Video Article

Amide Coupling Reaction for the Synthesis of Bispyridine-based Ligands and Their Complexation to Platinum as Dinuclear Anticancer Agents

Michael G. Apps[1], Ben W. Johnson[2], Oliver B. Sutcliffe[3], Sarah D. Brown[4], Nial J. Wheate[1]

[1]Faculty of Pharmacy, The University of Sydney

[2]School of Science and Health, University of Western Sydney

[3]Division of Chemistry and Environmental Science, School of Science and the Environment, Manchester Metropolitan University

[4]Nature Publishing Group

Correspondence to: Nial J. Wheate at nial.wheate@sydney.edu.au

URL: https://www.jove.com/video/51740
DOI: doi:10.3791/51740

Keywords: Chemistry, Issue 87, BBR3464, picoplatin, bispyridine, amide coupling, inorganic synthesis, cancer

Date Published: 5/28/2014

Citation: Apps, M.G., Johnson, B.W., Sutcliffe, O.B., Brown, S.D., Wheate, N.J. Amide Coupling Reaction for the Synthesis of Bispyridine-based Ligands and Their Complexation to Platinum as Dinuclear Anticancer Agents. *J. Vis. Exp.* (87), e51740, doi:10.3791/51740 (2014).

Abstract

Amide coupling reactions can be used to synthesize bispyridine-based ligands for use as bridging linkers in multinuclear platinum anticancer drugs. Isonicotinic acid, or its derivatives, are coupled to variable length diaminoalkane chains under an inert atmosphere in anhydrous DMF or DMSO with the use of a weak base, triethylamine, and a coupling agent, 1-propylphosphonic anhydride. The products precipitate from solution upon formation or can be precipitated by the addition of water. If desired, the ligands can be further purified by recrystallization from hot water. Dinuclear platinum complex synthesis using the bispyridine ligands is done in hot water using transplatin. The most informative of the chemical characterization techniques to determine the structure and gross purity of both the bispyridine ligands and the final platinum complexes is ^{1}H NMR with particular analysis of the aromatic region of the spectra (7-9 ppm). The platinum complexes have potential application as anticancer agents and the synthesis method can be modified to produce trinuclear and other multinuclear complexes with different hydrogen bonding functionality in the bridging ligand.

Video Link

The video component of this article can be found at https://www.jove.com/video/51740/

Introduction

Platinum anticancer drugs remain one of the most widely used family of agents in the treatment of human cancer[1]. Despite their success, they are limited in their application by severe dose-limiting side effects[2-4]. The limited doses that can be administered to patients also means that tumors can develop resistance[5]. As such, new drugs continue to be developed to improve the side effect profile and overcome acquired resistance, like phenanthriplatin[6] and phosphaplatin[7].

In the late 1990s, a trinuclear platinum drug was developed, BBR3464 (**Scheme 1**)[8], that is up to 1,000x more cytotoxic *in vitro* than the leading platinum drug, cisplatin. BBR3464 is also able to overcome acquired resistance in a panel of human cancer cell lines[9]. Unfortunately, the increased activity of BBR3464 is matched by 50- to 100- fold higher toxicity, which limits its use[10-12]. It is also easily degraded in the body, meaning little of the drug reaches cancer nuclei intact[9].

Picoplatin is a mononuclear platinum-based drug that contains a 2-methyl-pyridine ligand (**Scheme 1**)[13]. The methyl group of this drug protects it from attack by biological nucleophiles; in particular cysteine and methionine containing peptides/proteins[14-16]. As such, the drug is quite stable and has a much higher concentration that reaches cancer nuclei compared with both BBR3464 and cisplatin[17]. Its reduced reactivity also means picoplatin has a higher maximum tolerated dose compared with BBR3464 and cisplatin[10,18,19].

This project therefore sought to combine the properties of BBR3464 and picoplatin to produce new drugs that are able to overcome acquired resistance that display improved biological stability and less severe side-effects (*e.g.*, **Figure 1**). In doing so, a range of dinuclear platinum complexes were prepared with bispyridine bridging ligands[20]. The ligands are made using amide coupling reactions with isonicotinic acid, or its derivatives like 2-methyl-isonicotinic acid, variable length diaminoalkanes. Reaction of one mole equivalent of the ligands with two mole equivalents of transplatin yields the desired platinum complexes (**Scheme 1**).

