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Preparation of diazenecarboxamide–carboplatin conjugates by click chemistry

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ABSTRACT

An alternative, mild and highly efficient synthetic approach to platinum complexes with bioactive carrier ligands features a platinum-complex-tolerant copper(I)-catalyzed 1,3-dipolar cycloaddition. As demonstrated by the preparation of novel diazenecarboxamide–carboplatin conjugates, this approach is superior to other methodologies.

1. Introduction

Hybrid molecules with a dual mode of action are receiving considerable interest in the area of drug discovery [1]. An important example in this context is the development of platinum complexes that have bioactive carrier ligands. These ligands are capable of modulating the resistances that tumour cells acquire during chemotherapy. This effect has recently been illustrated by ethacraplatin, where the glutathione-S-transferase (GST)-inhibitor, ethacrynic acid, was attached to platinum [2] and the platinum complexes of nitrofuran carbohydrazide, a human thioredoxin reductase inhibitor [3]. In connection with this we have recently observed that diazenecarboxamides [4] are powerful modulators of intracellular glutathione (GSH) and show promise as bioactive ligands to platinum in anticancer treatments [5,6]. It should be noted that the tumour resistances are multifunctional processes in which GSH and GST often constitute the main cellular defence. Thus, a need for convenient, synthetic methodologies to access different conjugates with platinum complexes has emerged.

A general scheme to access the above platinum hybrid molecule involves the synthesis of a bioactive, organic scaffold, which is functionalized with a mono- or polydentate coordination site through a suitable linker. This then serves as an organic host for a guest metal ion. In the final step of the reaction sequence, the latter is appended to the metal (Scheme 1, *Strategy a*). However, this approach has several disadvantages: first, it is linear and, second, it cannot be used for the preparation of complexes that should consist of fragile organic scaffolds, which do not tolerate the potentially forcing reaction conditions required in the final coordination step.

One of the promising alternatives to the above is a nonlinear approach in which both the organic scaffold and the desired metal complex are preassembled and then, using an appropriate 'click' [7] reaction, subsequently combined into the final target molecules (Scheme 1, *Strategy b*).

Cu(I)-catalyzed terminal alkyne–azide cycloaddition (CuAAC) has recently emerged as a powerful 'click' reaction, known for its exquisite selectivity and high yields [8,9]. There are, however, only a handful examples demonstrating CuAAC reactions with complexes of different transition metals as substrates [10,11]. To the best of our knowledge, in platinum coordination spheres it has only been exercised with organoplatinum compounds [11].

2. Experimental

The reagents were purchased from Aldrich, whereas the $K_2[PtCl_4]$ was purchased from Alfa Aesar, and used as received. NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer at 302 K. Chemical shifts are reported in parts per million (ppm). ^{1}H NMR spectra were referenced to internal Me_4Si. ^{13}C NMR spectra are referenced to the residual DMSO-d_6 (39.5 ppm) or DMF-d_7 peak (30.1 ppm), unless otherwise noted. ^{195}Pt NMR spectra are referenced to $Na_2[PtCl_6]$ (325 mg/0.65 mL D_2O), as an external standard at 0 ppm. Coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on a Q-TOF Premier instrument. Infrared spectra were recorded on a BIO-RAD Excalibur Series spectrophotometer using samples in potassium bromide discs. Elemental analyses (C, H, N) were performed with a Perkin–Elmer 2400

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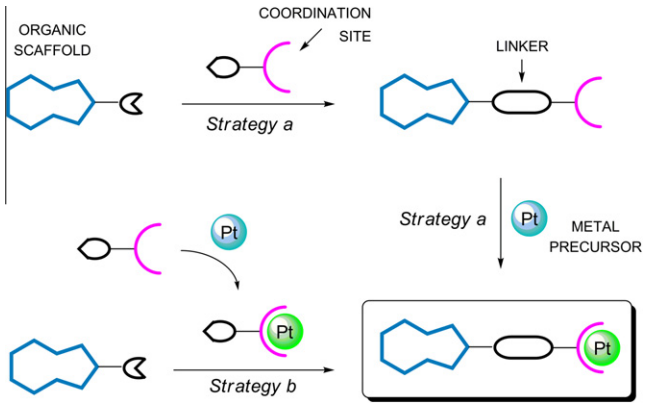


Scheme 1. Strategies to platinum complexes involving organic scaffold.

Series II CHNS/O Analyzer. Melting points were determined on a Kofler block and are uncorrected.

