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Note

Expeditious syntheses of two carbohydrate-linked cisplatin analogs

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Abstract

The syntheses of two carbohydrate-linked cisplatin analogs, namely lacto- and α-Gal-cisplatin, are described. A fusion enzymatic approach was used to create the diazido-α-Gal trisaccharide intermediate in the synthesis of α-Gal-cisplatin. © 2002 Published by Elsevier Science Ltd.

Keywords: Cisplatin analog; Chemo-enzymatic synthesis; Fusion enzyme

Since it was first discovered to have antitumour activity in the 1960s,[1] cisplatin, or *cis*-DDP, *cis*-$[PtCl_2(NH_3)_2]$, has been developed as one of the most widely used drugs for the treatment of human cancers.[2] The activity of cisplatin in cancer chemotherapy has been attributed to the 1,2-intrastrand addition of cisplatin at adjacent guanine residues of DNA. This facilitates the binding of HMG-domain proteins to DNA and thus shields the adduct from excision repair.[3]

The efficacy of cisplatin therapy has nevertheless been limited by two major factors.[4] One is its toxic side effects. The other is platinum resistance, either inherent (limited activity against many common human cancers) or acquired (reduced efficacy upon repeated treatment). These facts have stimulated a worldwide search for cisplatin analogs[5] that either have a better therapeutic index, a broader antitumour spectrum, or the ability to overcome tumor cell resistance to cisplatin. However, few analogs have shown definite advantages over cisplatin, especially in terms of reduced drug resistance.

Of particular interest in this field is the development of platinum complexes with biologically important ligands[6] because of their reduced toxicity. Although carbohydrates play a key role in various biological processes,[7] their usage in platinum-based cancer chemotherapy has remained virtually unexplored; only a few examples of platinum complexes of diamino-dideoxy carbohydrates (coordination through the amino groups) have been reported.[8] To capitalize on the fundamental roles of carbohydrates, and particularly the facilitated transport of monosaccharides in mammalian cells,[9] we have designed a novel class of carbohydrate-linked cisplatin analogs in which an intact carbohydrate moiety is connected to platinum through an appropriate linker. The platinum part is to exert antitumor activity, whereas the carbohydrate moiety is expected to provide enhanced water solubility, cell penetration, and drug–receptor interaction of the complex. With the presence of an intact carbohydrate moiety, together with the expeditiousness of the preparation, these carbohydrate-linked cisplatin analogs are anticipated to be advantageous over the aforementioned platinum complexes of diamino-dideoxy carbohydrates. Our model compound gluco-cisplatin (**1**), with its structure unambiguously confirmed by single-crystal X-ray determination, has demonstrated comparable antitumor activity to cisplatin.[10] Herein, we report the syntheses of two other carbohydrate-linked cisplatin analogs, namely lacto- (**2**) and α-Gal-cisplatin (**3**), the synthesis of the latter using a chemoenzymatic approach using a unique fusion enzyme.

