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The β-glucuronyl-based prodrug strategy allows for its application on β-glucuronyl-platinum conjugates

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Abstract—The use of platinum drugs in antitumour therapy is well established. An important drawback of these chemotherapeutics is the lack of selectivity for tumour cells, usually resulting in severe toxic side effects. A glucuronyl-platinum conjugate was designed and synthesised to test the compatibility of platinum compounds with β-glucuronidase-based prodrug therapy. Instantaneous cleavage of the β-glucuronic bond in the glucuronyl-platinum conjugate was observed upon addition of β-glucuronidase resulting in Pt^{II}(dach)(4-hydroxybenzylmalonate) and glucuronic acid.

Platinum cytostatics are recognised as an important class of drugs in cancer chemotherapy.[1,2] An important drawback of these chemotherapeutics is the lack of selectivity for tumour cells, usually resulting in severe toxic side effects. Since the discovery of the antitumour activity of cisplatin,[3] a plethora of platinum derivatives have been developed in the quest for a platinum drug that, in comparison with cisplatin, has a broader activity spectrum, can overcome drug resistance, has less toxic side effects, and is better soluble in water. A number of these platinum compounds overcome some of the limitations of cisplatin.[2,4] Most of the well-known platinum anticancer complexes have the general formula *cis*-$PtX_2(NHR_2)_2$, in which R is an organic fragment and X is a leaving group, such as chloride, or (chelating bis)-carboxylate.[5] Currently three drugs are used in routine clinical treatments: cisplatin, carboplatin and oxaliplatin.[6] Several others are under investigation in Phase-I and Phase-II clinical trials.

Selective generation of a cytotoxic drug from an inactive prodrug is feasible with antibody-directed enzyme prodrug therapy (ADEPT), or with prodrug monotherapy (PMT).[7–10] To the best of our knowledge, only one example of a platinum complex designed for ADEPT, a platinum-cephalosporine prodrug in combination with prokaryotic β-lactamase, has been reported.[11]

In this study, the compatibility of platinum compounds towards β-glucuronidase-based prodrug therapy has been investigated. β-Glucuronidase has proven to be a versatile activating enzyme in prodrug therapy.[12] As an endogenous enzyme, β-glucuronidase will not elicit immune responses, which may appear upon application of exogenous enzymes in ADEPT. Additionally, extracellular β-glucuronidase is predominantly present in necrotic areas in high local concentrations.[13]

The design of β-glucuronyl-platinum conjugate **12** is based on the following considerations. The attachment of a glucuronide unit to a platinum derivative greatly enhances the solubility of the platinum compound in aqueous media and probably leads to a reduced cell-membrane permeability of the prodrug. Release of the polar glucuronic acid moiety by β-glucuronidase affords a neutral platinum species that can enter the tumour cell. As diamine dicarboxylate complexes are potent platinum drugs,[14–16] a functionalised platinum(II)(dach) malonate complex was selected as the releasable moiety. To avoid ineffective enzymatic hydrolysis as a result of a too small distance between the glucuronide and the platinum centre,[17] an aromatic linker was installed between the platinum and the glucuronic acid moieties.

