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Note

Synthesis of two maleimide derivatives of *cis*-configurated platinum(II) complexes for the preparation of chemoimmunoconjugates

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Abstract

By attaching maleimide groups to anticancer drugs, derivatives are obtained which bind selectively to thiolated carrier proteins. Two maleimide ester derivatives of platinum(II) complexes which consist of an active *cis*-configurated platinum(II) moiety and a maleimide group capable of binding to thiolated carriers were prepared, i.e. *N*-(*O*-(3-maleimidobenzoyl)-2-hydroxyethyl)-1,2-diaminoethanedichloroplatinum(II) (**7**) and *N*-(*O*-(2-(4-maleimidophenyl)acetyl)-2-hydroxyethyl)-1,2-diaminoethanedichloroplatinum(II) (**8**). **7** and **8** were characterized through ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, elemental analysis and mass spectrometry (FAB). © 1998 Elsevier Science S.A.

Keywords: Platinum complexes; Cisplatin; Maleimide complexes; Chemoimmunoconjugates

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II)), a potent antineoplastic drug in the treatment of testicular carcinomas, ovarian carcinomas, tumors of the head and neck, and bladder tumors, does have serious side-effects due to its reactions with cellular components of healthy tissues by which it exerts its cytotoxicity [1]. These include nephrotoxicity, ototoxicity, myelotoxicity, peripheral neuropathy, nausea and vomiting.

The development of so-called 'second generation platinum complexes', e.g. carboplatin (*cis*-diammine-(1,1-cyclobutanedicarboxylato)platinum(II)), has, in part, contributed to altering the toxicity profile of platinum compounds [2].

A novel strategy to overcome the toxicity of cytotoxic platinum complexes to normal tissue — thereby increasing the therapeutic index of these agents — is to attach the active moiety of platinum complexes to carrier proteins which exhibit a significant uptake in tumor tissue.

Such an approach could be of clinical benefit considering that the dose-limiting factor of cisplatin is its high nephrotoxicity [3] and that cisplatin, once bound to serum proteins (approximately 90% of injected cisplatin are protein-bound three hours postinfusion [4]), does not retain its antitumor activity [5a,b].

An effective method of preparing such chemoimmunoconjugates is to introduce a maleimide group into the drug, which then binds selectively to sulfhydryl groups of carrier proteins through its carbon double bond [6]. Recently, we have synthesized a number of maleimide compounds for this purpose [7].

Hence, we have developed maleimide ester derivatives of platinum(II) complexes which consist of an active *cis*-configurated platinum(II) moiety and a maleimide group capable of binding to thiolated carriers.

2. Experimental

2.1. General

M.p.: Büchi 530; ^{1}H NMR and ^{13}C NMR: Bruker AM 400, Bruker WM 250 (internal standard: TMS); FAB-MS: Finnigan-MAT 312; elemental analysis: Perkin-Elmer elemental analyzer 240; FT-IR spectroscopy: Perkin-Elmer 16 PC; silica gel chromatography on silica gel 60 (0.063–0.100 mm) from Merck AG; TLC: silica coated plates 60 F_{254} from Merck AG; organic solvents: p.a. grade and a gift from BASF; 1 M HCl in ether from Aldrich; other organic or inorganic compounds: Merck AG. The maleimide spacers were prepared previously [7].

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2.2. *N-(2-Hydroxyethyl)-N,N'-bis-(tert-butyloxycarbonyl)-1,2-diaminoethane (2)*

N-(2-Hydroxyethyl)-ethylenediamine (**1**) (20.8 g, 200 mmol) was dissolved in 100 ml anhydrous methylene chloride. A solution of di-tert-butyldicarbonate (47.96 g, 220 mmol) in 200 ml anhydrous methylene chloride was added dropwise within 3 h at room temperature. After being stirred for further 9 h at room temperature the solution was diluted with 100 ml anhydrous ether, extracted with 150 ml water, dried over Na_2SO_4, and evaporated in vacuo. The residue was dissolved in a minimal amount of ethyl acetate and chromatographed on a silica gel column (ethyl acetate/hexane 1/2). Yield: 30.0 g, 98.6%; white crystals, m.p. 64°C. *Anal.* Calc. for $C_{14}H_{28}N_2O_5$: C, 55.26; H, 9.21; N, 9.21. Found: C, 55.50; H, 9.18; N, 9.02%. ^{1}H NMR ($CDCl_3$): δ 5.0 (s, 1H, N*H*), 3.7 (s, 2H, OCH_2), 3.5 (s, 1H, O*H*), 3.3 (bs[1], 6H, 3 N=CH_2), 1.5/1.4 (2s, 18H, 6 CH_3). ^{13}C NMR ($CDCl_3$): δ 156.80 (2 *C*=O), 79.45 (quart. *C*), 61.88 (OCH_2), 51.83/48.56/39.47 (3 NCH_2), 28.66 (6 CH_3).

