# Monitoring the Reactions of the Anticancer Drug Carboplatin with the Chemopreventive Agent Selenomethionine by Electrospray Mass Spectrometry and [$^1$H,$^{15}$N] HSQC NMR Spectroscopy

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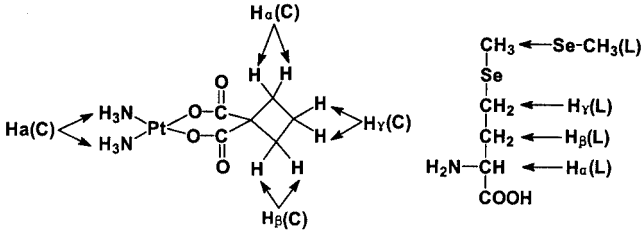
**Keywords:** Antitumor agents / Platinum / L-Selenomethionine / Mass spectrometry / NMR spectroscopy

The reactions of the second-generation platinum anticancer drug carboplatin with L-selenomethionine (L-Se-MetH) and L-methionine (L-MetH) (abbreviations used: L-Se-MetH: L-selenomethionine; L-Se-Met: deprotonated L-selenomethionine; L-MetH: L-methionine; L-Met: deprotonated L-methionine) were studied by ESMS and 2D [$^1$H,$^{15}$N] HSQC NMR spectroscopy. The combination of the two techniques provided the unambiguous assignment of [Pt($NH_3$)$_2$(CBDCA-*O*)(L-Se-MetH-*Se*)] (**1**), the long-lived ring-opened adduct, [Pt($NH_3$)(CBDCA-*O*)(L-Se-Met-*Se*,*N*)] (**2**), [Pt(CBDCA-*O*,*O*)(L-Se-MetH-*Se*,*N*)] (**3**) and [Pt(L-Se-Met-*Se*,*N*)$_2$] (**4**). The reaction of carboplatin with L-MetH is very similar to that with L-Se-MetH, except for a slower reaction rate. Interestingly, we observed the dimer and polymer forms of carboplatin in solution by electrospray mass spectrometry. This work demonstrates that carboplatin differs from cisplatin in that both reactions with L-Se-MetH and L-MetH form [Pt(L-Se-Met-*Se*,*N*)$_2$] or [Pt(L-Met-*S*,*N*)$_2$] as the dominant adducts.


## Introduction

Carboplatin, [Pt($NH_3$)$_2$(CBDCA)] (CBDCA: cyclobutane-1,1-dicarboxylate, Scheme 1), is now a widely used second-generation platinum anticancer drug. The major advantage of carboplatin compared with cisplatin ([$PtCl_2(NH_3)_2$]) [1] is its much lower toxicity, which may be attributed to its low reactivity caused by the presence of the chelating CBDCA ligand. [2]




**Carboplatin** **L-Se-MetH**

Scheme 1. Schematic drawing of carboplatin and L-Se-MetH and the labeling scheme used in the $^1$H NMR spectra

The in vivo activation of carboplatin has been a subject of much interest. [3,4] Although early studies suggested that carboplatin is merely a prodrug of cisplatin, [5] the aquation rate is too slow to account for its in vivo activity. [6] The amino acid L-methionine (L-MetH) has been shown to react rapidly with carboplatin to form long-lived ring-opened complexes. [7] It has been therefore postulated that L-methionine may play a role in the mechanism of the reaction of the platinum drug. [8] More interestingly, a similar ring-opened complex has been detected in the urine of animals treated with carboplatin. [9] Since the complex [Pt(Met-*S*,*N*)$_2$] was identified as the in vivo metabolite of cisplatin, many studies on the interactions of platinum drugs with methionine and its derivatives have been carried out. [10–14]

L-Selenomethionine (L-Se-MetH, Scheme 1), the selenium analogue of L-methionine, is currently on clinical trials as a potential cancer chemopreventive agent in reducing both environmentally and genetically determined cancer. [15,16] It was also recently reported that L-Se-MetH is able to protect against cisplatin-induced renal and other toxicities in mice and rats. [17] We have recently investigated the reactions between cisplatin and L-selenomethionine, and demonstrated that the combination of ESMS and NMR spectroscopy could provide valuable information on the mechanism of the reactions. [18] Here, we extend our study to the interactions of L-selenomethionine and the second-generation anticancer drug carboplatin. The reaction be-

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tween carboplatin and L-methionine is also carried out in order to compare the results with those studied by NMR spectroscopy.[7]

## Results and Discussion

### Results

#### *(1) ESMS Investigation of Carboplatin in Solution: Detection of Dimer and Polymer Forms*

A solution of carboplatin (2.68 mmol/mL) was studied at pH = 5.00 (no buffer solution was used) by ESMS. As shown in Figure 1, apart from the positive ion of protonated carboplatin ($m/z$ = 372.1) and {[Pt($NH_3$)$_2$(CBDCA)] + $Na^+$} ($m/z$ = 394.1), three pairs of peaks at $m/z$ = 743.1, 765.1; 1114.1, 1136.1; and 1486.0, 1508.0 were observed. These ions correspond to the formation of the dimer, trimer, and tetramer forms of carboplatin, respectively, and their corresponding sodium adducts (Table 1). These forms of carboplatin have never been detected previously in solution by other methods, although they may be formed only during the ionization process (vide infra). As can be seen from Figure 1, the intensity of the peaks decrease with the increase in the molecular size.

#### *(2) The Time-Dependent Reaction of Carboplatin with L-Se-MetH in a 1:1 Molar Ratio*

The reaction between carboplatin and L-Se-MetH was monitored for 22 h. The first ESMS spectrum was measured 2 min after mixing of the solutions of carboplatin and L-Se-MetH. Figure 2 shows the representative spectra of the reaction recorded after 25 min, 3 h and 22 h. As noted, all the isotopic peaks were separated by 1 unit and can be assigned to complexes with one positive charge. After 2 min, two new peaks (peak 1 and 12) appeared in the spectrum. Peak 1 at $m/z$ = 567.9 can be assigned to