Keywords: Platinum; Anticancer drugs; Prodrug therapy; β-Glucuronidase; Glucuronide prodrugs; ADEPT; PMT.
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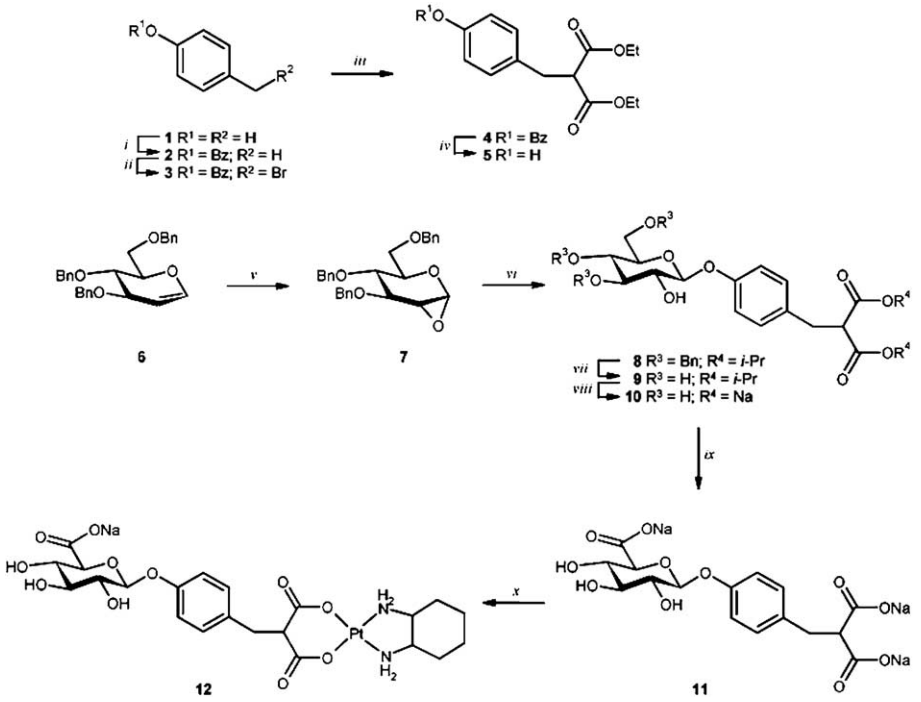


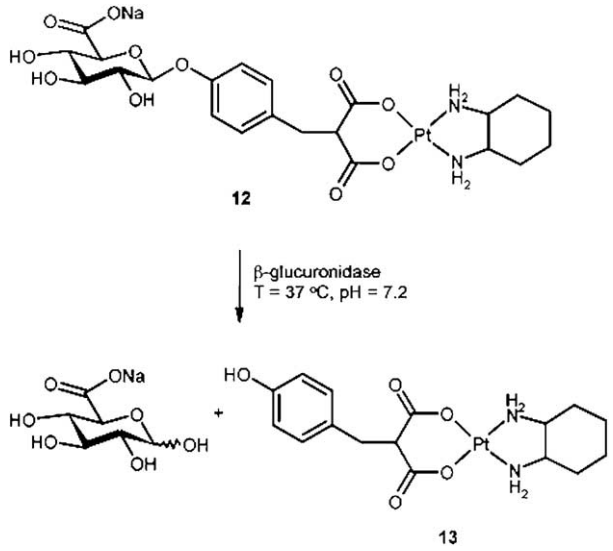
Scheme 1. Reagents and conditions: (i) BzCl, pyridine, 97%; (ii) NBS, AIBN, $CC1_4$, reflux, 50%; (iii) diethyl malonate, KO*t*Bu, 18-crown-6, THF, 67%; (iv) KO*t*Bu, ethanol, 56%; (v) 3,3-dimethyldioxirane, acetone/CH_2C1_2 (4/1, v/v); (vi) **5**, KO*t*Bu, 18-crown-6, *i*-PrOH/THF (1/1, v/v), 61% over two steps; (vii) 20% Pd/C, H_2, *t*-BuOH/H_2O (1/1, v/v), 73%; (viii) 1 M NaOH, dioxane; (ix) NaBr, TEMPO, NaOC1, H_2O, pH 10.8, 76% over two steps; (x) $Pt^{II}(dach)(NO_3)_2$, H_2O, 33%.

The assembly of glucuronyl-platinum conjugate **12** is depicted in Scheme 1. The aglycone **5** was prepared from 4-cresol (**1**) in four steps. Benzoylation of 4-cresol (**1**) gave compound **2** (97%). Reaction of **2** with *N*-bromosuccinimide and a catalytic amount of AIBN in refluxing tetrachloromethane yielded bromide **3** in 50% yield. Dropwise addition of bromide **3** to the anion of diethylmalonate, prepared in situ with 1 equiv KO*t*Bu in the presence of a catalytic amount of 18-crown-6 in dry THF, afforded malonate **4** in 67% yield. Removal of the benzoyl protective group was effected with EtOH/KO*t*-Bu to give aglycone **5** in 56% yield. Coupling of glucal **6** with glycosyl acceptor **5** was accomplished using a modification of the two-step procedure for stereoselective generation of aryl β-glycosides described by Danishefsky et al.[18] Thus, α-1,2-anhydroglucoside **7**[19] prepared by stereoselective oxidation of glucal **6** with 3,3-dimethyldioxirane[20] was added to a solution of aglycone **5**, KO*t*Bu (1 equiv) and 18-crown-6 (1 equiv) in isopropanol/THF (1/1, v/v) to afford β-aryl glucoside **8** (61% over two steps). Use of KO*t*Bu in the presence of isopropanol resulted in transesterification, yielding isopropylester **8**, instead of the corresponding ethyl ester. The nature of the base and solvent in the glycosylation reaction proved to be important, since the coupling did not proceed with K_2CO_3 in refluxing acetone, as described for other β-aryl glucosides.[18]