2.3. *N-(O-(3-Maleimidobenzoyl)-2-hydroxyethyl)-N,N'-bis-(tert-butyloxycarbonyl)-1,2-diaminoethane (3)*

3-Maleimidobenzoic acid chloride (6.82 g, 29 mmol), dissolved in 100 ml THF, was added dropwise to a solution of **2** (8 g, 26.3 mmol) in 50 ml THF and 4 ml triethylamine. After being stirred for 8 h at room temperature a precipitate was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in a minimal amount of ethyl acetate and chromatographed on a silica gel column (ethyl acetate/hexane 1/1). Yield: 9.6 g, 72.6%; yellow oil. *Anal.* Calc. for $C_{25}H_{33}N_3O_8$: C, 59.64; H, 6.56; N, 8.35. Found: C, 60.04; H, 6.77; N, 8.24%. ^{1}H NMR ($CDCl_3$): δ 8.1–7.5 (m, 4H, Ph-*H*), 6.9 (s, 2H, *HC*=C*H*), 5.0 (s, 1H, N*H*), 4.4 (s, 2H, HOCH_2), 3.6/3.5/3.4 (3s, 6H, 3 NCH_2), 1.4 (s, 18H, 6 CH_3). ^{13}C NMR ($CDCl_3$): δ 169.09 (*C*=O), 165.38 (Ph*C*OO), 134.35 (HC=CH), 131.63/131.21/130.37/129.29/128.98/127.10 (Ph-*C*), 80.38 (quart. *C*), 63.37 (OCH2), 46.66/46.45/39.46 (3 NCH_2), 28.41 (6 CH_3).

2.4. *N-(O-(2-(4-Maleimidophenyl)acetyl)-2-hydroxyethyl)-N,N'-bis-(tert-butyloxycarbonyl)-1,2-diaminoethane (4)*

4-Maleimidophenylacetic acid (5 g, 21.65 mmol) and **2** (6.58 g, 21.65 mmol) were dissolved in a mixture of 15 ml methylene chloride and 45 ml THF. After adding 26.4 mg (0.2165 mmol) dimethylaminopyridine (DMAP), a solution of 4.9 g (23.82 mmol) *N,N'*-dicyclohexyl-carbodiimide (DCC), dissolved in 25 ml THF, was added dropwise at 0°C. The intensive red solution was stirred for 24 h, the formed precipitate removed by filtration and the filtrate evaporated to dryness in vacuo. The oily residue was dissolved in a minimal amount of ethyl acetate and chromatographed on a silica gel column (ethyl acetate/hexane 3.5/3). Yield: 4.8 g, 42.9%; pale yellow crystals, m.p. 87°C. *Anal.* Calc. for $C_{26}H_{35}N_3O_8$: C, 60.35; H, 6.77; N, 8.12. Found: C, 60.77; H, 7.4; N, 8.75%. ^{1}H NMR ($CDCl_3$): δ 7.4 (d, 2H, 2 NCC*H*), 7.2 (d, 2H, 2 CH_2CC*H*), 6.8 (s, 2H, *HC*=C*H*), 4.9 (s, 1H, N*H*), 4.2 (s, 2H, OCH_2), 3.7 (s, 2H, Ph-CH_2), 3.4/3.2 (2s, 6H, 3 NCH_2), 1.5/1.4 (2s, 18H, 6 CH_3). ^{13}C NMR ($CDCl_3$): δ 170.83 (*C*=O), 169.45 (PhCH_2*C*OO), 134.25 (H*C*=*C*H), 130.13/129.80/126.22/126.09 (Ph-*C*), 80.29 (quart. *C*), 63.42 (O*C*H_2), 47.38/46.62/40.89 (3 NCH_2), 39.32 (PhCH_2), 28.41 (6 *C*H_3).

