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Synthesis and anti-cancer activity of dinuclear platinum(II) complexes containing bis(thioalkyl)dicarba-*closo*-dodecaborane(12) ligands†

Susan L. Woodhouse,[b] Erin J. Ziolkowski[a] and Louis M. Rendina*[a]

[a] *School of Chemistry, The University of Sydney, Sydney, NSW, 2006, Australia. E-mail: rendina@chem.usyd.edu.au; Fax: 61 2 9351 3329; Tel: 61 2 9351 4781*

[b] *School of Chemistry and Physics, The University of Adelaide, Adelaide, South Australia, 5005, Australia*

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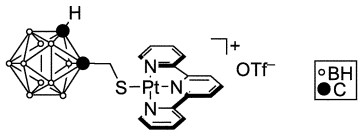
A series of novel, dinuclear (2,2′:6′,2″-terpyridine)-platinum(II) complexes containing bis(thioalkyl)-dicarba-*closo*-dodecaborane(12) (carborane) ligands were prepared and characterised, and their preliminary anti-cancer characteristics have been determined *in vitro*; the complexes are the first examples of bis-intercalator complexes containing a boron-rich carborane cage.

Platinum(II)–trpy (trpy = 2,2″:6′,2″-terpyridine) complexes have attracted considerable attention in recent years for their interesting biological properties, including anti-cancer activity.[1,2] Dinuclear platinum(II)–trpy complexes linked by simple dithioalkyl ligands with varying chain lengths have been shown to bifunctionally intercalate DNA with higher affinities for DNA over their mononuclear analogues.[2,3] Indeed, the linking of two DNA intercalating moieties to form a 'bis-intercalator' frequently increases the binding affinity of the species due to a decrease in the dissociation rate in comparison with the mono-intercalator.[3–9] Some bis-intercalators are also known to exhibit potent anti-cancer activity against numerous tumour cell lines.[5,10–14] In many cases, a variation in the rigidity or length of the linker between the two intercalating moieties within a bis-intercalator can alter the mode of binding and the ability of the compound to bis-intercalate.[5,8,9,15–17] In the case of diplatinum(II)–trpy complexes with short linkers, mono- or bis-intercalation is found to occur, with one base-pair separating the two intercalating chromophores in the latter. Intermediate-linked centres bifunctionally intercalate across a short range, such as two base-pairs. The longest linked metal centres tend to show mixed mono- and bis-intercalation. In the case of mono-intercalation, the non-intercalated chromophore may bind externally to the DNA or aggregate with a chromophore from an adjacent molecule.[2] As found for the corresponding mononuclear platinum(II)–trpy species, dinuclear complexes require a GC base pair at the intercalation site and display growth inhibition of L1210 murine leukaemia cells.[2] The mode of anti-cancer activity is thought to be due to DNA intercalation of the platinum(II)–trpy moiety and consequent interruption to cellular processes, action on topoisomerases[7,12,18,19] or disruption of the cell membrane causing cell lysis.[2]

Recent work in our group[20,21] has led to the development of a series of thioalkylcarborane ligands and their corresponding platinum(II)–trpy complexes for potential application in Boron Neutron Capture Therapy (BNCT).[22,23] These complexes, *e.g.* **1**, have demonstrated a propensity to intercalate calf-thymus DNA and display *in vitro* anti-cancer activity.[20,21] It was anticipated that the use of carboranes containing two platinum(II)–trpy chromophores would decrease the dissociation of the complexes from DNA in comparison with the mono-intercalators,[2,3] thereby allowing the compound to persist near the DNA for longer periods and thus maximising cellular damage by the products of thermal neutron capture involving the ^{10}B nucleus. Herein we report the synthesis of a series of novel bis(thioalkyl)carborane ligands and their dinuclear platinum(II)–trpy complexes, the first examples of potential bis-intercalator molecules containing a carborane. Preliminary *in vitro* anti-cancer screening of the complexes against L1210 leukaemia and its cisplatin-resistant variant (L1210/DDP) is also reported.