2.1. 2-Azidopropane-1,3-diamine dihydrochloride (*4*)

Into a solution of *tert*-butyl 2-azidopropane-1,3-diyldicarbamate [12] (315 mg, 1.00 mmol) in ethyl acetate (3 mL), aqueous hydrochloric acid (1.4 mL, 6 M) was added. The reaction mixture was stirred for 8 h and then chilled at 5 °C overnight. The resulting crystals were filtered off and washed several times with a few millilitres of ethyl acetate until the crystals were no longer sticky, to give the pure product **4** (175 mg, 93%) as white needles, mp 227–231 °C. δ_H (300 MHz, DMSO-d_6) 2.84–3.00 (m, 2H), 3.10–3.24 (m, 2H), 4.22–4.33 (m, 1H), 8.50 (br s, 6H). δ_C (75 MHz, DMSO-d_6) 40.9, 57.6. ν_{max}(cm^{-1}) 3000, 2135, 1473, 1281, 959. *Anal.* Calc. for $C_3H_{11}Cl_2N_5$: C, 19.16; H, 5.90; N, 37.24. Found: C, 19.12; H, 5.98; N, 36.91%.

2.2. cis-[Pt(2-azidopropane-1,3-diamine)Cl_2] (*2a*)

DBU (152 mg, 1.00 mmol) was added into a solution of **4** (94 mg, 0.50 mmol) in DMF (1 mL), followed by *cis*-[Pt(DMSO)$_2$Cl$_2$] [13] (224 mg, 0.50 mmol). The reaction mixture was stirred for 2 days. After the addition of water (2 mL) and cooling at 5 °C for a few hours, the precipitate was collected by filtration and washed with water (2 × 2 mL) to give **2a** (90 mg, 0.24 mmol) as a grey solid, mp >300 °C. δ_H (300 MHz, DMF-d_7) 2.80–3.10 (m, 4H), 4.18–4.40 (m, 1H), 5.00–5.48 (m, 4H). δ_C (75 MHz, DMF-d_7) 54.9, 59.4. δ_{Pt} (64 MHz, DMF-d_7) −2286. ν_{max}(cm^{-1}) 3234, 3123, 2108, 1587, 1259. *m/z* (ESI+) 403.7 ([M+Na]$^+$, 100), 346.7 (50). HRMS (ESI−) Calc. for $C_3H_8{}^{35}Cl_2N_5{}^{194}Pt^-$ [M−H]$^-$: 377.9784. Found: 377.9781. *Anal.* Calc. for $C_3H_9Cl_2N_5Pt$: C, 9.45; H, 2.38; N, 18.38. Found: C, 9.83; H, 2.76; N, 17.99.

2.3. cis-[Pt(2-azidopropane-1,3-diamine)(CBDCA)] (*2b*)

To a solution of $K_2[PtCl_4]$ (2.08 g, 5.00 mmol) in water (35 mL) was added KI (8.30 g, 50.0 mmol) and the reaction mixture was stirred for 1 h. A solution of **4** (940 mg, 5.00 mmol) in aqueous NaOH (400 mg, 10.0 mmol in 7.5 mL of water) was added dropwise to the reaction mixture, which was then stirred at 60 °C for 5 min. The reaction mixture was chilled to 0 °C and the precipitated brown solid was filtered off and washed successively with small amounts of ice-cold water, methanol and diethyl ether to give *cis*-[Pt(2-azidopropane-1,3-diamine)I_2] (2.74 g, 4.86 mmol, 97% yield from $K_2[PtCl_4]$). This compound was suspended in water (100 mL) and a water solution of $AgNO_3$ (1.53 g, 9.00 mmol in 15 mL of water) was added dropwise, under stirring. The stirring was continued for 3 h. The produced silver iodide was removed by filtration. To the filtrate was added a water (8 mL) solution of 1,1-cyclobutanedicarboxylic acid (649 mg, 4.50 mmol) and NaOH (360 mg, 9.00 mmol). The reaction mixture was stirred overnight, filtered and the solvent of the filtrate was removed on a rotary evaporator. The residue was suspended in water (5 mL) and chilled on an ice bath. A grey solid precipitated, which was collected by filtration and dried to afford pure **2b** (1.51 g, 3.34 mmol, 67% from $K_2[PtCl_4]$), mp >300 °C (H_2O). δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.6, 2H), 2.45–2.75 (m, 8H), 3.91 (m, 1H), 5.20–5.50 (m, 4H, D_2O exchangeable). δ_C (75 MHz, D_2O, 1,4-dioxane as external standard at δ 66.7 ppm) 15.4, 30.4, 31.9, 45.1, 56.4, 58.4, 181.9. δ_{Pt} (64 MHz, D_2O) −1954. ν_{max}(cm^{-1}) 3175, 3100, 2101, 1602, 1380, 1255. *m/z* (ESI+) 475.1 ([M+Na]$^+$, 100), 453.1 ([M+H]$^+$, 28), 396.1 (62), 222.1 (94), 94.1 (50). HRMS (ESI+) Calc. for $C_9H_{16}N_5O_4{}^{195}Pt^+$ [M+H]$^+$: 453.0850. Found: 453.0863.