2.5. *N-(O-(3-Maleimidobenzoyl)-2-hydroxyethyl)-1,2-diaminoethane dihydrochloride (5) and N-(O-(2-(4-male-imidophenyl)acetyl)-2-hydroxyethyl)-1,2-diaminoethane dihydrochloride (6)*

5 or **6** (12 mmol) was dissolved in 60 ml 1 M HCl/ether and stirred for 96 h during which time a precipitate was formed. The precipitate was collected by filtration, washed several times with anhydrous ether and dried in vacuo.

5: 2.93 g, 65%; m.p. 176°C. *Anal.* Calc. for $C_{15}H_{19}N_3O_4Cl_2$: C, 47.89; H, 5.05; N, 11.17; Cl, 18.86. Found: C, 47.97; H, 5.42; N, 11.03; Cl, 17.63%. ^{1}H NMR (DMSO-d_6): δ 9.9 (s, 2H, NH_2'), 8.5 (s, 3H, NH_3'), 8.2–7.6 (m, 4H, Ph-*H*), 7.2 (s, 2H, *HC*=C*H*), 4.6 (t, 2H, OCH_2), 3.6–3.2 (m, 6H, 3 NCH_2). ^{13}C NMR (DMSO-d_6): δ 169.67 (*C*=O), 164.81 (*C*OO), 134.76 (HC=CH), 131.92/131.78/129.94/129.24/129.05/127.96 (Ph-*C*), 60.57 (OCH$_2$), 45.52/44.38/35.17 (3 NCH$_2$).

6: 3.42 g, 73%; m.p. 212°C. *Anal.* Calc. for $C_{16}H_{21}N_3O_4Cl_2$: C, 49.24; H, 5.39; N, 10.77; Cl, 18.18. Found: C, 49.01; H, 5.68; N, 10.53; Cl, 17.88%. ^{1}H NMR (DMSO-d_6): δ 9.9 (s, 2H, NH_2'), 8.5 (s, 3H, NH_3'), 7.4 (d, 2H, 2 NCC*H*), 7.3 (d, 2H, 2 CH_2CC*H*), 7.2 (s, 2H, *HC*=C*H*), 4.4 (t, 2H, OCH_2), 3.9 (s, 2H, Ph-CH_2), 3.4/3.2 (2s, 6H, 3 NCH_2). ^{13}C NMR (DMSO-d_6): δ 170.82 (*C*=O), 169.85 (*C*OO), 134.58 (HC=CH), 130.17/129.84/126.55/126.40 (Ph-*C*), 59.68 (O*C*H_2), 45.44/44.28/35.14 (3 N*C*H_2), 39.39 (Ph*C*H_2).