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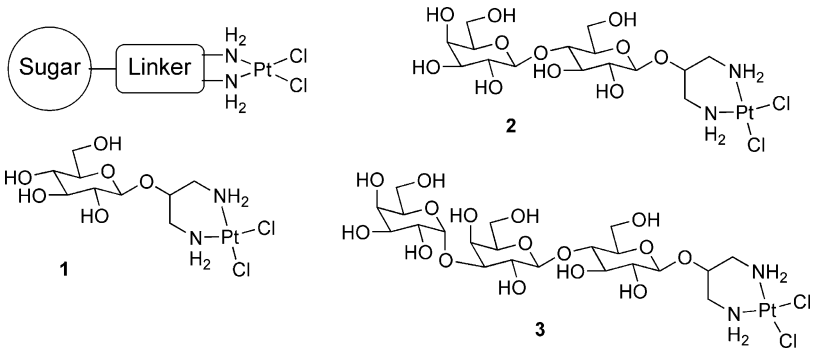
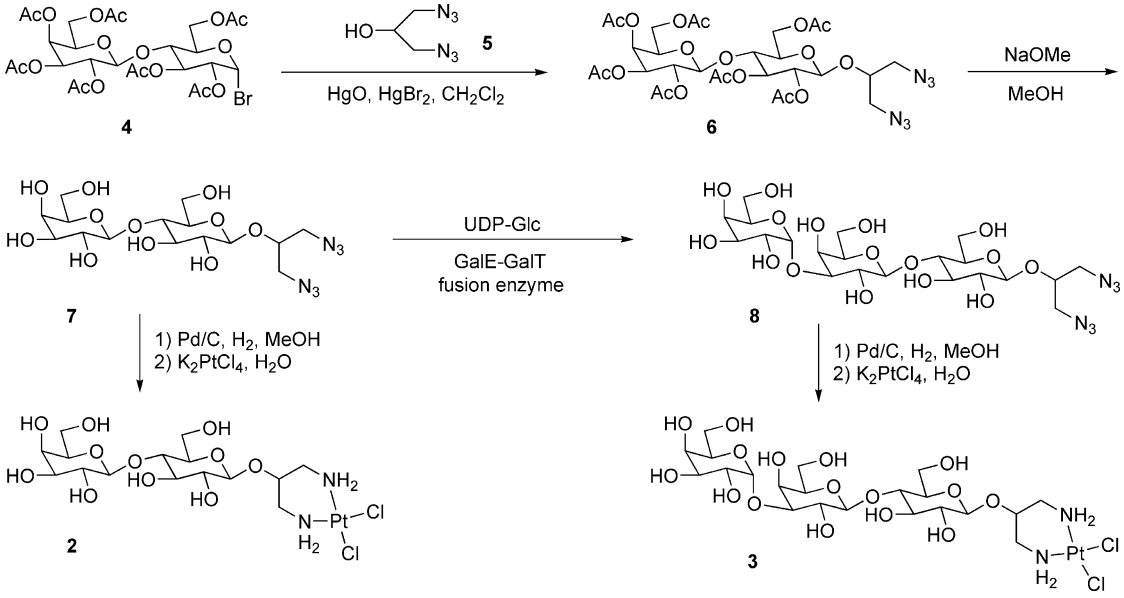


(Structures 1–3.)



Scheme 1.

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ), apparent multiplicities and coupling constants in Hz for compounds **1**, **2** and **3**

	^{1}H		^{13}C	
	From sugar	From linker	From sugar	From linker
1 [a]	4.39 (d, 8.0, 1 H), 3.70 (dd, 12.5, 2.0,	4.23 (m, 1 H)	102.2, 76.5, 76.2, 73.7, 70.1,	74.1
	1 H), 3.55 (dd, 12.5, 5.0, 1 H), 3.34–	2.86 (m, 2 H)	61.2	46.2
	3.23 (m, 3 H), 3.17 (dd, 9.5, 8.0, 1 H)	2.68 (m, 2 H)		45.1
2	4.57 (d, 7.5, 1 H), 4.41 (d, 8.0, 1 H),	4.38 (m, 1 H)	103.7, 102.2, 78.9, 76.1,	74.2
	3.88 (m, 2 H), 3.78–3.58 (m, 8H), 3.47	3.00 (m, 2 H)	75.5, 74.9, 73.5, 73.3, 71.8,	46.1
	(t, 8.8, 1 H), 3.35 (t, 7.8, 1 H)	2.82 (m, 2 H)	69.4, 61.9, 60.7	45.1
3	4.99 (d, 3.5, 1 H), 4.46 (d, 8.0, 1 H),	4.26 (m, 1 H)	103.5, 102.1, 96.1, 78.9,	74.2
	4.38 (d, 8.0, 1 H), 4.05–4.03 (m, 2 H),	2.90 (m, 2 H)	77.8, 75.7, 75.3, 74.9, 73.3,	45.8
	3.87 (m, 1 H), 3.81–3.78 (m, 2 H), 3.72–	2.70 (m, 2 H)	71.5, 70.2, 69.9, 69.8, 68.8,	
	3.46 (m, 12 H), 3.25 (t, 8.5, 1 H)		65.5, 61.6(5), 61.5(7), 60.5	44.8

[a] Data from Ref. 10.