2.4. General procedure for the preparation of *3*

A mixture of the appropriate propargyl-appended diazenecarboxamide **1** (0.250 mmol), *cis*-[Pt(2-azidopropane-1,3-diamine)Cl_2] (**2a**, 113 mg, 0.250 mmol), $CuSO_4{\cdot}5H_2O$ (6.2 mg 0.025 mmol, 10 mol%) and granular copper (60.3 mg, 0.950 mmol, 3.8 equiv.) in DMF (1 mL) was stirred at room temperature for the time indicated in Table 1. *Isolation procedure for **3a–c,e,f,h,i,k**:* Two drops of saturated aqueous ammonium hydrogen carbonate solution were added to the reaction mixture. The resulting mixture was stirred for a few minutes and subjected to column chromatography on silica gel. After an initial elution with a mixture of dichloromethane and methanol (in a ratio of 10:1, followed by 1:1) to remove the DMF, pure product was isolated by subsequent elution with methanol. *Isolation procedure for **3d,g,j**:* The reaction mixture was diluted with DMF (10 mL) and ammonium hydrogen carbonate (70 mg) was added. The mixture was heated at the boiling point until the product dissolved. After filtration through a pad of Celite the filtrate was concentrated on a rotary evaporator (to ca. 0.5 mL) and cooled down to room temperature. The product was precipitated by the addition of methanol (20 mL), collected by filtration and washed with methanol (2 mL) and diethyl ether (2 mL). Pure products **3** were obtained by these two methods in the yields indicated in Table 1.

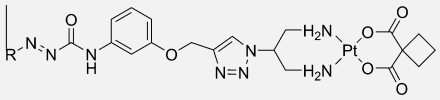
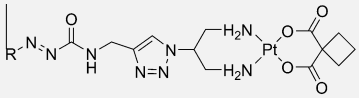
2.4.1. Complex *3a*

Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.6, 2H), 2.57–2.78 (m, 6H), 2.89–3.05 (m, 2H), 4.66 (br t, *J* 9.0, 1H), 5.16 (s, 2H), 5.40–5.60 (m, 2H), 5.78–5.93 (m, 2H), 6.85–6.96 (m, 1H), 7.28–7.38 (m, 2H), 7.47 (br s, 1H), 8.05 (d, *J* 8.6, 2H), 8.12 (d, *J* 8.6, 2H), 8.39 (s, 1H), 11.06 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 47.4, 55.6, 58.0, 61.1, 106.4, 110.5, 112.3, 123.4 (q, *J* 273), 123.5, 123.7, 126.9 (q, *J* 3.8), 130.0, 132.5 (q, *J* 31.7), 138.9, 142.9, 153.2, 158.4, 159.3, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1958. ν_{max}(cm^{-1}) 3140, 1735, 1613, 1335, 1122, 852. *m/z* (ESI−) 798.2 ([M−H]$^-$, 100). HRMS (ESI−) Calc. for $C_{26}H_{26}F_3N_8O_6{}^{194}Pt^-$ ([M−H]$^-$): 797.1554. Found: 797.1564. *Anal.* Calc. for $C_{26}H_{27}F_3N_8O_6Pt$: C, 39.05; H, 3.40; N, 14.01. Found: C, 38.71; H, 3.51; N, 13.62.