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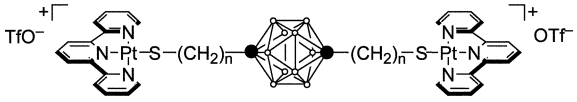

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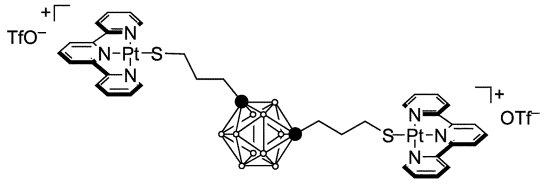

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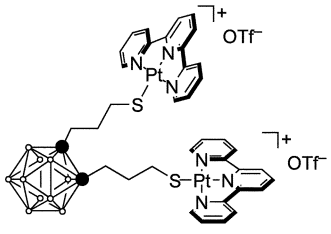

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There exists no precedent for the synthesis of the desired bridging bidentate bis(thioalkyl)carborane ligands required in the preparation of the target complexes **2–5**. In a related

† Electronic supplementary information (ESI) available: Synthetic procedures and characterisation data for the bis(thioalkyl)carborane ligands and complexes **2–5**. See http://dx.doi.org/10.1039/b508340k

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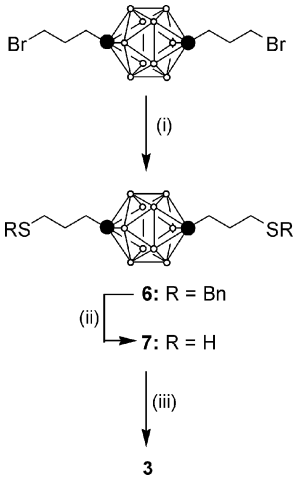
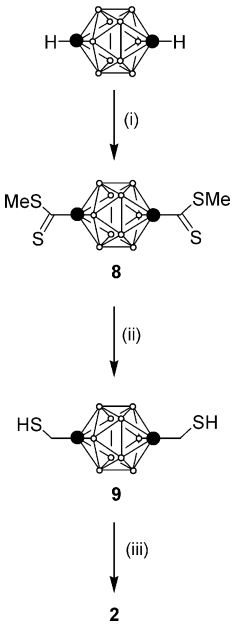
procedure to that reported for the monodentate thioalkylcarborane ligands,[23] treatment of the benzylthiolate anion with 1,12-bis(bromopropyl)-1,12-carborane, for example, afforded the S-benzyl protected carborane **6** in 96% yield (Scheme 1). This method was also successfully applied to the synthesis of the corresponding 1,2- (90%) and 1,7-carborane isomers (95%). The S-benzyl groups in **6** were readily cleaved by treatment with freshly sublimed $AlCl_3$ in C_6H_6 at 50 °C to afford the bis(thiopropyl)-1,12-carborane ligand **7** in reasonable yield (50%) (Scheme 1).[23] Similar reaction conditions afforded the 1,2- (54%) and 1,7-carborane isomers (37%). Small quantities (<5%) of unidentified impurities were detected by ^{1}H NMR spectroscopy and may be the result of thiol oxidation but the products could not be successfully purified by recrystallisation, distillation, sublimation or chromatography methods. Characterisation of the bis(thiopropyl)carborane ligands was confirmed, however, by means of ESI-MS, with molecular ion peaks detected at *m/z* 292. The bis(thiomethyl)-1,12-carborane ligand **9** was prepared from the corresponding dithioester derivative **8** by reduction with $BH_3{\cdot}SMe_2$ (Scheme 2). The preparation of **8** followed a related procedure to that reported by Nachman *et al.*[24] for the preparation of the corresponding thiomethyl-1,12-carborane derivative from 1,12-carborane using two equivalents of ^{n}BuLi, MeI and CS_2 in the presence of CuBr and LiBr.



Scheme 1 *Reagents*: (i) NaOEt, BnSH; (ii) $AlCl_3$; (iii) [Pt(MeCN)(trpy)](OTf)$_2$.



Scheme 2 *Reagents*: (i) ^{n}BuLi, CuBr/LiBr, CS_2, MeI; (ii) $BH_3{\cdot}SMe_2$, conc. HCl; (iii) [Pt(MeCN)(trpy)](OTf)$_2$.