Protocol

1. Synthesis of the *N,N'*-(alkane-1,n-diyl)diisonicotinamide

1. Dry a single neck or three-neck round bottom flask in an oven (100 ºC, 1 hr) to ensure all moisture is removed.
2. Add solid isonicotinic acid, or its derivative, to the flask along with a magnetic stirring bar. If the diaminoalkane ligand(s) are solids at room temperature, then 0.5 mole (to the number moles of isonicotinic acid) is added to the flask at this stage.
3. Cap the neck(s) of the flask with rubber septa and replace the air in the flask with nitrogen either through a continuous nitrogen stream or through the use of nitrogen filled balloons.
4. Use a hypodermic needle and a syringe to add anhydrous dimethylformamide or dimethylsulfoxide (4 ml per 500 mg of isonicotinic acid or 2-methyl-isonicotinic acid) to dissolve the solid. If the solids do not dissolve easily, then heat the solution gently.
5. Add 7 mole equivalents (to the amount of isonicotinic acid used) of triethylamine (weak base) and 0.5 mole equivalents of the diaminoalkane. If the solution is a liquid at room temperature, then add 1.5 mole equivalents.
6. Add one mole equivalent of 1-propylphosphonic anhydride (coupling agent) with continuous stirring and allow the reaction to complete over 5-12 hr.

2. Purification of the Ligands

1. For bispyridine ligands made using diaminoalkane ligands with 10 or more methylene groups, wait for the products to precipitate from solution as the reaction progresses.
2. For bispyridine ligands made using diaminooctane, precipitate the product by adding ~40 ml of water.
3. For bispyridine made using diaminoalkanes of two to six methylene groups, add ~40 ml of water and allows the compounds to crystallize over 1-3 days.
4. Collect each bispyridine ligand by vacuum filtration and recrystallize from approximately 400-500 ml of boiling water per 200 mg of ligand. Note: More water is needed for the longer bispyridine ligands due to their much reduced water solubility.
5. Add NaOH and KOH (pH 9) to the solution to ensure that the compounds are free bases upon recrystallization.

3. Synthesis and Purification of the Dinuclear Platinum Complexes

1. Fully dissolve *trans*-diamminodichloridoplatinum(II), transplatin, in hot (70-80 ºC) water (150 ml per 200 mg of transplatin) to produce a clear strongly yellow colored solution.
2. Add a 0.5 mole equivalent of the bispyridine ligand and stir the solution at temperature until the ligand dissolves (clear solution). Wait for the solution to turn near colorless, turn off the heat, and stir at room temperature for a few additional hours.
3. Remove the solvent by rotary evaporation, which will yield a yellow colored powder.
4. Purify, the platinum complex(es) by dissolving in a minimum amount of warm water (~50 ºC). If remaining yellow or white colored solids are present, then filter these off.
5. Add acetone to the solution until a white precipitate is formed which appears to be a polymeric form of the metal complexes and represents up to 10% of the reaction product. Continue the addition of acetone (~extra 20-30 ml) until no more precipitate appears. Note: this white precipitate is an impurity.
6. Remove this precipitate by filtering the contents through nylon filter paper (0.2 µm pore size) and rotary evaporating the remaining solution to dryness, which will yield a pure product. Note: If necessary, then additional acetone precipitation steps can be performed until the complex is pure.