2.4.2. Complex *3b*

Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.70 (quintet, *J* 7.7, 2H), 2.60–2.75 (m, 6H), 2.86–3.05 (m, 2H), 4.65 (br t, *J* 10.5, 1H), 5.15 (s, 2H), 5.40–5.60 (m, 2H), 5.75–6.92 (m, 2H), 6.88–6.98 (m, 1H), 7.30–7.40 (m, 2H), 7.48 (s, 1H), 8.48 (s, 1H), 11.36 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 47.4, 55.6, 58.0, 61.2, 106.5, 110.9, 112.5, 123.5, 130.0, 138.7, 142.9, 158.1, 158.4, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3430, 3205, 1648, 1611, 1518, 1030. *m/z* (ESI−) 820.1 ([M−H]$^-$, 100), 624.1 (96), 409.1 (90). HRMS (ESI−) Calc. for $C_{25}H_{25}F_5N_8O_6{}^{194}Pt^-$ [M−H]$^-$: 819.1209. Found: 819.1215.

Table 1
Diazenecarboxamide–platinum conjugates **3a–k** (Scheme 2).

Entry	Reaction time (h)	Product **3**	R	Yield[a] (%)
N-Aryl analogues				
1	3	**3a**	4-CF_3–C_6H_4	65
2	3.5	**3b**	C_6F_5	49
3	5	**3c**	4-Cl–C_6H_4	65
4	3.5	**3d**	4-NO_2–C_6H_4	41
N-Alkyl analogues				
5	3	**3e**	4-CF_3–C_6H_4	81
6	2	**3f**	4-Cl–C_6H_4	84
7	12	**3g**	4-NO_2–C_6H_4	47
8	3	**3h**	4-CH_3–C_6H_4	75
9	6	**3i**	4-CH_3O–C_6H_4	93
10	12	**3j**	C_6H_5	44
11	3	**3k**	4-F–C_6H_4	73

[a] Pure product.

*2.4.3. Complex **3c***

Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.6, 2H), 2.57–2.78 (m, 6H), 2.89–3.05 (m, 2H), 4.66 (br t, *J* 10.3, 1H), 5.15 (s, 2H), 5.40–5.60 (m, 2H), 5.78–5.92 (m, 2H), 6.85–6.96 (m, 1H), 7.28–7.38 (m, 2H), 7.46 (br s, 1H), 7.75 (d, *J* 7.9, 2H), 7.97 (d, *J* 7.9, 2H), 8.39 (s, 1H), 10.96 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 47.4, 55.6, 58.0, 61.1, 106.3, 110.4, 112.3, 123.4, 124.8, 129.9, 138.2, 139.0, 143.0, 149.7, 158.4, 159.6, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1959. ν_{max}(cm^{-1}) 3142, 1614, 1595, 1386. *m/z* (ESI−) 365.1 ($[M]^-$, 100), 582.7 (30). HRMS (ESI−) Calc. for $C_{25}H_{27}{}^{35}ClN_8O_6{}^{194}Pt^-$ $[M-H]^-$: 763.1291. Found: 763.1300. *Anal.* Calc. for $C_{25}H_{27}ClN_8O_6Pt$: C, 39.20; H, 3.55; N, 14.63. Found: C, 38.81; H, 3.55; N, 14.33.

*2.4.4. Complex **3d***

Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.69 (quintet, *J* 7.5, 2H), 2.60–2.77 (m, 6H), 2.90–3.06 (m, 2H), 4.66 (br t, *J* 10.2, 1H), 5.15 (s, 2H), 5.40–5.60 (m, 2H), 5.77–5.92 (m, 2H), 6.90 (br s, 1H), 7.30–7.40 (m, 2H), 7.46 (br s, 1H), 8.15 (br s, 2H), 8.38 (s, 1H), 8.50 (br s, 2H), 11.14 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 47.4, 55.6, 58.0, 61.2, 123.4, 142.9, 177.3. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3441, 3096, 1737, 1632, 1611, 1375, 1347. *m/z* (ESI−) 776.2 ($[M]^-$, 100), 624.1 (74), 461.1 (40), 341.1 (71). HRMS (ESI−) Calc. for $C_{25}H_{26}N_9O_8{}^{194}Pt^-$ $[M-H]^-$: 774.1531. Found: 774.1557.

*2.4.5. Complex **3e***

Ochre solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 9.0, 2H), 2.54–2.75 (m, 6H), 2.85–3.05 (m, 2H), 4.53 (d, *J* 6.0, 2H), 4.60 (br t, *J* 9.0, 1H), 5.40–5.60 (m, 2H), 5.77–5.92 (m, 2H), 8.01 (s, 4H), 8.22 (s, 1H), 9.21 (br t, *J* 6.0, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.2, 30.5, 35.3, 47.4, 55.5, 57.9, 122.1, 123.4, 123.6 (q, *J* 273), 126.8 (q, *J* 3.9), 132.2 (q, *J* 31.1), 144.0, 153.2, 162.2, 177.3. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3221, 1710, 1629, 1325, 1129, 1065, 854. *m/z* (ESI+) 730.1 ($[M+Na]^+$, 100), 708.1 ($[M+H]^+$, 17). HRMS (ESI+) Calc. for $C_{20}H_{23}F_3N_8O_5{}^{194}PtNa^+$ $[M+Na]^+$: 730.1289. Found: 730.1296.