Deprotection of **8** was effected by hydrogenolysis of the benzyl groups and subsequent saponification of the isopropyl ester functions to provide **10**. The resulting glucoside **10** was selectively oxidised at C-6 using TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical)[21–23] with sodium hypobromite as the regenerating oxidant to give **11**. β-Glucuronide **11** was purified by gel filtration on a Fractogel TSK HW40 (S) column and isolated in 34% overall yield, starting from glucal **6**.

In the next stage, the target compound **12** was assembled by reaction of $Pt^{II}(dach)(NO_3)_2$ with the malonate function of glucuronide **11**. Although chromatographic purification of platinum complexes has been reported to be problematic due to potential hydrolysis and side-product formation in the dilute aqueous solutions used as the mobile phase,[24] Pt^{II}(glucuronide) complex **12** was successfully purified by gel filtration on a Fractogel TSK HW40 (S) column. The appropriate fractions were lyophilised immediately after collection. The structure of **12** was established by ^1H and ^{195}Pt NMR spectroscopy and mass spectrometry.[25]

The enzymatic cleavage of the β-glucuronyl-platinum conjugate (**12**) by β-glucuronidase (Scheme 2) was carried out in the NMR tube[26] at 37 °C and pD 7.2. Compound **12** was dissolved in a deuterated phosphate buffer (pD 7.2) at 37 °C. After recording the ^1H NMR spectrum at 37 °C, a β-glucuronidase buffer solution was added. The ^1H NMR spectrum was taken immediately after addition. This revealed the virtually instantaneous formation of glucuronic acid and platinum compound **13** (Fig. 1). The identity of the products formed after enzymatic hydrolysis was confirmed by comparison with glucuronic acid and a separately prepared synthetic standard of platinum complex **13**.[27]



Scheme 2. Reagents and conditions: Deuterated phosphate buffer (pD 7.2), β-glucuronidase, 37 °C.

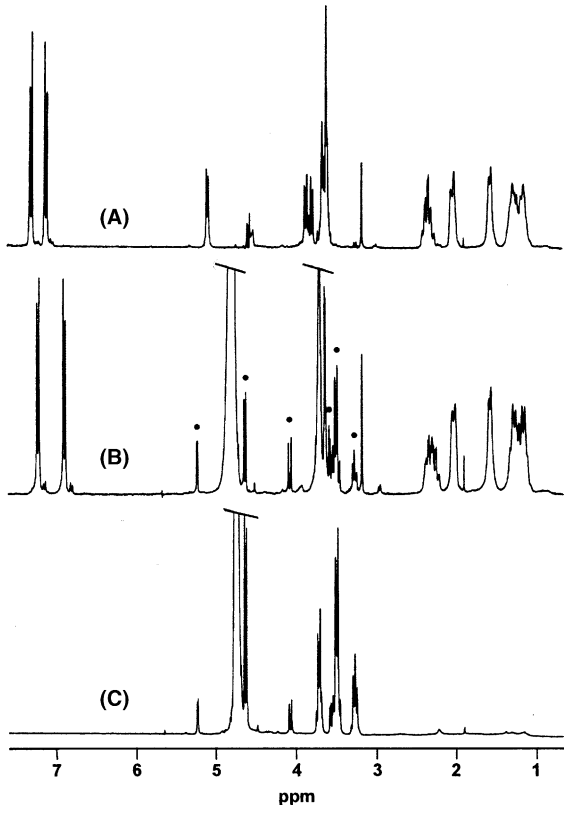


Figure 1. ^{1}H NMR spectra at 37 °C and pD 7.2 depicting (A) β-glucuronyl-platinum conjugate **12** in the absence of β-glucuronidase, (B) β-glucuronidase-mediated release of platinum compound **13** with release of glucuronic acid (●) and (C) glucuronic acid.