Representative Results

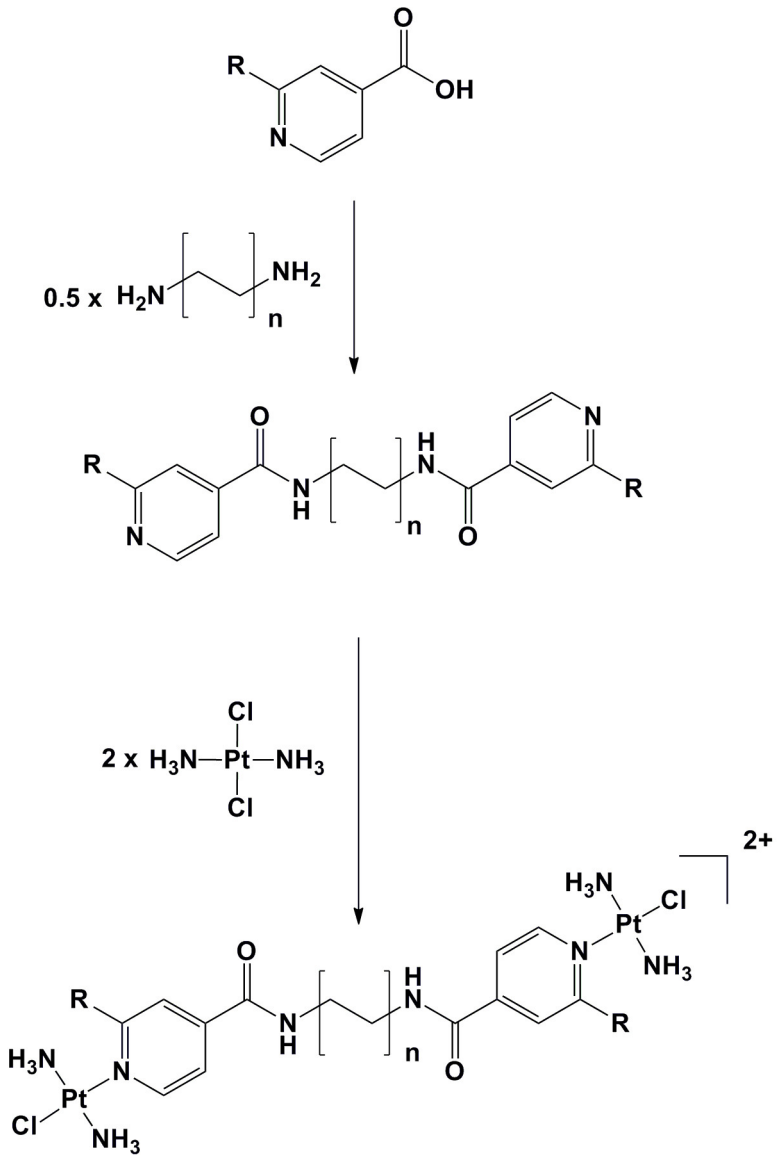
The bispyridine ligands and their respective dinuclear platinum complexes are characterized by ^{1}H, ^{13}C and ^{195}Pt NMR (**Tables 1** and **2**), and electrospray ionization mass spectroscopy. Accurate melting points can be determined using differential scanning calorimetry and purity is best determined by elemental analysis for C, H and N percentage content. Of most use is ^{1}H NMR as it is quick and easy to use, giving results within minutes of isolation of the final products with resonances that can definitively demonstrate successful amide coupling and platinum coordination (**Tables 1 and 2**).

Isonicotinic acid has three resonances; two doublets in the aromatic region (7 to 9 ppm) and a very broad resonance around 13 ppm for the carboxylic acid proton. The diaminoalkane proton resonances are all located in the aliphatic region between 1 and 4 ppm. As the length of the diaminoalkane chain increases many of the methylene resonances become equivalent, and as such, fewer peaks are observed in the aliphatic region than would be expected; although they are significantly more intense and can be assigned loosely by their integrations (**Tables 1** and **2**). For example see **Figures 1** and **2** for the chemical structure and ^{1}H NMR spectra of the *N,N'*-(octane-1,8-diyl)bis(isonicotinamide), biao, ligand. Due to symmetry in the molecule five aliphatic resonances would normally be expected for biao; but the four inner most methylene peaks are all magnetically equivalent and show up as one large resonance at ~1.2 ppm.

The amine proton resonance of the uncoupled diaminoalkane chain is located in the aliphatic region and is the most important resonance as it moves significantly downfield, into the aromatic region, upon coupling to the carboxylic acid. The subsequent amide proton resonance is seen around 8.7 ppm for all of these ligands, as a relatively broad triplet resonance (**Figure 2**).