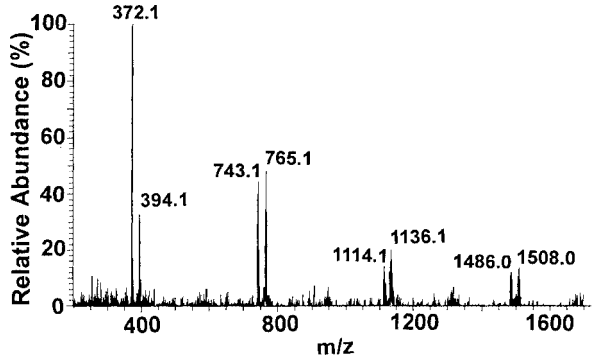



Figure 1. ESMS spectrum of a carboplatin solution (2.68 mmol/mL) at pH = 5.00

[Pt($NH_3$)$_2$(CBDCA)(L-Se-MetH)]$^+$ (**1**), in which L-Se-MetH is likely to be coordinated to Pt via the selenium atom and the CBDCA chelate ring is opened. This is confirmed by 2D [$^1$H,$^{15}$N] HSQC NMR spectroscopy (vide infra). Peak 12 ($m/z$ = 939.9) can be assigned to {[Pt($NH_3$)$_2$(CBDCA)]$_2$ ($m/z$ = 742.5) + L-Se-MetH + $H^+$}. The calculated molecular mass (Table 2) and isotopic distribution match perfectly with the formulae (Figure 1 of Supporting Information). It is worth noting that the intensity of the peak assigned to the carboplatin dimer ($m/z$ = 743.1, 765.1) decreased with time. However, it could still be seen at the end of the reaction (Figure 2c). The intensity of peak 1 increased with time for the first 3 h, then decreased after 4 h, and finally disappeared after 22 h.

Peak 2 ($m/z$ = 553.0), which is assigned to [Pt($NH_3$)(CBDCA)(L-Se-MetH)]$^+$ (**2**), appeared 15 min after the reaction began. The intensity of this peak increased gradually, but decreased after 9 h of the reaction. Peak 3 ($m/z$ = 532.3) appeared after 50 min with a low intensity, and is assigned to [Pt(CBDCA)(L-Se-MetH)]$^+$ (**3**). It finally disappeared completely after 12 h of the reaction. Peak 4 ($m/z$ = 587.0), assigned to [Pt(L-Se-MetH)(L-Se-

Table 1. Observed and calculated molecular masses of the complexes for the reaction of carboplatin with L-Se-MetH

| Peak | Compound | Observed mass[a] | Calculated mass |
|---|---|---|---|
| 1 | [Pt($NH_3$)$_2$(CBDCA)(L-Se-MetH)] + $H^+$ | 564.0−569.9 | 568.4 |
| 2 | [Pt($NH_3$)(CBDCA)(L-Se-MetH)] + $H^+$ | 548.1−556.1 | 551.3 |
| 3 | [Pt(CBDCA)(L-Se-MetH)] + $H^+$ | 531.1−538.2 | 533.3 |
| 4 | [Pt(L-Se-MetH)(L-Se-Met)]$^+$ | 582.9−590.9 | 587.0 |
| 5 | [Pt($NH_3$)(L-Se-MetH)(L-Se-Met)]$^+$ | − | 603.3 |
| 6 | [Pt(L-Se-Met)$_2$] + $Na^+$ | 604.9−612.9 | 609.0 |
| 7 | [Pt($NH_3$)$_2$(L-Se-Met)]$^+$ | 421.9−427.9 | 424.2 |
| 8 | [Pt($NH_3$)$_2$(CBDCA)] + $H^+$ | 371.1−375.2 | 372.3 |
| 9 | [Pt($NH_3$)$_2$(CBDCA)] + $Na^+$ | 392.1−397.1 | 394.3 |
| 10 | [Pt($NH_3$)$_2$(CBDCA)]$_2$ + $H^+$ | 741.1−747.1 | 743.5 |
| 11 | [Pt($NH_3$)$_2$(CBDCA)]$_2$ + $Na^+$ | 763.1−769.1 | 765.5 |
| 12 | [Pt($NH_3$)$_2$(CBDCA)]$_2$ + L-Se-MetH + $H^+$ | 933.9−943.9 | 939.6 |
| 13 | [Pt(L-Se-MetH)(L-Se-Met)]$^+$ + carboplatin | 952.9−964.9 | 958.3 |
| 14 | [Pt($NH_3$)$_2$(CBDCA)]$_3$ + $H^+$ | 1112.0−1118.2 | 1114.2 |
| 15 | [Pt($NH_3$)$_2$(CBDCA)]$_3$ + $Na^+$ | 1132.1−1140.1 | 1136.2 |
| 16 | [Pt($NH_3$)$_2$(CBDCA)]$_4$ + $H^+$ | 1482.9−1491.9 | 1489.9 |
| 17 | [Pt($NH_3$)$_2$(CBDCA)]$_4$ + $Na^+$ | 1502.7−1512.7 | 1511.9 |

[a] The Peaks are separated by $m/z$ = 1 in the mass region indicated.

Met)]$^+$ (**4**), began to appear after 1 h 40 min. It had the highest relative intensity after 3 h 10 min (Figure 2b) and became the dominant complex in the final stage of the reaction (Figure 2c). Peaks 6 ($m/z$ = 608.8) and 7 ($m/z$ = 423.1), assigned to the adduct of complex **4** + $Na^+$ (Figure 2 of the Supporting Information) and complex [$Pt(NH_3)_2$(L-Se-MetH)]$^+$ (**7**), respectively, began to appear after 3 h and could be observed until the end of the reaction (Figures 2b and c). Peak 13 at $m/z$ = 956.9 in the final stage of the reaction (see Figure 2c) can be assigned to {[$Pt(NH_3)_2$(CBDCA)] + [Pt(L-Se-Met)$_2$] + $H^+$} (Table 1). As shown in Figure 2c, after 22 h of the reaction less products were observed for this reaction than for the reaction of cisplatin with L-Se-MetH,[18] and the major species is peak 4 which corresponds to [Pt(L-Se-MetH)(L-Se-Met)]$^+$ (**4**). This result is rather similar to the reaction of cisplatin with L-Se-MetH in a 1:2 molar ratio, in which the bis(chelated) adduct was the dominant product. No further changes were observed in the ESMS spectra after 22 h. In contrast to the reaction of cisplatin, no dinuclear adducts were observed during the course of the reaction.