Table 1 IC_{50} (μM) values ($n = 2$) for **1–5**. IC_{50} values were determined using a Coulter Counting (CC) assay

Cell line	Cisplatin	1	2	3	4	5
L1210	0.5	1.6	24.5	5.3	7.4	0.9
L1210/DDP	6.9	0.9	26.5	7.0	10	0.8

Addition of the labile precursor [Pt(trpy)(MeCN)](OTf)$_2$ to **7** in DMF solution resulted in an immediate and dramatic colour change from pale-yellow to deep purple, indicating substitution of the S-donor ligand for the MeCN ligand had readily occurred to afford **3** in 61% yield (Scheme 1). Similar reaction conditions were successfully applied to the synthesis of **2** (32%, Scheme 2), **4** (87%) and **5** (84%). The identities of **2–5** were confirmed by microanalysis and multinuclear (^{1}H, ^{13}C, ^{11}B and ^{195}Pt) NMR spectroscopy. 2D-NMR (COSY, HMBC and HMQC) experiments were performed to allow complete assignment of all ^{1}H and ^{13}C{^{1}H} NMR signals for the complexes. In particular, coordination of the thiol ligand to the platinum(II) centre in **3**, for example, was confirmed by a shift in the ^{13}C{^{1}H} NMR spectrum from δ 23.8 (CH_2SH) to δ 30.2 (CH_2SPt) and a characteristic peak in the ^{195}Pt{^{1}H} NMR spectrum at δ −3206 which is characteristic of a PtN_3S core.[22,23,25] Despite repeated attempts no molecular ion peaks were detected for **2–5** in the ESI-MS; only peaks resulting from fragmented ions were detected, *e.g.* $[PtS(trpy)]^+$.

The dinuclear complexes **2–5** were screened against the L1210 murine leukaemia cell line and its cisplatin-resistant variant L1210/DDP.[26] Cisplatin was used as the control compound, and the mononuclear platinum-carborane complex **1** was also examined in order to ascertain any differences in cytotoxicity between related mono- and di-nuclear species. The concentrations of complexes required to achieve 50% inhibition of cell growth (IC_{50}) are presented in Table 1.

The relative cytotoxicities of **1–5** are similar in both the cisplatin-sensitive L1210 and cisplatin-resistant L1210/DDP cell lines. This clearly indicates that the mechanism of cytotoxicity by **1–5** is not affected by the cisplatin resistance mechanism(s) operating in the L1210/DDP cell line. In comparison with cisplatin, **1** and **5** are considerably more potent in the cisplatin-resistant L1210/DDP line. In contrast, **2–4** are not as active. It appears that the inclusion of 1,7- and 1,12-carborane moieties in **2–4** adversely affects the ability of these complexes to exert a cytotoxic effect. Although subtle electronic effects associated with the nature of the carborane cage may explain the observed differences in biological activity, these results are more likely to be consistent with the poor aqueous solubility of **2–4** which is largely attributed to the low polarity of these complexes. It appears that the significantly higher cytotoxicity of **5**, compared to **3** and **4**, results from its somewhat higher solubility in aqueous solution. Indeed, while all the complexes prepared in this work are freely soluble in DMF, **1** and **5** are also soluble in MeOH and, unlike **2–4**, do not precipitate out of solution upon dilution with water. Any precipitation of the complexes from solution during the 48 h incubation period with the tumour cell lines would prevent the full cytotoxic effect from being exerted, thus resulting in the observation of low cytotoxicity.

The exact basis of the cytotoxicity exhibited by the complexes cannot be established with any certainty until their exact mechanism(s) of action is determined, but both DNA- and protein-binding are possible as has been observed with related complexes.[2,3,27,28] It is also feasible that the complexes embed themselves within the cell membrane, possibly by a membrane-spanning mechanism for the larger bis(thiopropyl) derivatives **3–5**, which may ultimately lead to cell death due to lysis.[2]

In conclusion, the first examples of dinuclear platinum(II)–trpy complexes containing 1,2-, 1,7- and 1,12-carboranes were prepared and fully characterised by microanalysis and multinuclear (^{1}H, ^{13}C, ^{11}B and ^{195}Pt) 1D- and 2D-NMR spectroscopy.

All complexes were screened against the L1210 leukaemia cell line and its cisplatin-resistant variant, thus allowing a direct comparison of their IC_{50} values with those of cisplatin and the mononuclear species **1**. We are currently exploring methods for improving the aqueous solubility of the complexes and we are also determining their DNA-binding characteristics, cell uptake and intra-cellular distribution. The results of this work will be reported in due course.

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