Coordination of the platinum group to the bispyridine ligands is observed through selective shifts of the ligand's aromatic resonances (0.15 ppm downfield shift of the Ha resonances and 0.07 ppm upfield shift of the Hb resonances) and the observance of platinum coupling on the doublet resonance for the Ha protons (**Figure 3**). Coordination of the platinum groups to the bispyridine ligand can also be observed easily

using ^{195}Pt NMR. The chemical shift of a platinum resonance is directly related to the types of atoms coordinated[21]. Chlorido ligands shift ^{195}Pt resonances downfield (towards 0 ppm) and am(m)ino ligands shift the resonances upfield (towards -2,500 ppm). The dinuclear platinum complexes synthesized here show a single resonance around -2,300 ppm due to the 3 x am(m)ino and 1 x chlorido coordination state (**Figure 4**)[22]. Unreacted transplatin impurity would show as a resonance around -2,100 ppm whereas if two ligands reacted with a single transplatin molecule (4 x am(m)ino environment) then this impurity would be upfield of -2,400 ppm[22].



Scheme 1. The chemical structures of the platinum drugs picoplatin and BBR3464 and the general synthetic scheme for the synthesis of the bispyridine ligands and their respective dinuclear platinum complexes; R = H or CH_3; n = 1-6. Counter ions for the platinum complexes have been omitted; by using the method described here they are dichloride salts.

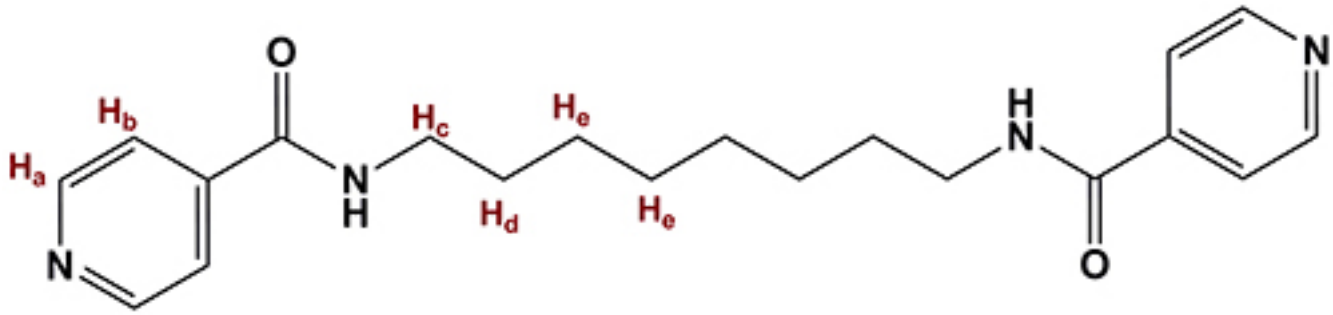


Figure 1. The chemical structure of the *N,N′*-(octane-1,8-diyl)bis(isonicotinamide) ligand, biao, and the proton numbering scheme used for assignment of the resonances in the ^{1}H NMR. Note the equivalence of the four methylene protons (He) in the center of the molecule.