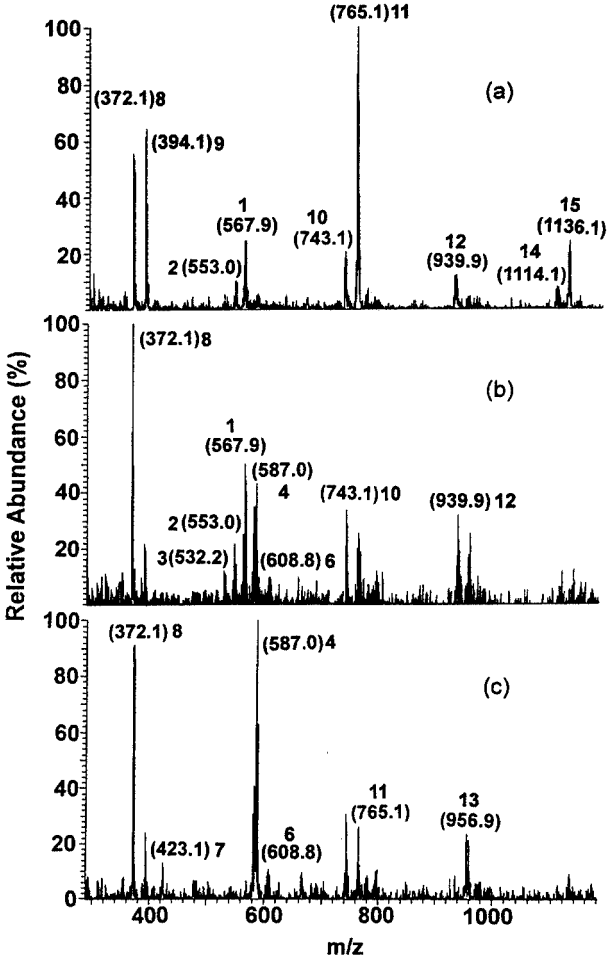



Figure 2. ESMS spectra of the reaction of L-Se-MetH with carboplatin (1:1, 310 K), recorded at different reaction times: (a) 25 min, (b) 3 h, (c) 22 h

### *(3) ESMS Study of the Time-Dependent Reaction of Carboplatin with L-MetH in a 1:1 Molar Ratio*

The above reaction was conducted in order to compare the results with those previously reported.[7] Three typical ESMS spectra recorded after 40 min, 12 h and 38 h of the reaction are shown in Figure 3. Three new peaks (1′, 8′ and 9′) appeared in the spectra immediately after mixing the two reactants. Peak 1′ ($m/z$ = 520.9) corresponds to the ring-opened complex [$Pt(NH_3)_2$(CBDCA)(L-MetH)]$^+$ (**1′**), peak 8′ ($m/z$ = 669.9) is assigned to {[$Pt(NH_3)_2$(CBDCA)(L-MetH)] + L-MetH}$^+$ (Figure 3 of the Supporting Information) and peak 9′ ($m/z$ = 892.0) to {[$Pt(NH_3)_2$(CBDCA)]$_2$ + L-MetH}$^+$ (Figure 4 of the Supporting Information). In these two latter complexes, the existing status of L-MetH is not clear. The hydrogen bonding between the carboxylate and amino groups may play a role in the formation of the two adducts. Peak 1′ remained to be the dominant species for ca. 15 h and then began to decrease in intensity after 18 h, and finally disappeared after 38 h. Peak 2′ ($m/z$ = 504.1), which is assigned to the complex [$Pt(NH_3)$(CBDCA)(L-MetH)]$^+$ (**2′**), appeared after 40 min of the reaction (Figure 3a), and its intensity increased in the following 8 h, then decreased after 10 h. Peak 3′ ($m/z$ = 486.1) is assigned to [Pt(CBDCA)(L-MetH)]$^+$ (**3′**) and appeared after 50 min but remained with a low intensity until it disappeared after 24 h. Peak 7′ ($m/z$ = 377.1), which is assigned to [$Pt(NH_3)_2$(L-Met)]$^+$ (**7′**), appeared after 2 h, reached its maximum intensity after 10 h and then

Table 2. Observed and calculated molecular masses of the complexes for the reaction of carboplatin with L-MetH

| Peak | Compound | Observed mass[a] | Calculated mass |
|---|---|---|---|
| 1′ | [$Pt(NH_3)_2$(CBDCA)(L-MetH)] + $H^+$ | 520.0−524.0 | 521.5 |
| 2′ | [$Pt(NH_3)$(CBDCA)(L-MetH)] + $H^+$ | 503.1−507.1 | 503.4 |
| 3′ | [Pt(CBDCA)(L-MetH)] + $H^+$ | 486.2−490.1 | 486.4 |
| 4′ | [Pt(L-MetH)(L-Met)]$^+$ | 491.1−495.1 | 492.5 |
| 5′ | [$Pt(NH_3)$(L-Met)(L-MetH)]$^+$ | − | 509.5 |
| 6′ | [Pt (L-Met)$_2$] + $Na^+$ | 513.1−517.1 | 514.5 |
| 7′ | [$Pt(NH_3)_2$(L-Met)]]$^+$ | 376.1−379.1 | 377.4 |
| 8′ | [$Pt(NH_3)_2$(CBDCA)(L-MetH)] + $H^+$ + MetH | 668.9−672.9 | 670.7 |
| 9′ | [$Pt(NH_3)_2$(CBDCA)]$_2$ + L-MetH + $H^+$ | 890.1−898.1 | 892.7 |
| 10′ | [Pt(L-MetH)(L-Met)]$^+$ + carboplatin | 861.9−867.9 | 863.8 |

[a] The Peaks are separated by $m/z$ = 1 in the mass region indicated.

remained constant for 38 h. Peak 4′ ($m/z$ = 491.2), which appeared after 3 h and is assigned to $[Pt(L\text{-MetH})(L\text{-Met})]^+$ (**4′**), increased in intensity with time and became the major species in the final stage. It should be noted that complex $[Pt(NH_3)(L\text{-MetH})(L\text{-Met})]^+$ (**5′**) which is similar to complex **5** and should have an $m/z$ value of 509.5 was not observed in this reaction. Similar to peak 6, peak 6′ at $m/z$ = 514.1 can be assigned to complex **4′** + $Na^+$. Peak 10′ at $m/z$ = 863.2 (see Figure 3c) can be assigned to $\{[Pt(NH_3)_2(CBDCA)] + [Pt(L\text{-Met})_2] + H^+\}$ (Table 2). Similar to the reaction with L-Se-MetH, the species observed after 38 h were the unchanged carboplatin and the adducts corresponding to peaks 4′ and 7′. From the ESMS results, it can be seen that the final intensity of the peak assigned to carboplatin is much weaker than that of peak 8 in the reaction with L-Se-MetH.