The glycosyl acceptor **5** was obtained from commercially available 1,3-dibromo-2-propanol by reaction with sodium azide. Glycosylation of **5** with peracetylated lactosyl bromide **4** was promoted by $HgO/HgBr_2$ in CH_2Cl_2 to give glycoside **6**, and subsequent deacetylation under Zemplén conditions afforded compound **7**. After hydrogenation, the resultant diamino ligand was treated with equimolar potassium tetrachloroplatinate (K_2PtCl_4) in water to give **2**, which remained dissolved in the reaction solution and was isolated by a gel-filtration column using Bio-Gel P2 resin (67% yield, Scheme 1).

An enzymatic galactosylation[11] approach to the diazido-αGal epitope **8** was employed in the synthesis of α-Gal-cisplatin **3**. Our laboratory recently developed recombinant fusion proteins which join the UDP-Gal 4-epimerase and α-(1→3)-galactosyltransferase (α1,3GalT) together.[12] UDP-Glc was then used as the donor to avoid the high cost associated with the direct use of UDP-Gal. α-Gal trisaccharide **8** was obtained in a comparable 57% yield after gel filtration. Again **8** was hydrogenated to give the trisaccharide chelate ligand, and coordination with potassium tetrachloroplatinate in water afforded α-Gal-cisplatin **3**, which was also purified by a gel-filtration column (84%).

Although crystallization of complexes **2** and **3** has not been successful, the complex formations were confirmed by comparison of NMR spectra (^1H, ^{13}C) with those of gluco-cisplatin (Table 1), by HRMS of **2** (Anal. Calcd for $[MK]^+$ 719.0513. Found 719.0506) and **3** (Anal. Calcd for $[MK]^+$ 881.1041. Found 881.1055) and by ^{195}Pt NMR spectra (δ −2329.110 and −2330.445, respectively). Characteristic NMR peaks, which are consistent with those for gluco-cisplatin (**1**), occur at δ 4.38, 3.00, 2.82 (^1H) and 74.2, 46.1, 45.1 (^{13}C) for complex **2**, and at δ 4.26, 2.90, 2.70 (^1H) and 74.2, 45.8, 44.8 (^{13}C) for complex **3**. The ^{195}Pt NMR chemical shifts for complex **2** and complex **3** were also consistent for cis-coordinated nitrogen Pt (II) species.

In summary, we have shown an expeditious route to the synthesis of two carbohydrate-linked cisplatin analogs. With the use of a unique fusion enzyme, the methodology provides considerably accelerated access to trisaccharide complexes such as **3**.

1. Experimental

General methods.—^1H and ^{13}C NMR spectra were recorded on a Varian VXR 400 MHz or a Varian Unity 500 MHz spectrometer. ^{195}Pt NMR experiments were performed on a Varian Unity 500 MHz spectrometer and referenced to an external sample of 0.1 M K_2PtCl_4 in D_2O (chemical shift δ −1623 ppm vs. Na_2PtCl_6, resonating at δ 0 ppm). Mass spectra were run at the mass spectrometry facility at Wayne State University or University of California at Irvine. Thin-layer chromatography (TLC) utilized precoated Silica Gel-60 F_{254} (E. Merck), with detection by charring with 5% H_2SO_4 in MeOH. Liquid column chromatography was performed with J.T. Baker silica gel (40 μm).