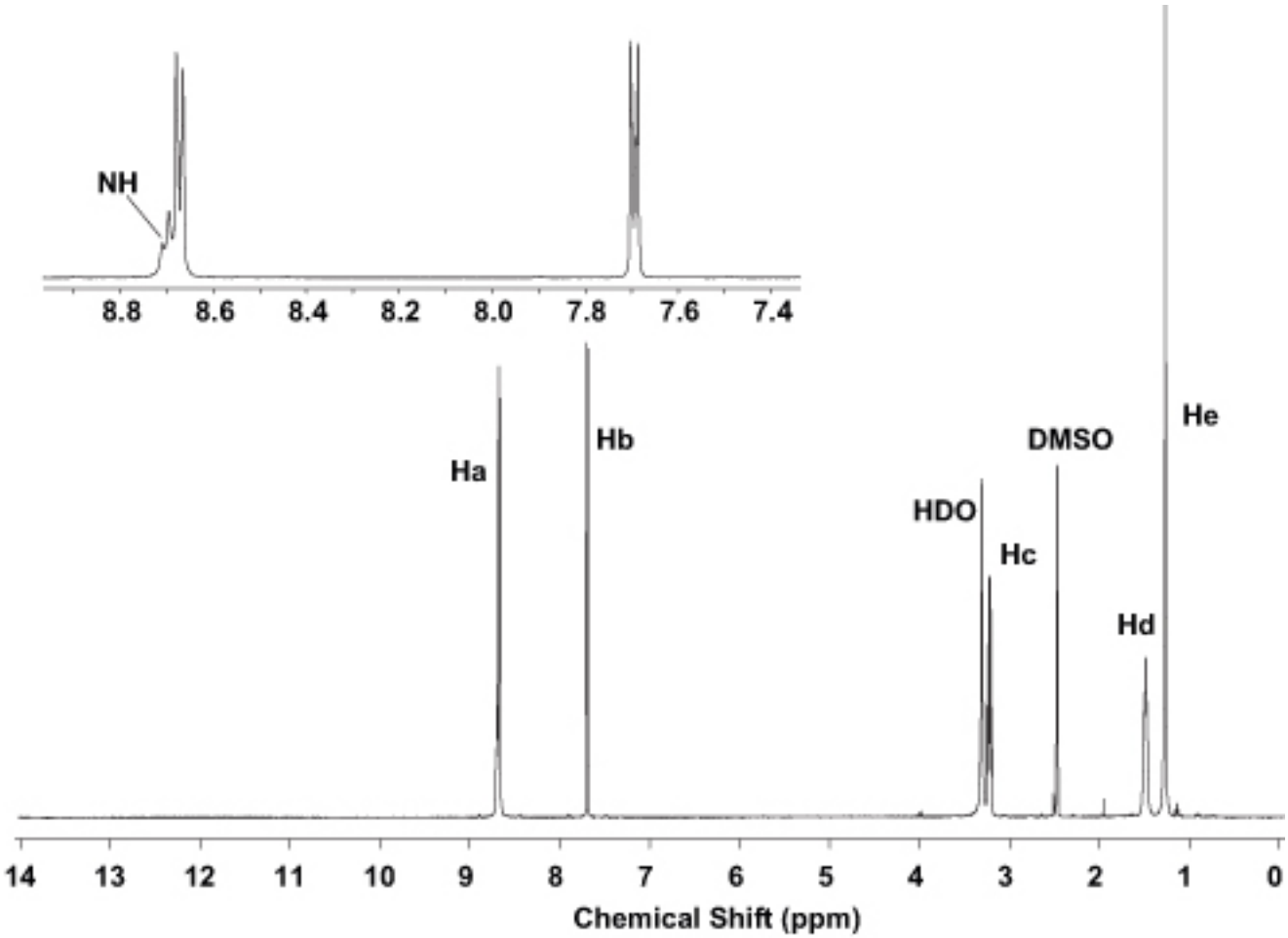


Figure 2. The ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of *N,N′*-(octane-1,8-diyl)bis(isonicotinamide), biao. Note the location of the triplet amide resonance at around 8.7 ppm, up from 1-2 ppm for the diaminooctane amine resonance before coupling.