*2.4.6. Complex **3f***

Ochre solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.9, 2H), 2.54–2.77 (m, 6H), 2.85–3.05 (m, 2H), 4.50 (d, *J* 6.0, 2H), 4.66 (br t, *J* 9.0, 1H), 5.35–5.51 (m, 2H), 5.75–5.95 (m, 2H), 7.70 (d, *J* 8.7, 2H), 7.85 (d, *J* 8.7, 2H), 8.20 (s, 1H), 9.11 (br t, *J* 5.8, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 35.4, 47.4, 55.6, 58.0, 122.1, 124.6, 129.8, 137.9, 144.2, 149.6, 162.4, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3164, 1721, 1659, 1490, 1366, 845. *m/z* (ESI+) 697.1 ($[M+Na]^+$, 100), 675.1 ($[M+H]^+$, 13), 638.6 (23). HRMS (ESI+) Calc. for $C_{19}H_{23}ClN_8O_5{}^{194}PtNa^+$ $[M+Na]^+$: 696.1025. Found: 696.1044.

*2.4.7. Complex **3g***

Dark brown solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.2, 2H), 2.55–2.80 (m, 6H), 2.85–3.05 (m, 2H), 4.53 (d, *J* 5.4, 2H), 4.66 (br s, 1H), 5.35–5.60 (m, 2H), 5.75–5.95 (m, 2H), 8.03 (d, *J* 8.7, 2H), 8.22 (s, 1H), 8.45 (d, *J* 8.7, 2H), 9.25 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.5, 35.4, 47.4, 55.6, 58.0, 122.2, 123.8, 125.2, 144.0, 149.6, 154.2, 162.1, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1958. ν_{max}(cm^{-1}) 3181, 1717, 1651, 1621, 1527, 1346, 864. *m/z* (ESI+) 707.1 ($[M+Na]^+$, 30), 385.1 ($[M+H]^+$, 8), 325.2 (66), 234.2 (74), 231.1 (65), 114.0 (100). HRMS (ESI+) Calc. for $C_{19}H_{23}N_9O_7Na^{195}Pt^+$ $[M+Na]^+$: 707.1266. Found: 707.1288.

*2.4.8. Complex **3h***

Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 9.0, 2H), 2.42 (s, 3H), 2.54–2.77 (m, 6H), 2.85–3.05 (m, 2H), 4.50 (d, *J* 6.0, 2H), 4.63 (br t, *J* 9.0, 1H), 5.35–5.51 (m, 2H), 5.75–5.95 (m, 2H), 7.73 (d, *J* 8.0, 2H), 7.75 (d, *J* 8.0, 2H), 8.19 (s, 1H), 9.02 (br t, *J* 6.0, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 21.1, 30.3, 30.6, 38.7, 47.5, 55.6, 58.0, 122.1, 123.0, 130.1, 143.9, 144.4, 149.2, 162.7, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3211, 1714, 1635, 1368, 1154. *m/z* (ESI+) 676.2 ($[M+Na]^+$, 100), 654.2 ($[M+H]^+$, 61), 216.1 (51). HRMS (ESI+) Calc. for $C_{20}H_{27}N_8O_5{}^{194}Pt^+$ $[M+H]^+$: 654.1752. Found: 654.1774. *Anal.* Calc. for $C_{20}H_{26}N_8O_5Pt$: C, 36.76; H, 4.01; N, 17.15. Found: C, 36.71; H, 3.98; N, 16.88.