1,3-Diazido-2-propyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**6**).—Heptaacetyl α-D-lactosyl bromide (**4**, 1.24 g, 1.77 mmol) and 1,3-diazido-2-propanol (**5**, 0.5 g, 3.54 mmol) were added to a previously flame-dried flask containing 2 g of 4 Å molecular sieves (MS) and anhyd CH_2Cl_2 (30 mL). After the resulting suspension was stirred for 30 min, HgO (0.38 g, 1.77 mmol) and $HgBr_2$ (0.13 g, 0.35 mmol) were added. The mixture was then stirred in the dark at ambient temperature for 48 h, passed through a Celite-packed glass funnel, and washed with satd aq $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography using silica gel (1:1 EtOAc–hexane) to afford **6** (0.71 g, 53%) as a crystalline solid: ^1H NMR (500 MHz, $CDCl_3$): δ 5.28 (d, *J* 2.5 Hz, 1 H), 5.14 (t, *J* 9.2 Hz, 1 H), 5.04 (dd, *J* 10.2, 7.8 Hz, 1 H), 4.91 (dd, *J* 10.5, 3.5 Hz, 1 H), 4.85 (dd, *J* 9.5, 8.0 Hz, 1 H), 4.64 (d, *J* 8.0 Hz, 1 H), 4.56 (dd, *J* 12.2, 1.8 Hz, 1 H), 4.47 (d, *J* 8.0 Hz, 1 H), 4.08–3.98 (m, 3 H), 3.85–3.74 (m, 3 H), 3.57 (m, 1 H), 3.45 (dd, *J* 13.0, 5.0 Hz, 1 H), 3.34 (dd, *J* 13.2, 7.2 Hz, 1 H), 3.30–3.23 (m, 2 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 × 3 H), 1.90 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.9(9), 170.9(5), 170.8, 170.7, 170.4, 170.3, 169.7, 101.7, 101.3, 79.0, 76.6, 73.5 (m), 72.3, 71.6, 71.3, 69.8, 67.3, 62.0, 61.5, 53.1, 52.9, 21.5, 21.4, 21.3(6), 21.3 (m), 21.1.

1,3-Diazido-2-propyl β-D-*galactopyranosyl*-(1→4)-β-D-*glucopyranoside* (**7**).—To a solution of **6** (0.708 g, 0.93 mmol) in abs MeOH (30 mL) was added sodium methoxide to adjust the pH to 9. The mixture was stirred overnight at rt and then neutralized with Dowex 50WX2-100 (H^+) resin. The resin was filtered off, and the filtrate was concentrated to give **7** (0.42 g, 97%) as a crystalline solid: ^1H NMR (500 MHz, D_2O): δ 4.48 (d, *J* 7.5 Hz, 1 H), 4.29 (d, *J* 7.5 Hz, 1 H), 3.99 (m, 1 H), 3.84–3.19 (m, 16 H); ^{13}C NMR (125 MHz, D_2O): δ 103.6, 102.4, 78.9, 77.5, 76.0, 75.5, 75.0, 73.5, 73.2, 71.6, 69.2, 61.7, 60.7, 52.7, 52.2.

cis-*Dichloro[2-(*β-D-*galactopyranosyl*-(1→4)-β-D-*glucopyranosidyl)propane-1,3-diamino]platinum* (**2**).—A solution of **7** (0.14 g, 0.3 mmol) in MeOH was hydrogenated overnight at rt and 45 psi in the presence of Pd/C (40 mg). The mixture was filtered through a pad of Celite, and the MeOH was removed under reduced pressure. The residue was redissolved in water and lyophilized to give the diamino compound (0.12 g, 96%). A solution of the diamino compound (103 mg,

0.25 mmol) and K_2PtCl_4 (103 mg, 0.25 mmol) in water (5 mL) was stirred at rt for 2 days and concentrated in vacuo. Chromatography of the residue on a gel-filtration column with Bio-Gel P2 afforded **2** (114 mg, 67%). ^{1}H NMR (500 MHz, D_2O): δ 4.57 (d, J 7.5 Hz, 1 H), 4.41 (d, J 8.0 Hz, 1 H), 4.38 (m, 1 H), 3.88 (m, 2 H), 3.78–3.58 (m, 8 H), 3.47 (t, J 8.8 Hz, 1 H), 3.35 (t, J 7.8 Hz, 1 H), 3.01–2.98 (m, 2 H), 2.85–2.79 (m 2 H); ^{13}C NMR (125 MHz, D_2O): δ 103.7, 102.2, 78.9, 76.1, 75.5, 74.9, 74.2, 73.5, 73.3, 71.8, 69.4, 61.9, 60.7, 46.1, 45.1; ^{195}Pt NMR (107.1 MHz): δ −2329.110; HR MALDI m/z [M + K$^+$] Anal. Calcd for $C_{15}H_{30}Cl_2KN_2PtO_{11}$: 719.0513. Found 719.0506.