In conclusion, we have demonstrated for the first time, that the β-glucuronidase-based prodrug therapy is feasible for platinum complexes. The virtually instantaneous cleavage of the synthesised conjugate (**12**) by β-glucuronidase demonstrates the potential of platinum-glucuronide conjugates as prodrugs. Currently, we are working on the next step towards clinically interesting platinum prodrugs through developing β-glucuronyl-platinum conjugates based on highly potent platinum drugs in combination with traceless linkers.

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25. Purification of platinum complex **12** was performed by gel filtration on a Fractogel TSK HW40 (S) column, by elution with triethylammonium hydrogencarbonate buffer (0.15 M in methanol/water, 1/9, v/v) at a flow rate of 1.5 mL/min. To avoid side product formation, the volatile mobile phase was removed by lyophilisation to give **12** in 33% yield). ^{1}H NMR (300 MHz, deuterated phosphate buffer, pD 7.06) δ (relative to TMS) = 1.09–1.25 (m, 4H, cyclohexane CH_2), 1.51–1.53 (m, 2H, cyclohexane CH_2), 1.96–2.00 (m, 2H, cyclohexanyl H_{eq}-3, -6), 2.17–2.35 (m, 2H, cyclohexane *H-1*, *H-2*), 3.55–3.66 (m, 5H, *H-2*, *H-3*, *H-4*, CH_2CH), 3.70–3.76 (m, 1H, CH$_2$C*H*), 3.78–3.88 (m, 1H, *H-5*), 5.06 (d, 1H, $J = 6.8$, *H-1*), 7.08 (d, 2H, $J = 8.6$, C*H* arom), 7.27 (d, 2H, $J = 8.6$, C*H* arom). ^{195}Pt NMR (300 MHz, D_2O) δ (relative to K_2PtCl_4, external ref.) = −1895. Calculated Mass: 714. FAB: $(M+H)^+ = 715$, $(M+Na)^+ = 737$.
26. β-Glucuronidase (EC 3.2.1.31 from *Escherichia coli* (type X-A)) was purchased from Sigma Chemical Company. Deuterated phosphate buffer was prepared by dissolving Na_2HPO_4 (7.06 mg, 50×10^{-3} mmol) and KH_2PO_4 (6.78 mg, 50×10^{-3} mmol) in D_2O, lyophilised three times and dissolved in 2 mL of D_2O. To 0.4 mg (~3720 units) β-glucuronidase (9300 units mg^{-1} solid for phenolphthalein glucuronide) was added 0.3 mL of deuterated phosphate buffer (pH 7.2, 50 mM). Glucuronyl-platinum complex **12** (1.74 mg, 2.44×10^{-3} mmol) was dissolved in 400 μL deuterated phosphate buffer (50 mM, pH 7.2) at 37 °C. After taking the ^{1}H NMR spectrum at 37 °C, 180 μL β-glucuronidase buffer solution was added. The ^{1}H NMR spectrum was taken immediately.
27. Reference compound **13** was prepared by reaction of Pt^{II}(dach)$(NO_3)_2$ with bis-sodium 4-hydroxybenzylmalonato (deprotected **5**) in 41% yield. ^{1}H NMR (deuterated phosphate buffer, pH 7.06, 37 °C) δ (relative to TMS) = 1.01–1.29 (m, 4H, cyclohexane CH_2), 1.49–1.63 (m, 2H, cyclohexane CH_2), 1.92–2.08 (m, 2H, cyclohexanyl H_{eq}-3, -6), 2.22–2.42 (m, 2H, cyclohexane *H-1*, *H-2*), 3.52–3.62 (m, 2H, CH_2 CH), 3.68–3.76 (m, 1H, CH$_2$C*H*), 6.90 (d, 2H, $J = 7.1$, C*H* arom), 7.24 (d, 2H, $J = 7.1$, C*H* arom). ^{195}Pt NMR (300 MHz, deuterated phosphate buffer) δ (relative to K_2PtCl_4, external ref.) = −1879. Calculated Mass: 517. ESI/MS: $(M+Na)^+ = 540$.