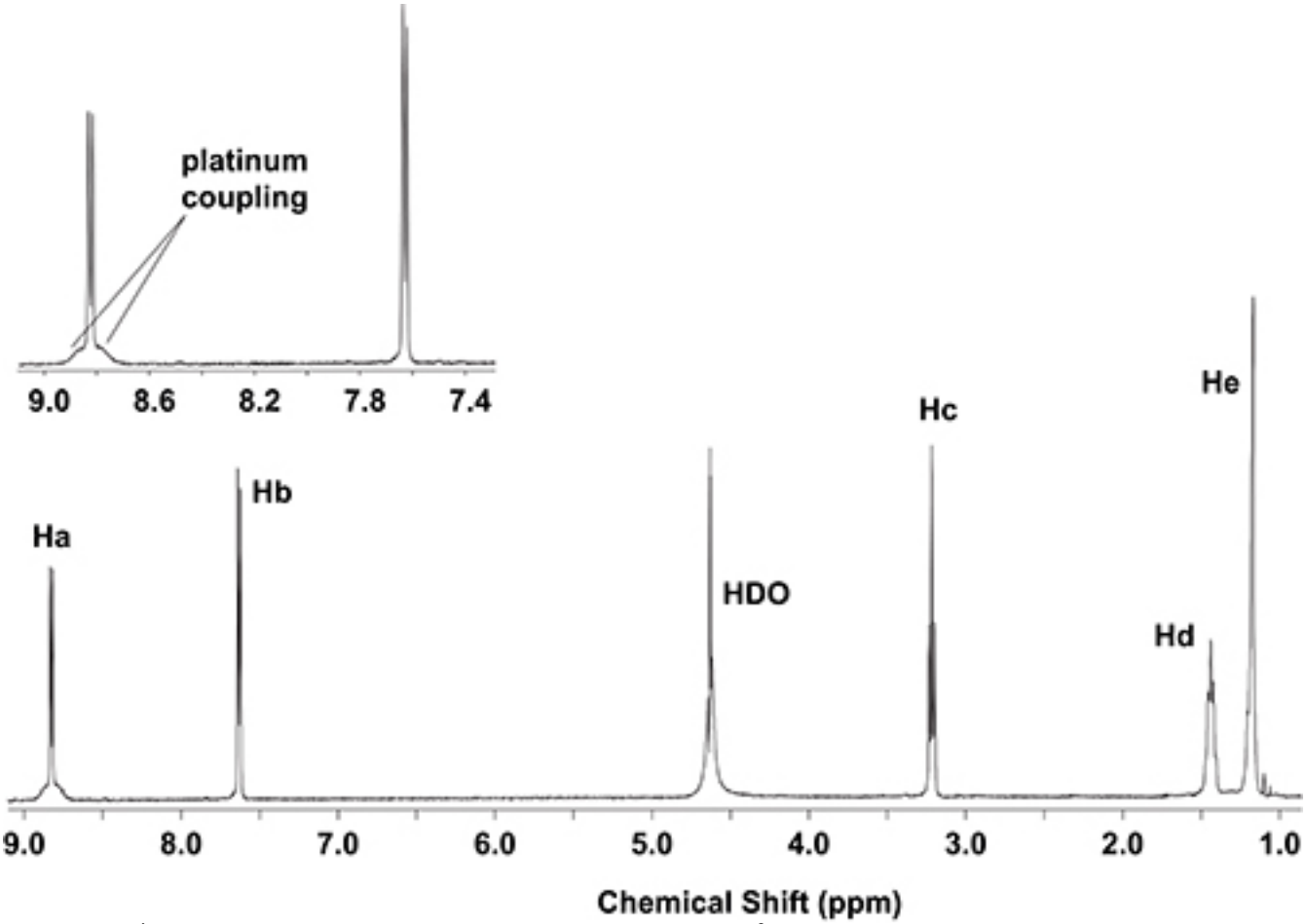


Figure 3. The ^{1}H NMR (D_2O, 400 MHz) spectrum of trans-[{Pt(NH_3)$_2$Cl}$_2$μ-biao]$^{2+}$. Note the platinum coupling on the aromatic Ha resonance at 8.82 ppm.

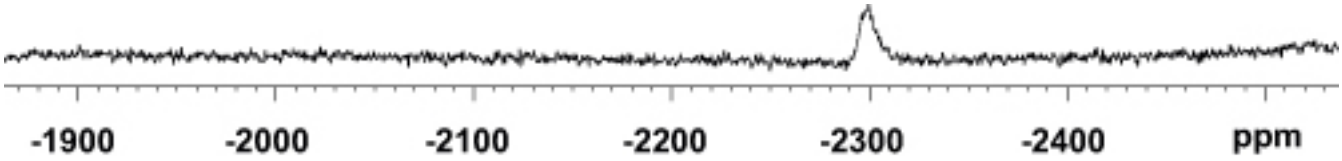


Figure 4. The ^{195}Pt NMR (D_2O, 400 MHz) spectrum of trans-[{Pt(NH_3)$_2$Cl}$_2$μ-biao]$^{2+}$. Note the single broad resonance around -2,300 ppm which is consistent with a PtN_3Cl environment; N = am(m)ine.

Ligand	Proton assignment, chemical shift (ppm), splitting pattern and integration					
	NH	Ha	Hb	Hc	Hd	He
N,N′-(octane-1,8-diyl)bis(isonicotinamide) (biao)	8.70, 2H, t	8.67, 4H, d	7.69, 4H, d	3.21, 4H, q	1.48, 4H, m	1.26, 8H, m
N,N′-(decane-1,10-diyl)bis(isonicotinamide) (biade)	8.69, 2H, t	8.67, 4H, d	7.69, 4H, d	3.21, 4H, q	1.48, 4H, m	1.24, 12H, m
N,N′-(dodecane-1,12-diyl)bis(isonicotinamide) (biado).	8.69, 2H, t	8.67, 4H, d	7.70, 4H, d	3.22, 4H, q	1.23, 4H, m	1.21, 16H, m

Table 1.The ^{1}H NMR characterization data of the bispyridine ligands in DMSO-d_6 at 400 MHz. d = doublet; t = triplet; q = quartet; m = multiplet.