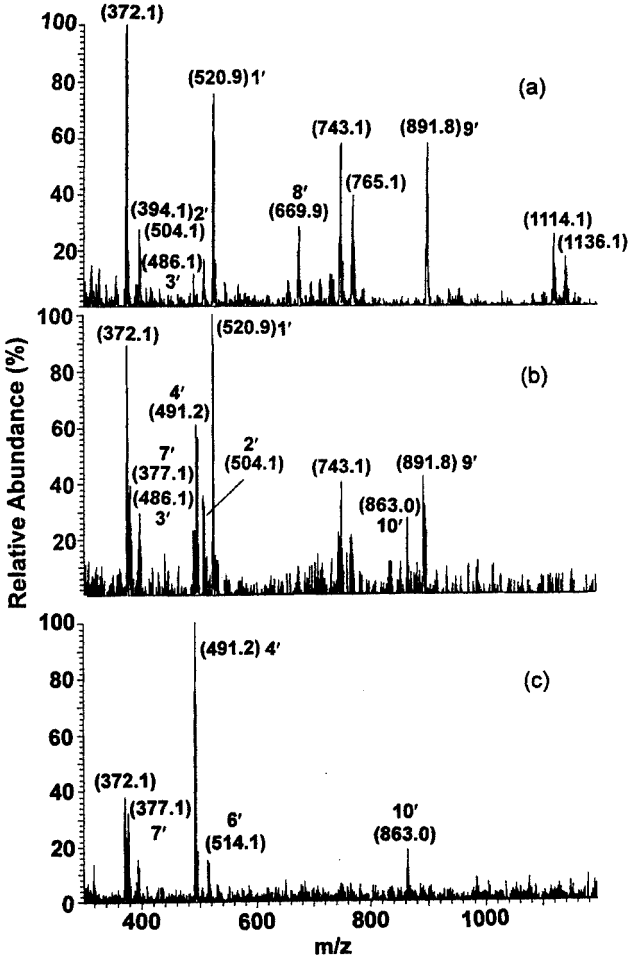



Figure 3. ESMS spectra of the reaction of L-MetH with carboplatin (1:1, 310 K), recorded at different reaction times: (a) 40 min, (b) 12 h, (c) 38 h

We also performed the reactions of carboplatin with L-Se-MetH or L-MetH at 290 K; the spectra recorded after 7 d are shown in Figure 4. As can be seen, complex **7** and **7′** were more abundant than in the reactions at 310 K, suggesting their lower stability at higher temperatures.

### *(4) NMR Spectroscopic Study of the Reaction of Carboplatin with L-Se-MetH in a 1:1 Molar Ratio*

The reaction was initially monitored by $^1H$ NMR spectroscopy. The spectra recorded at 40 min, 5 h and 10 h are

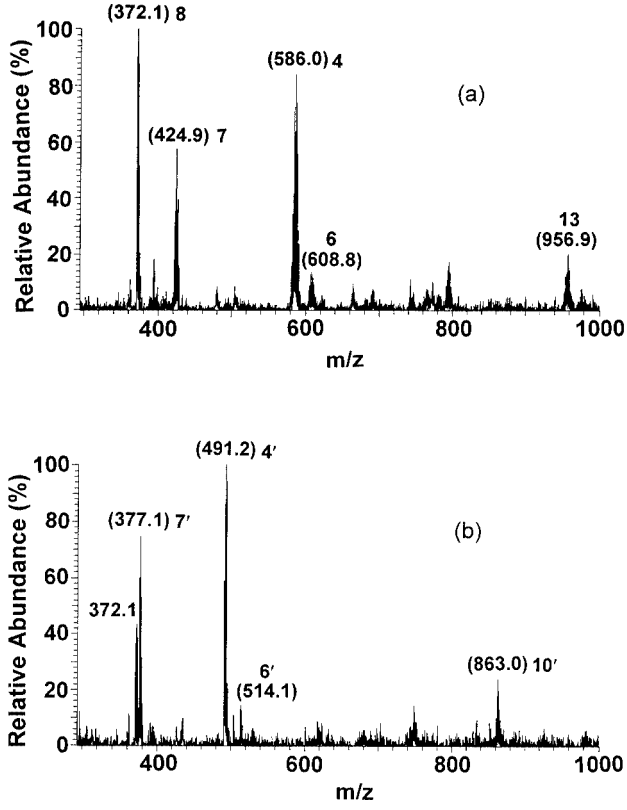



Figure 4. (a) Final ESMS spectra of the reaction of carboplatin with L-Se-MetH (1:1, 290 K); (b) final ESMS spectra of the reaction of carboplatin with L-MetH (1:1, 290 K)

shown in Figure 5. Initially, apart from the signals of free L-Se-MetH and carboplatin, four new singlets appeared between δ = 2.55 and 2.65 ppm. These signals can be assigned to the $CH_3$ group of the *Se*-bound L-Se-MetH. During the course of the reaction, the signals assigned to free carboplatin and L-Se-MetH decreased in intensity, whilst the signals assigned to free CBDCA increased (Figures 4b, c). After 22 h of the reaction, the signal of the $CH_3$ group of free L-Se-MetH disappeared completely, while the signals for carboplatin could still be observed (Figure 5 of the Supporting Information). These data are consistent with those observed by ESMS.

The same reaction was then monitored by 2 D [$^1H$,$^{15}N$] HSQC NMR spectroscopy. Figure 6 shows the spectra of the reaction recorded after 2 h, 10 h and 22 h. After 1 h, apart from the cross-peak of carboplatin at δ = 4.19/−82.0 ppm (Table 3), four new cross-peaks at δ = 4.33/−84.0 ppm (1a), δ = 4.30/−84.0 ppm (1a′), δ = 4.28/−45.5 ppm (1b), and δ = 4.28/−45.3 ppm (1b′) appeared. The $^{15}N$ chemical shifts of 1a, 1a′ are consistent with species that have an