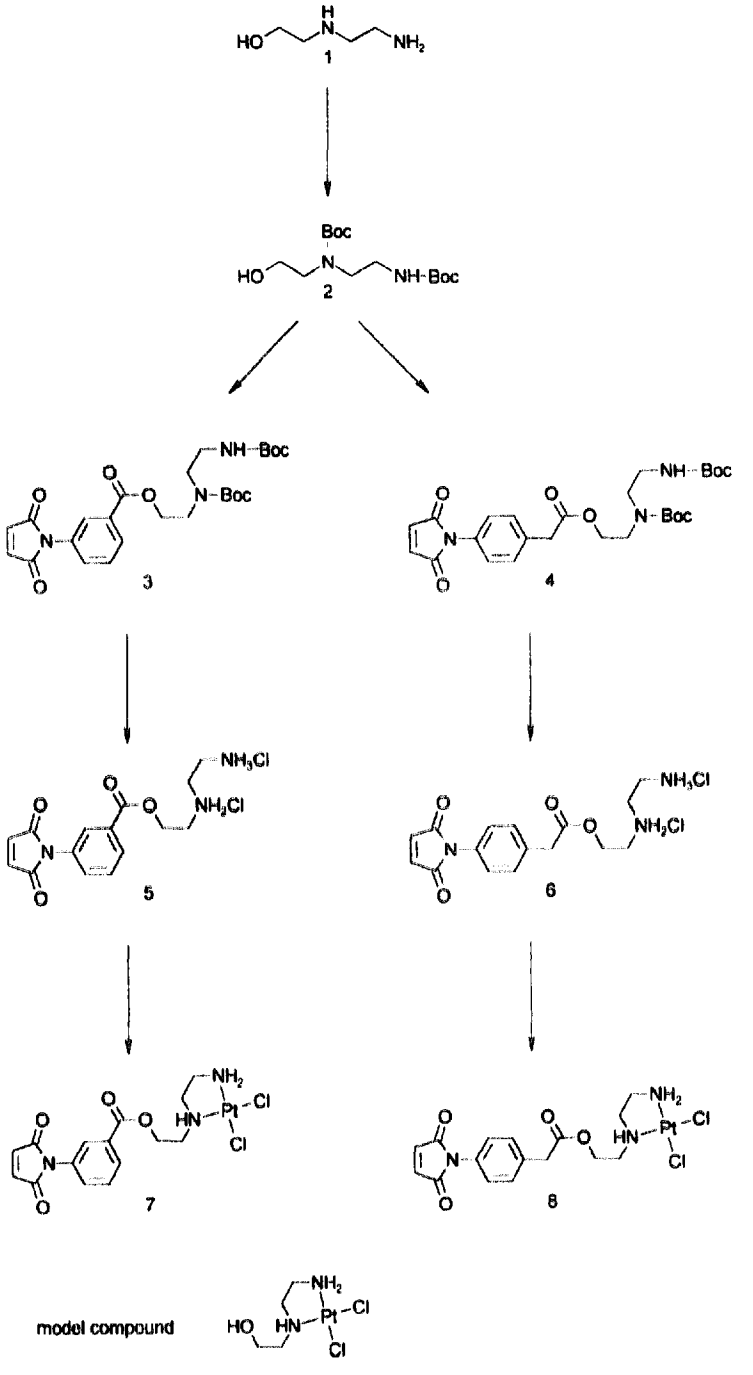
2.6. *N-(O-(3-Maleimidobenzoyl)-2-hydroxyethyl)-1,2-diaminoethanedichloroplatinum(II) (7) and N-(O-(2-(4-maleimidophenyl)acetyl)-2-hydroxyethyl)-1,2-diaminoethanedichloroplatinum(II) (8)*

5 or **6** (1 mmol) was dissolved in 5 ml of 20% THF in water and added dropwise to a solution of K_2PtCl_4 (0.415 g, 1 mmol) dissolved in the same amount of 20% THF in water. After being stirred for 1 h the yellow precipitate was collected by filtration, washed several times first with 20% THF in water and then with anhydrous ether and dried in high vacuum.

[1] bs = broad singlet.

7: 0.39 g, 68%; m.p. > 250°C. *Anal.* Calc. for $C_{15}H_{17}N_3O_4PtCl_2$: C, 31.63; H, 2.99; N, 7.38; Pt, 34.29; Cl, 12.46. Found: C, 31.19; H, 3.37; N, 6.79; Pt, 33.35; Cl, 12.95%. FT-IR (CsI): ν(Pt–Cl) = 348 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.1–7.6 (m, 4H, Ph–*H*), 7.2 (s, 2H, *H*C=C*H*), 6.3 (m, 2H, NH_2), 4.6 (m, 2H, OCH_2), 4.5 (m, 1H, N*H*), 3.5/2.9/2.7 (3m, 6H, NCH_2). ^{13}C NMR (DMSO-d_6): δ 169.64 (*C*=O), 164.74 (*C*OO), 134.75 (H*C*=*C*H), 131.94/131.52/130.24/129.30/128.59/127.50 (Ph–*C*), 61.70 (OCH_2), 54.34/49.57/45.22 (3 NCH_2); MS-FAB, 3 kV, nitrobenzylalcohol (rel. intensity): *m/z* 570 ($M^+ + 1$, 10), 534 ($M^+ - Cl$, 17), 499 ($M^+ - 2Cl$, 2).