*2.4.9. Complex **3i***

Ochre solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.3, 2H), 2.54–2.77 (m, 6H), 2.85–3.05 (m, 2H), 3.88 (s, 3H), 4.49 (d, *J* 5.4, 2H), 4.62 (br s, 1H), 5.35–5.66 (m, 2H), 5.75–5.96 (m, 2H), 7.15 (d, *J* 8.9, 2H), 7.86 (d, *J* 8.9, 2H), 8.18 (s, 1H), 8.97 (br t, *J* 5.0, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.6, 35.4, 47.4, 55.6, 55.8, 57.9, 114.8, 122.1, 125.3, 144.5, 145.2, 162.6, 163.4, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1958. ν_{max}(cm^{-1}) 3220, 3154, 1656, 1626, 1502, 1258, 1145. *m/z* (ESI+) 692.2 ($[M+Na]^+$, 100), 670.2

([M+H]$^+$, 19), 216.1 (33), 94.1 (32). HRMS (ESI+) Calc. for $C_{20}H_{27}N_8O_6{}^{194}Pt^+$ [M+H]$^+$: 670.1701. Found: 670.1716.

2.4.10. Complex ***3j***

Dark brown solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 8.0, 2H), 2.40–2.66 (m, 6H), 2.88–3.08 (m, 2H), 4.50 (d, *J* 5.8, 2H), 4.62 (br t, *J* 10.8, 1H), 5.40–5.55 (m, 2H), 5.75–5.94 (m, 2H), 7.58–7.70 (m, 3H), 7.82–7.89 (m, 2H), 8.20 (s, 1H), 9.07 (br t, *J* 5.6, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.6, 35.4, 47.4, 55.6, 58.0, 122.1, 122.8, 129.6, 133.2, 144.3, 151.1, 162.7, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1957. ν_{max}(cm^{-1}) 3442, 3204, 2950, 1715, 1661, 1632, 1362. *m/z* (ESI+) 662.1 ([M+Na]$^+$, 12), 344.1 (100). HRMS (ESI+) Calc. for $C_{19}H_{25}N_8O_5{}^{195}Pt^+$ [M+H]$^+$: 640.1596. Found: 460.1600.

2.4.11. Complex ***3k***

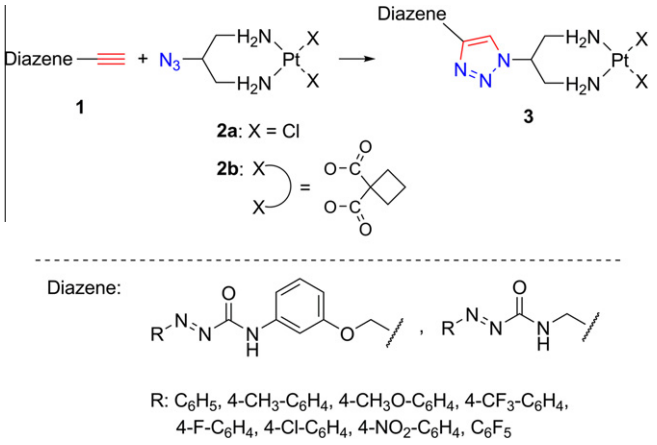
Orange solid, mp >300 °C. δ_H (300 MHz, DMSO-d_6) 1.67 (quintet, *J* 7.6, 2H), 2.55–2.77 (m, 6H), 2.88–3.06 (m, 2H), 4.50 (s, 2H), 4.64 (br t, *J* 9.0, 1H), 5.40–5.62 (m, 2H), 5.75–5.95 (m, 2H), 7.47 (d, *J* 8.8, 2H), 7.88–7.99 (m, 2H), 8.20 (s, 1H), 9.09 (br s, 1H). δ_C (75 MHz, DMSO-d_6) 15.0, 30.3, 30.6, 35.4, 47.5, 55.6, 57.9, 116.6, 116.9, 122.1, 125.4, 125.6, 144.3, 147.8, 162.5, 177.4. δ_{Pt} (64 MHz, DMSO-d_6) −1958. ν_{max}(cm^{-1}) 3460, 3159, 1717, 1655, 1628, 1501, 1364, 1226, 1138, 850. *m/z* (ESI−) 656.1 ([M−H]$^-$, 100). HRMS (ESI−) Calc. for $C_{19}H_{22}FN_8O_5{}^{194}Pt^-$ [M−H]$^-$: 655.1324. Found: 655.1340.

3. Results and discussion

Our plan for the preparation of diazenecarboxamide–platinum conjugates, as introduced in Scheme 1 *Strategy b*, is depicted in Scheme 2. Relatively inert terminal alkyne and azido moieties should be easily introduced into the organic molecules to afford the prerequisite diazenecarboxamides **1** and 2-azidopropane-1,3-diamine. The latter is coordinated to platinum(II) into **2**. In the key step both 'click' partners are combined by the CuAAC reaction into the target molecule **3**.