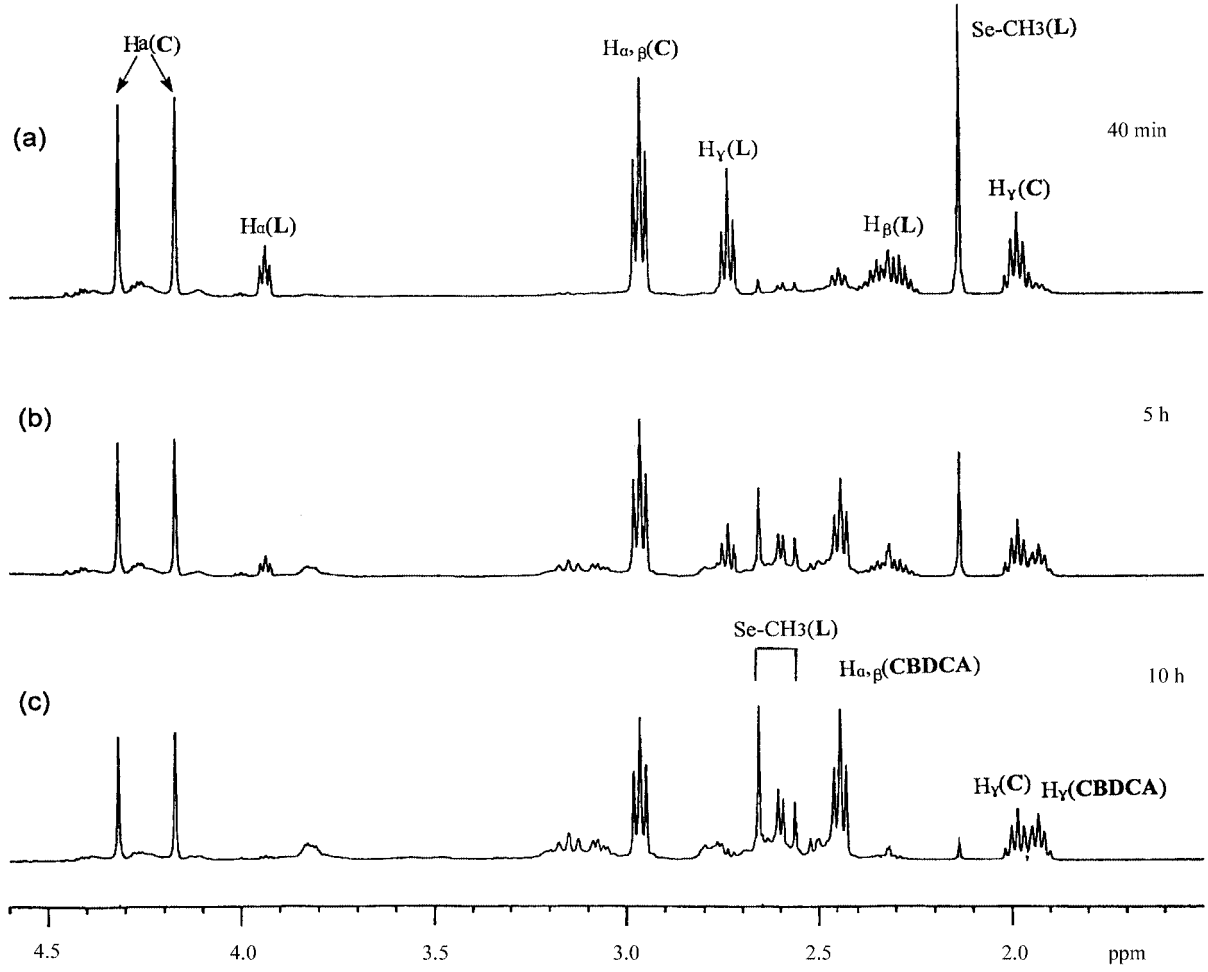



Figure 5. $^{1}H$ NMR spectra of the reaction of $^{15}N$-carboplatin with L-Se-MetH in a 1:1 molar ratio and at 310 K, recorded at different reaction times: (a) 40 min, (b) 5 h, (c) 10 h

amine group *trans* to the oxygen atom, and the $^{15}N$ chemical shifts of 1b, 1b′ are consistent with species that have an amine group *trans* to the selenium atom. It is likely that these cross-peaks correspond to the formation of $[Pt(NH_3)_2(CBDCA)(L\text{-Se-MetH})]$, which contains a monodentate CBDCA and L-Se-MetH. This agrees well with the ESMS results. The splitting of the cross-peaks (1a and 1a′; 1b and 1b′) in the $^{1}H$ dimension is similar to that observed in the reaction of carboplatin and L-MetH,[7,19] which can be explained by the slow inversion of the methyl group at the coordinated chiral selenium atom of L-Se-MetH. In the following 3 h, these two cross-peaks increased in intensity, and then disappeared after 14 h. After 1.5 h of the reaction, a new cross-peak at $\delta = 4.16/-67.0$ ppm (2a) appeared, which has a $^{15}N$ chemical shift in the region of the $NH_3$ group *trans* to N. After ca 2.5 h, the cross-peaks at $\delta = 4.44/-42.1$ (2b) and $\delta = 4.41/-42.4$ (2b′), which have the $NH_3$ group *trans* to the selenium atom, began to appear. These cross-peaks were observed even after 22 h. Comparing these with the result of the mass spectrum, cross-peaks 2a and 2b, 2b′ can be assigned to complex $[Pt(NH_3)_2(L\text{-Se-Met})]$ (**7**). However, from the structure of complex **7**, the cross-peak 2a should be split into a doublet in the $^{1}H$ dimension, similar to the cross-peaks 2b, 2b′, due to the slow inversion of the methyl group at the chiral selenium atom. Therefore, we performed the NMR experiment at 283 K (Figure 5 of the Supporting Information). In the spectrum, cross-peak 2a became two cross-peaks with different chemical shifts in the $^{1}H$ dimension. The intensity of cross-peaks 2a and 2a′ was equal to that of 2b and 2b′. This showed that the two pairs of cross-peaks are related to the same species. The earlier appearance of cross-peak 2a than cross-peaks 2b, 2b′ may be due to the splitting of cross-peaks 2b, 2b′.

### *(5) NMR Spectroscopic Study of the Reaction between L-MetH and Carboplatin*

An NMR investigation of the reaction between L-MetH and carboplatin was carried out, monitored by 2 D [$^{1}H$,$^{15}N$] HSQC NMR spectroscopy. The results obtained could then be compared with the ESMS results and those of a previous NMR study at a different pH. Figure 7 shows the spectra recorded after 2 h, 12 h and 38 h. After 1 h of the reaction,