8: 0.33 g, 57%; m.p. > 250°C. *Anal.* Calc. for $C_{16}H_{19}N_3O_4PtCl_2$: C, 32.93; H, 3.26; N, 7.2; Pt, 33.46; Cl, 12.16. Found: C, 32.64; H, 3.54; N, 7.31; Pt, 33.00; Cl, 11.97%. FT-IR (CsI): ν(Pt–Cl) = 338 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.4 (d, 2H, 2 NCC*H*), 7.3 (d, 2H, 2 CH_2CC*H*), 7.2 (s, 2H, *H*C=C*H*), 6.3 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 170.65 (*C*=O), 169.84 (*C*OO), 134.60 (H*C*=*C*H), 133.71/130.19/129.95/126.59 (Ph–*C*),



Scheme 1.

61.18 (OCH_2), 54.31/49.38/45.12 (3 NCH_2), 30.59 ($PhCH_2$); MS-FAB, 3 kV, nitrobenzylalcohol (rel. intensity): m/z 584 ($M^+ + 1$, 12), 548 ($M^+ - Cl$, 19), 513 ($M^+ - 2Cl$, 4).

3. Results and discussion

The method of preparing the platinum(II) complexes **7** and **8** is depicted in Scheme 1. The starting compound *N*-(2-hydroxyethyl)-ethylenediamine (**1**) was first reacted with di-tert-butyldicarbonate. Interestingly, in the isolated product **2** the BOC group was introduced at the primary amine as well as at the secondary amine position. The rationale for introducing the BOC group was firstly to obtain the maleimide esters **3** and **4** in high yields, and secondly to prevent the primary amine group reacting spontaneously with the double bond of the maleimide group. The BOC group was removed by dissolving **3** or **4** in anhydrous ether/HCl upon which the hydrochlorides **5** and **6** precipitated. To the thus obtained hydrochlorides an equivalent amount of K_2PtCl_4 in a mixture of THF/water was added to yield the yellow-colored platinum complexes **7** and **8**.

All synthesized compounds were characterized through ^1H and ^{13}C NMR spectroscopy, elemental analysis and/or mass spectrometry. NMR spectroscopy of the relevant complexes **7** and **8** reveals the characteristic peaks of the introduced maleimide group at 7.2 ppm (strong singlet) for the proton signals of the double bond in the ^1H NMR spectra and at 134–135 and 168–170 ppm for the carbon atoms of the double bond and carbonyl group in the ^{13}C NMR spectra.

The MS-FAB spectra of the two complexes **7** and **8** show a characteristic molecular ion peak ($M^+ + 1$) at 570 and 584, respectively, and peaks at 534 or 548 and 499 or 513, corresponding to $M^+ - Cl$ and $M^+ - 2Cl$, are distinctly visible. FT-IR spectroscopy shows a distinct band at $\nu = 348$ cm^{-1} for **7** and $\nu = 338$ cm^{-1} for **8**, which is the characteristic region for Pt–Cl stretching vibrations [8].

Based on the above data and the elemental analyses of **7** and **8**, their structures are postulated as shown in Scheme 1.

7 and **8** were designed so as to incorporate a maleimide group which reacts rapidly with sulfhydryl groups [6]. In order to demonstrate that the sulfhydryl group reacts significantly faster with the maleimide groups of **7** and **8** than with their *cis*-configurated platinum(II) moieties, the model compounds **5**, **6** and **9** (**9** prepared according to [9]; see Scheme 1) were incubated with cysteine in 0.9% saline in a ratio of 1:1 (c[Pt] = 300 μM, pH 6) at room temperature. The concentration of sulfhydryl groups was determined with Ellman's reagent ($\epsilon_{412} = 13\,600$ M^{-1} cm^{-1} [10]). Whereas **5** and **6** reacted quantitatively with cysteine within a few minutes, we observed no loss of sulfhydryl groups for **9** during 60 min. In accordance with our results it has been reported that cisplatin also reacts slowly with cysteine in 0.9% saline, the reaction being complete after 4–5 days [11].

Hence, **7** and **8** exhibit suitable properties for binding the active *cis*-configurated platinum(II) moiety to thiolated carrier proteins.

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