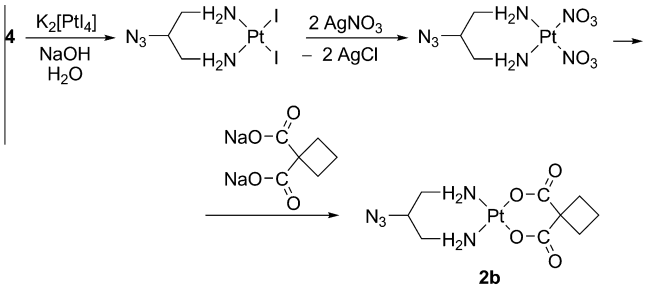
3.1. Propargyl-appended diazenecarboxamides

Two types of propargyl-appended diazenecarboxamides **1** were selected: *N*-aryl and *N*-alkyl. Their rational and synthesis have been described in [14,15].



Scheme 2. Synthesis of diazenecarboxamide (shortly "diazene")–platinum conjugates **3** by platinum tolerant CuAAC.

Scheme 3. Synthesis of cisplatin analogue **2a**.



Scheme 4. Synthesis of carboplatin analogue **2b**.

3.2. Azido-appended platinum(II) complexes

For the azido functionalized diamine bidentate ligand we selected 2-azidopropane-1,3-diamine dihydrochloride (**4**), which was obtained from *tert*-butyl 2-azidopropane-1,3-diyldicarbamate [12] by standard BOC deprotection chemistry using hydrochloric acid. Through this ligand, amine platinum(II) complexes of formulae [Pt(diamine)Cl$_2$] (**2a**) and [Pt(diamine)(CBDCA)] (**2b**, CBDCA = 1,1-cyclobutanedicarboxylate) were prepared.

The synthesis of **2a** was accomplished by the reaction of **4** with *cis*-[PtCl$_2$(DMSO)$_2$] [13] (DMSO = dimethyl sulfoxide) in the presence of 1,8-diazabiciclo[5.4.0]undek-7-en (DBU), albeit in a moderate 48% yield (Scheme 3). The ^{195}Pt NMR chemical shift for **2a** of δ −2286 ppm is consistent with the *cis*-diamino-dichloridoplatinum(II) complex [16]. With the exception of the DMSO and the *N*,*N*-dimethylformamide (DMF), the complex **2a** turned out to be virtually insoluble in water and in most common organic solvents, which diminishes its value in the synthesis of potential biologically active compounds. Thus, no attempts were made to improve its yield.

The preparation of a more soluble carboplatin analogue **2b** turned out to be less straightforward. In general, several methods for the synthesis of amine platinum(II) dicarboxylate complexes have been developed. As reported by Lin and co-workers [17], the most general protocols include (i) the reaction of the dimethyl sulfoxide platinum complex [Pt(DMSO)$_2$(dicarboxylate)] with diamines, (ii) the reaction of the dichloroplatinum complex [Pt(diamine)Cl$_2$] with the disilver salt of dicarboxylic acid and (iii) the transformation of the dichloridoplatinum complex [Pt(diamine)Cl$_2$] with silver nitrate into the dinitratoplatinum complex [Pt(diamine)(NO$_3$)$_2$], followed by a reaction with the disodium salt of dicarboxylic acid. Water was used as the reaction solvent in the above approaches.

We unsuccessfully employed different reaction conditions to react **4** with *cis*-[Pt(DMSO)$_2$(CBDCA)]. As subsequently reported by Lin and Pierpont [18], and more recently by the group of Navarro-Ranninger and co-workers [19], the reaction is strongly temperature and concentration dependent. Unfortunately, conducting the reaction between **4** and *cis*-[Pt(DMSO)$_2$(CBDCA)][1] at different temperatures and concentrations, as well as by employing different

[1] *cis*-[Pt(DMSO)$_2$(CBDCA)] was prepared as reported in Ref. [18].

bases to free-base the 2-azidopropane-1,3-diamine from its dihydrochloride salt **4**, only led to the formation of complex mixtures of unidentified products. Also unsuccessful were attempts to react **2a** with either the disilver salt of CBDCA or with silver nitrate. No product formation could be observed, which could be accounted for by the complete insolubility of **2a** in water.

