids and peptides. I. Cysteine and glutathione. *Inorg Chem Acta* 66:L41-L43, 1982
18. Ismail IM, Sadler PJ: ^{195}Pt- and ^{15}N-NMR studies of antitumor complexes. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*, Lippard SJ, ed. Washington, American Chemical Society, 1983, pp 171-190
19. Krohn KA, Mathis CA: The use of isotopic nitrogen as a biochemical tracer. In *Short-Lived Radionuclides in Chemistry and Biology*, Root JW, Krohn KA, eds. Washington, American Chemical Society, 1981, pp 233-249
20. Ell PJ, Holman BL, eds: *Computed Emission Tomography*, Oxford, Oxford University Press, 1982
21. Slegers G, Vandecasteele C, Sambre J: Cyclotron production of ^{13}N-labelled ammonia for medical use. *J Radioanal Chem* 59:585-587, 1980
22. Kauffman GB, Cowan DO: Cis- and trans-dichlorodiammineplatinum(II). In *Inorganic Synthesis*, Vol. 7, Kleinberg J, ed. New York, McGraw-Hill, 1963, pp 239-245
23. Lebedinskii VV, Golounya GA: Ammonia compounds of bivalent platinum. *Chem Abstr* 44:5257, 1950
24. Dhara SC: A rapid method for the synthesis of cis-$[Pt(NH_3)_2Cl_2]$. *Ind. J Chem* 8:193-194, 1970
25. De Spiegeleer B, Slegers G, Van Den Bossche W, et al: Quantitative analysis of cis-dichlorodiammineplatinum(II) by high-performance thin-layer chromatography. *J Chromatogr* 315:481-487, 1984
26. Riley CM, Sternson LA, Repta AJ: High-performance liquid chromatography of cis-dichlorodiammineplatinum(II) using chemically-bonded and solvent-generated ion exchangers. *J Chromatogr* 217:405-420, 1981
27. Henry RJ, ed: *Clinical Chemistry: Principles and Technics*. New York, Harper and Row, 1966, pp 266-270
28. Drew HD, Pinkard FW, Wardlaw W, et al: The Structure of the simple and mixed tetramminoplatinous dihalides. *J Prakt Chem* 1:1004-1016, 1932
29. Gmelin L, ed: *Handbuch der Anorganischen Chemie*, Berlin, Verlag Chemie, 1957, pp 52-60
30. Milner OI, Shipman GF: Colorimetric determination of platinum with stannous chloride. *Anal Chem* 27:1476-1478, 1955