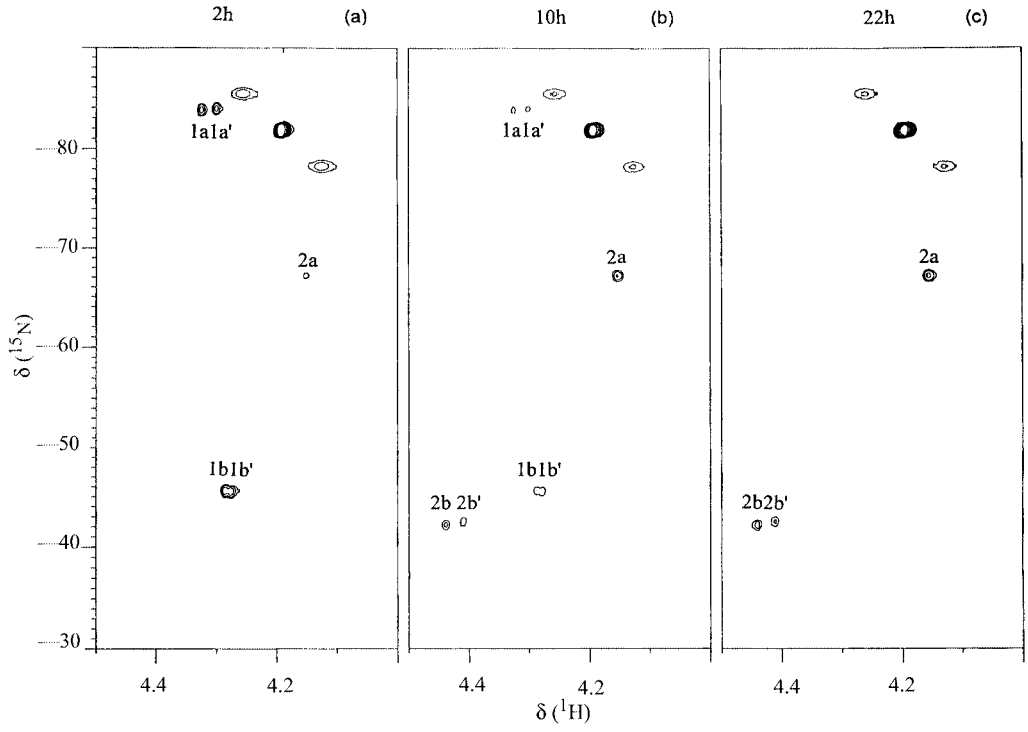



Figure 6. 2D [$^1$H,$^{15}$N] HSQC NMR spectrum of the reaction of $^{15}$N-carboplatin with L-Se-MetH in a 1:1 molar ratio at 310 K, recorded after (a) 2 h, (b) 10 h, (c) 22 h

Table 3. $^1$H and $^{15}$N NMR chemical shifts of carboplatin and its adducts with L-Se-MetH and L-MetH

| Complexes | δ($^1$H)/δ($^{15}$N) for Pt−$NH_3$ | Pt−$NH_3$ *trans* to |
|---|---|---|
| [Pt($NH_3$)$_2$(CBDCA)] | 4.19/−82.0 | O |
| [Pt($NH_3$)$_2$(CBDCA)(L-Se-Met)]$^+$ | 4.33/−84.0; 4.30/−84.0 | O |
| | 4.28/−45.5; 4.28/−45.3 | Se |
| [Pt($NH_3$)(L-Se-Met)(L-Se-MetH)]$^+$ | 4.16/−67.0 | N |
| | 4.44/−42.1; 4.41/−42.2 | Se |
| [Pt($NH_3$)$_2$(CBDCA)(L-Met)]$^+$ | 4.36/−79.5; 4.34/−79.5 | O |
| | 4.29/−46.0 | S |
| [Pt($NH_3$)$_2$(L-Met)]$^+$ | 4.21/62.6; 4.18/−62.5 | N |
| | 4.44/−43.0; 4.39/−42.5 | S |

apart from the cross-peak of carboplatin, two sets of new cross-peaks (1a, 1a′, 1b) with similar intensities appeared at δ = 4.36/−79.5 ppm (1a), δ = 4.34/−79.5 ppm (1a′), and δ = 4.29/−46.09 ppm (1b). These three cross-peaks (1a, 1a′, 1b) can be assigned to [Pt($NH_3$)$_2$(CBDCA)(L-MetH)] (**1′**), based on their known shifts.[20] The split of cross-peaks 1a and 1a′ can be attributed to the slow inversion of the coordinated chiral sulfur atom. The intensity of these peaks increased in the following 7 h and decreased after 8 h of the reaction. After 30 h, the peaks disappeared completely, which is consistent with the result by mass spectrometry. After ca. 7 h, two new cross-peaks at δ = 4.21/−62.6 ppm (2a) and δ = 4.44/−43.0 ppm (2b) appeared, and 2 h later another pair of cross-peaks could be seen at δ = 4.18/−62.5 ppm (2a′) and δ = 4.39/−42.5 ppm (2b′). From their $^{15}$N chemical shifts these cross-peaks (2a, 2a′ and 2b, 2b′) can be assigned to the $NH_3$ group *trans* to N and S, respectively. These two pairs of cross-peaks correspond to complex [Pt($NH_3$)$_2$(L-Met)] (**7′**), and the splitting of the cross-peaks in the $^1$H dimension can be attributed to the slow inversion of the methyl group at the coordinated chiral sulfur atom.[7] These adducts could be observed even after 38 h. The final product [Pt(L-Met)(L-MetH)]$^+$ (**4′**) which was observed in the ESMS study could not be observed in the 2 D [$^1$H,$^{15}$N] HSQC NMR study due to the elimination of ammonia. This result is different from those reported previously[7] in which, apart from the complex **7′**, complexes **1′** and **5′** were also observed even after 3 d at 293 K and at pH = 7.