1,3-Diazido-2-propyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**8**).—To a mixture of **7** (90 mg, 0.19 mmol), UDP-Glc (118 mg, 0.19 mmol), bovine serum albumin (BSA) (0.1%) and $MnCl_2$ (10 mM) in Tris–HCl (100 mM, pH 7.0, 3.8 mL) was added fusion enzyme GalE-GalT (1 U). The reaction mixture was shaken gently for 3 days at rt and then passed through a chloride anion-exchange column [Dowex 1 (Cl^-)]. The elute was concentrated and purified by gel-permeation chromatography on Bio-Gel P2 with doubly distilled water to give **8** (70 mg, 57%) as a white solid: ^{1}H NMR (400 MHz, D_2O): δ 5.13 (d, J 3.2 Hz, 1 H), 4.64 (d, J 8.0 Hz, 1 H), 4.52 (d, J 8.0 Hz, 1 H), 4.20–4.13 (m, 3 H), 4.00–3.92 (m, 3 H), 3.86–3.58 (m, 14 H), 3.53–3.46 (m, 2 H), 3.35 (t, J 8.2 Hz, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 103.0, 101.9, 95.6, 78.7, 77.4, 77.0, 75.2, 75.0, 74.6, 73.0, 71.0, 69.8, 69.5, 69.3, 68.4, 65.0, 61.2, 61.1, 60.3, 52.2, 51.8; HR FABMS m/z [M + Na$^+$] Anal. Calcd 651.2085. Found 651.2114.

cis-*Dichloro[2-(α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosidyl)propane-1,3-diamino]platinum* (**3**).—A solution of **8** (69 mg, 0.11 mmol) in MeOH was hydrogenated overnight at rt and 36 psi in the presence of Pd/C (20 mg). The mixture was filtered through a pad of Celite, and the MeOH was removed under reduced pressure. The residue was redissolved in water and lyophilized to give the diamino compound (63 mg, 99%). A solution of the diamino compound (63 mg, 0.11 mmol) and K_2PtCl_4 (46 mg, 0.11 mmol) in water (4 mL) was stirred at rt for 2 days and concentrated in vacuo. Chromatography of the residue on a gel-filtration column with Bio-Gel P2 afforded **3** (78 mg, 84%); ^{1}H NMR (500 MHz, D_2O): δ 4.99 (d, J 3.5 Hz, 1 H), 4.46 (d, J 8.0 Hz, 1 H), 4.38 (d, J 8.0 Hz, 1 H), 4.26 (m, 1 H), 4.05–4.03 (m, 2 H), 3.87 (m, 1 H), 3.81–3.78 (m, 2 H), 3.72–3.46 (m, 12 H), 3.25 (t, J 8.5 Hz, 1 H), 2.93–2.87 (m, 2 H), 2.74–2.67 (m, 2 H); ^{13}C NMR (125 MHz, D_2O): δ 103.5, 102.1, 96.1, 78.9, 77.8, 75.7, 75.3, 74.9, 74.2, 73.3, 71.5, 70.2, 69.9, 69.8, 68.8, 65.5, 61.6(5), 61.5(7), 60.5, 45.8, 44.8; ^{195}Pt NMR (107.1 MHz): δ −2330.445; HR MALDI m/z [M + K$^+$] Anal. Calcd for $C_{21}H_{40}Cl_2KN_2PtO_{16}$: 881.1041; Found 881.1055.

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