Thus, we surmised that employing the more soluble iodine complex, *cis*-[Pt(2-azidopropane-1,3-diamine)I_2], instead of **2a** should be advantageous (Scheme 4). This diiodidoplatinum complex was prepared from **4** and potassium tetraiodoplatinate [20]. A subsequent reaction with silver nitrate generated the dinitrato complex, which upon the addition of the disodium salt of CBDCA afforded pure **2b** in an appreciable 67% overall yield from **4**. Compound **2b** exhibits the ^{195}Pt NMR chemical shift of δ −1954 ppm, typical for the [Pt(diamine)CBDCA] coordination [19].

3.3. CuAAC reaction

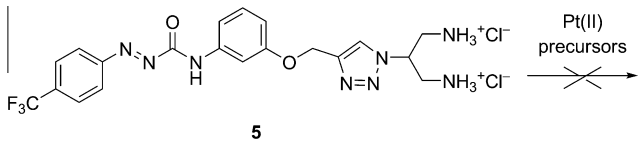
When attempting the CuAAC reaction in the presence of other metal complexes, several complications may arise, including redox reactions with redox-sensitive copper(I), and ligand-exchange reactions displacing the metal from the substrate complex by copper(I) or copper(II) species. These may lead to inhibition of the CuAAC reaction and product contamination, respectively.

In a classical CuAAC reaction the copper(I) catalyst is generated by the in situ reduction of copper(II) ions with ascorbic acid. Diazenecarboxamides, however, are sensitive to ascorbic acid and the problem of their interference in the redox process has previously been addressed by the use of a copper(0)/copper(II) couple. In addition, reaction solvents such as DMSO and DMF proved beneficial in the CuAAC reaction involving alkyne functionalized diazenecarboxamides as the reaction partners [14,15].

Adopting the above findings, in a typical procedure a mixture of alkyne **1**, azide **2b**, $CuSO_4{\cdot}5H_2O$ and granular copper in DMF was stirred in the presence of air for 3–12 h (Scheme 2). The crude reaction mixture was subjected to column chromatography on silica gel, which allowed facile removal of the DMF and isolation of the pure products **3** in good-to-excellent yields (Table 1). Moderate isolated yields were seen for the products **3d**,**g**,**j** (entries 4,7,10) with sparing solubility. An alternative chromatography-free isolation procedure in these instances was more appropriate, as described in Section 2. The addition of a saturated aqueous ammonium hydrogen carbonate solution to the reaction mixture, prior to the chromatography, coordinated the copper ions and improved the purification. Ammonium salts such as ammonium chloride are commonly used in the isolation procedures after CuAAC reactions to remove any adventitious copper ions from the products. For the synthesis of **3**, however, ammonium chloride was not appropriate, as it caused CBDCA exchange from the platinum. As a reaction solvent, DMSO worked similarly to DMF, but it could not be completely removed from the product using the above-mentioned chromatographic workup.

The ^{1}H and ^{13}C NMR spectra of the compounds **3a**–**k** featured resonances from both 'click' partners **1** and **2b**, along with the characteristic triazole H-5 proton (in the ^{1}H NMR spectra) appearing in the range of δ 8.18–8.39 ppm. The elemental analyses and/or mass spectra agreed with the suggested molecular formulae of **3**. By ^{195}Pt NMR spectroscopic analogy with **2b**, all the products **3** (δ −1957 to −1959 ppm) retained the [Pt(diamine)CBDCA] coordination sphere.

It is important to note that for the preparation of the diazenecarboxamide–platinum conjugates **3**, *Strategy b* is superior to *Strategy a* (Scheme 1). For example, attempts to coordinate compound **5** [14] to platinum(II) by using different platinum precursors and reaction conditions failed, including those described above for **2a** and **2b** (Scheme 5). As judged by the ^{1}H and ^{195}Pt NMR spectroscopy, complex mixtures of unidentified products were obtained. Under more forcing conditions, i.e., conducting the reactions at elevated temperatures, the diazenecarboxamide part of the molecule rapidly decomposed.



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Scheme 5. Attempts to coordinate diazenecarboxamide-appended propane-1,3-diamine to platinum(II) failed.

4. Conclusion

A mild and highly efficient synthetic route to diazenecarboxamide–carboplatin conjugates by copper-catalyzed 1,3-dipolar cycloaddition has been developed. The method complements the existing methodologies and can be extended to the preparation of other platinum complexes with potentially bioactive carrier ligands, especially for sensitive ligands. A screening of the subset of compounds **3** has been currently initiated against tumours, showing an activity similar to carboplatin.

Acknowledgements

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