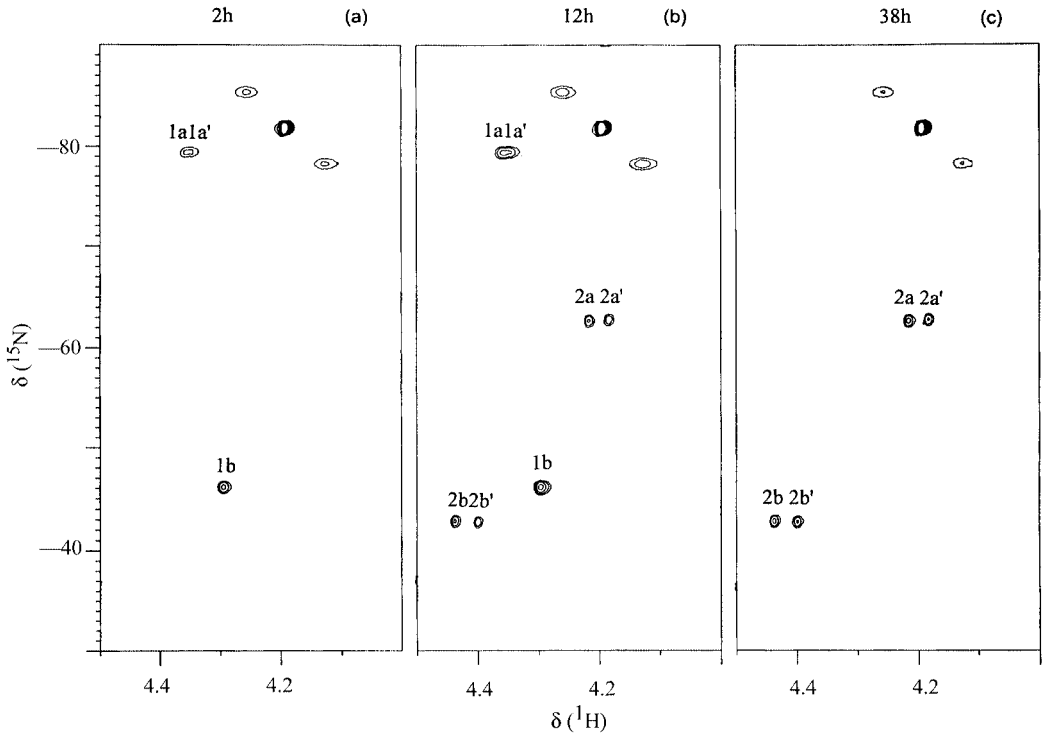



Figure 7. 2D [$^1$H,$^{15}$N] HSQC NMR spectrum of the reaction of $^{15}$N-carboplatin with L-MetH in a 1:1 molar ratio at 310 K, recorded after (a) 2 h, (b) 12 h, (c) 38 h

## Discussion

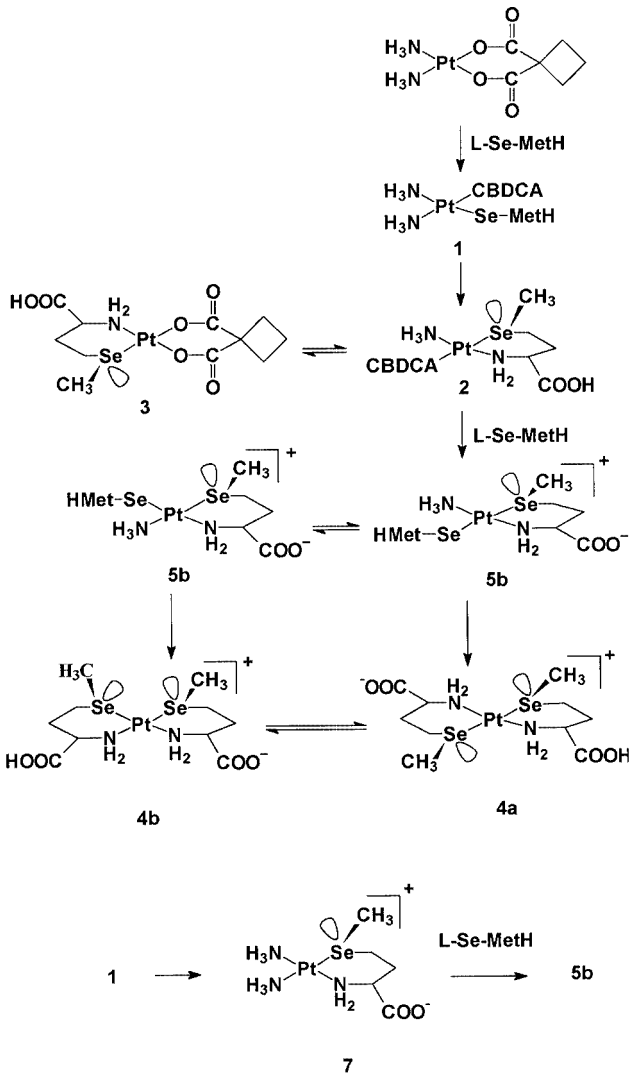
Our recent work on the investigation of the reaction of cisplatin with L-Se-MetH and L-MetH using the combined techniques of ESMS and 2D [$^1$H,$^{15}$N] NMR has further clarified the reaction pathways.[18] Here we extend our study to the reaction of the second-generation anticancer drug carboplatin with L-Se-MetH and L-MetH, and compared the different reactivities of the two drugs towards the two ligands.

We have detected the dimer, trimer and tetramer forms of carboplatin by ESMS. Although they are not stable in the presence of L-Se-MetH, the dimer form of carboplatin (either as the $H^+$ or $Na^+$ form) can be observed for quite a long period of time. Recently, it has been reported that polynuclear Pt complexes can be formed in aqueous solution.[21,22] However, it is possible that these complexes may be formed only in the capillary device of the mass spectrometer due to the high temperature.[23]

Based on the ESMS and 2D [$^1$H,$^{15}$N] NMR results, the reaction pathway between carboplatin and L-Se-MetH is summarized in Scheme 2.

As can be seen from the ESMS results, the reaction between L-Se-MetH and carboplatin initially gave rise to a ring-opened species [Pt($NH_3$)$_2$(CBDCA)(L-Se-MetH)] (**1**) which is very stable and can be observed for ca 18 h in the ESMS experiment (peak 1) and for ca 13 h in the 2D [$^1$H,$^{15}$N] HSQC NMR experiment (1a, 1a′; 1b, 1b′). The appearance of two pairs of cross-peaks 1a, 1a′, 1b, 1b′ may be due to the hydrogen bonding between the $NH_3$ group and the oxygen atom of CBDCA similar to that reported in *cis*-[Pt(CBDCA)($NH_3$)$_2$(L-Met-*S*)][7] and [Pt(en)(Me-Mal-O)(Ac-Met-*S*)].[24] The high *trans* effect of selenium labilizes the $NH_3$ group, and L-Se-MetH undergoes ring-closure to form [Pt(CBDCA)($NH_3$)(L-Se-Met-*N*,*Se*)] (**2**). The complex [Pt($NH_3$)$_2$(L-Se-Met-*Se*,*N*)] (**7**) is also formed. This species corresponds to the cross-peaks 2a and 2b, 2b′ in the 2D NMR spectrum. Most of complex **2** was converted into complex **5**, [Pt($NH_3$)(L-Se-MetH-*Se*)(L-Se-MetH-*Se*,*N*)], the rest was converted into complex **3**, [Pt(CBDCA)(L-Se-MetH-*Se*,*N*)] (peak 3 in the ESMS spectrum). Complex **3** could only be observed in the ESMS spectrum with a low intensity for ca. 9 h. However, complex **5** could be observed neither in the ESMS nor in the NMR spectrum, which may be due to its fast conversion into the final product, complex **4**. As can be seen from the ESMS data, the dominant adduct at the end of the reaction is [Pt(L-Se-Met-*Se*,*N*)$_2$] (complex **4**, peak 4 in the ESMS spectrum). There should be two geometrical isomers (*cis* and *trans*) of complex **4**. Previous studies on [Pt(L-Met-*S*,*N*)$_2$] showed that both isomers were present in solution, and that the dominant one was the *cis* isomer. A similar situation may occur in the current work. This result is different from that between cisplatin and L-Se-MetH. In the latter case the final product has one L-Se-MetH coordinated group, i.e. the final product is the complex [Pt($NH_3$)Cl(L-Se-MetH)], and there is a low abundance of complex **4**.[18]

Similarly, the reaction of carboplatin with L-MetH gives rise to [Pt(L-Met-*N*,*S*)$_2$] as the final adduct, which is differ-




Scheme 2. Reaction pathway of carboplatin with L-Se-MetH in a 1:1 molar ratio at pH = 5.40

ent from the previous 2D [$^1$H,$^{15}$N] NMR study.[7] However, it should be noted that the temperature and pH of the two studies are different. The lower temperature and high pH may facilitate the stabilization of the ring-opened adduct and slow down the rate of elimination of the ammonia ligand. It can be concluded from the comparison between the reactions of cisplatin and carboplatin with L-Se-MetH and L-MetH that under the same conditions the bis(chelated) adducts [Pt(L-Se-Met)$_2$] or [Pt(L-Met)$_2$] can be formed more easily in the reaction with carboplatin than in that the reaction with cisplatin.

It is interesting to note that under the same experimental conditions the inversion rate of Se−CH$_3$ in complex **7** is faster than that of S−CH$_3$ in complex **7′**. This is rather intriguing because a reversal of the inversion rate would be expected.

## Conclusion

The self-association of carboplatin in aqueous solution was detected by ESMS for the first time. From the ESMS and 2D [$^1$H,$^{15}$N] HSQC NMR results of this work, the major species [Pt(NH$_3$)$_2$(CBDCA)(L-SeMetH)] (**1**), [Pt(NH$_3$)$_2$(CBDCA)(L-MetH)] (**1′**); [Pt(NH$_3$)(CBDCA)-(L-SeMetH)] (**2**), [Pt(NH$_3$)(CBDCA)(L-MetH)] (**2′**); [Pt(CBDCA)(L-MetH)](**3**), [Pt(CBDCA)(L-MetH)] (**3′**); [Pt(NH$_3$)$_2$(L-SeMet)]$^+$ (**7**), [Pt(NH$_3$)$_2$(L-Met)]$^+$ (**7′**); [Pt (L-Se-Met)$_2$] (**4**) and [Pt(L-Met)$_2$] (**4′**) were found. Due to the elimination of the ammonia group and the abundance of the species, only complex **1**, **1′** and complex **7**, **7′** can be observed by 2D [$^1$H,$^{15}$N] HSQC NMR spectroscopy. A detailed pathway of the reaction was provided and compared with that of cisplatin. The bis(chelated) adducts [Pt(L-Se-Met)$_2$] or [Pt(L-Met)$_2$] can be formed more easily in the reaction with carboplatin than in the reaction with cisplatin under the same reaction conditions. It remains to be seen whether this observation can be related to in vivo activity and metabolism of the drug.

## Experimental Section

**General:** Carboplatin and [$^{15}$N]carboplatin were prepared and recrystallized according to a published procedure,[25] L-selenomethionine (L-Se-MetH) and L-methionine (L-MetH) were purchased from Sigma.

**Spectroscopy**

**ESMS:** Electrospray mass spectra were recorded using an electron mass spectrometer (ESMS, Finnigan) by loading 1.0 μL of solution into the injection valve of the LCQ unit, and then injecting into the mobile phase solution (50% of aqueous methanol) which was carried through the electrospray interface into the mass analyzer at a rate of 200μL min$^{-1}$. The voltage employed at the electrospray needles was 5 kV, and the capillary was heated to 200 °C. A maximum injection time of 200 ms, along with 10 scans was set. Positive ion mass spectra were obtained. The predicted isotope distribution patterns for each of the complexes were calculated using the Isopro 3.0 program.[26]

**NMR:** NMR spectroscopic data were acquired with 500-MHz Bruker DMX and DRX spectrometers. One-dimensional spectra were typically acquired with 128 transients and 32 K data points over a spectral width of 10 kHz by using standard pulse sequences. Two-dimensional [$^1$H,$^{15}$N] HSQC NMR (Heteronuclear Single Quantum Coherence) data were acquired and processed according to previously reported methods.[27] The $^{15}$N chemical shifts are externally referenced to 1.5 M $NH_4Cl$ in 1 M HCl. The indirect referencing method resulted in slightly different $^{15}$N chemical shifts to those in previous reports for similar species, and no corrections have been made.

**pH Measurements:** The pH measurements were carried out using a pHS-3C pH meter equipped with a Phoenix Ag/AgCl reference electrode, calibrated with phosphate buffer solutions at pH = 4.00 and 10.00. The values reported are those measured after the reactions were finished.

**Sample Preparations:** Samples for the ESMS studies were prepared using double-distilled water. Due to the limitation of the ESMS, no phosphate buffer solution was employed in these studies so as to avoid the potential blockage of the capillary of the instrument. (1) [Pt(NH$_3$)$_2$(CBDCA)] (1.00 mg) was dissolved in 1 mL of water. The pH of the solution was 5.00. The ESMS spectra were recorded

at this pH value. (2) $[Pt(NH_3)_2(CBDCA)]$ (1.00 mg) and L-Se-MetH (0.53 mg) were dissolved in 1 mL of water. The solution was monitored by ESMS for a period of 22 h at 310 K. The pH value after 22 h was 5.40. (3) $[Pt(NH_3)_2(CBDCA)]$ (1.00 mg) was mixed with L-MetH in a 1:1 molar ratio. The reaction was monitored by ESMS for 38 h at 310 K. The pH of the solution after 38 h was 5.41. All the reactions were carried out at 310 K unless otherwise stated. Samples for the NMR study were prepared as follows. $[Pt(NH_3)_2(CBDCA)]$ (2.68 mmol) and L-Se-MetH (2.68 mmol) or L-MetH (2.68 mmol) were dissolved in a 500-μL solution of 90% $H_2O$ and 10% $D_2O$. The final concentration of carboplatin and L-Se-MetH or L-MetH was 2.68 mmol/mL. To be consistent with the conditions used in the ESMS study, no buffer solution was employed in the NMR study. The reaction was also conducted at 310 K.

## Acknowledgments

We thank the National Natural Science Foundation of China for the Distinguished Young Scientists Fund (Grant No. 29925102) and the Major State Basic Research Development Program (Grant No. G200007750). We thank Prof. P. J. Sadler (Edinburgh) for access to his NMR spectrometer with which a part of the experiments were carried out.

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Received January 9, 2002
[I02019]