Metal complex	Proton assignment, chemical shift (ppm), splitting pattern and integration				
	Ha	Hb	Hc	Hd	He
trans-$[\{Pt(NH_3)_2Cl\}_2\mu$-biao$]^{2+}$	8.82, 4H, d	7.63, 4H, d	3.22, 4H, t	1.44, 4H, m	1.18, 8H, m
trans-$[\{Pt(NH_3)_2Cl\}_2\mu$-biade$]^{2+}$	8.83, 4H, d	7.63, 4H, d	3.22, 4H, t	1.43, 4H, m	1.13, 12H, m
trans-$[\{Pt(NH_3)_2Cl\}_2\mu$-biado$]^{2+}$	8.83, 4H, d	7.63, 4H, d	3.23, 4H, t	1.44, 4H, m	1.15, 8H, m 1.10, 8H, m

Table 2. The ^{1}H NMR characterization data of the dinuclear platinum complexes in D_2O at 400 MHz. d = doublet; t = triplet; q = quartet; m = multiplet.

Discussion

In this work dinuclear platinum complexes have been synthesized as potential anticancer agents. In doing so bispyridine bridging ligands were synthesized via an amide coupling reaction using isonicotinic acid and variable length diaminoalkanes. Previously the synthesis of bispyridine ligands and their methyl analogues with 2 to 8 methylene groups and their respective platinum complexes have been reported. In this paper, the synthesis and purification method has been revised making it faster and cheaper and have demonstrated this by synthesizing bispyridine ligands with 8, 10 and 12 methylene groups (the shortest with the eight methylene groups, biao, was made to compare the new purification method to the older method). Dinuclear platinum complexes were also made using these ligands.

The synthesis of the bispyridine ligands was completed in anhydrous solvent and under an inert nitrogen atmosphere using triethylamine as a weak base and propylphosphonic anhydride as the coupling agent. Either DMF or DMSO can be used as the solvent, although the isonicotinic acid dissolves better in the DMSO than it does in the DMF. For either solvent, gentle heating under a running stream of hot water can be used to aid dissolution.

This old method required extended reaction times (several days) and a multiple step purification process including liquid/liquid extraction against diethyl ether and neutralization with $NaHCO_3$, which is now eliminated. The ligand synthesis reactions are now completed in a matter of hours at room temperature after all the reactants are added together in one step. The bispyridine ligands precipitate from solution with the addition of water (for ligands with 8 or less methylene groups) and precipitate from solution upon formation for the 10 and 12 methylene group ligands. All the ligands can be recrystallized from boiling water and in some instances their slow recrystallization can yield crystals suitable for X-ray diffraction.

The dinuclear platinum complexes are made by reacting two moles of transplatin to one mole of the bispyridine ligands. The trans-labializing effect of the chlorido ligands ensures that the major product is coordination of the platinum drugs through a single site on the bispyridine ligands (the nitrogen atom of the ring). The dinuclear platinum complexes have good solubility as dication salts in both water, DMF and DMSO.

Purification is achieved through fractional precipitation using acetone. The unidentified impurity precipitates before the product and is removed by filtration through narrow pore filter paper. It's important to note that the acetone precipitation works better with increasing amount of product to be purified. At amounts less than 200 mg, the method does not work as well and repeated acetone precipitations can be required. For amounts around 200 mg or more we've found that single acetone precipitation steps are generally only required.

Whilst this paper details the synthesis of specific ligands and platinum complexes, the techniques used here can be applied to synthesize a much wider range of ligands and multinuclear platinum complexes. For example, with suitable protective groups other diaminoalkane ligands, such as spermine and spermidine, can be used to make bispyridine ligands. Non-symmetrical bispyridine ligands could also be made using isonicotinic acid on one end and 2-methyl-isonicotinic acid on the other end. These non-symmetric ligands could be generated through the use of FMOC/BOC-protection on one end of the diaminoalkane chains. The unprotected amine of the chain can be reacted with an isonicotinic derivative using the amide coupling reactions described. The protecting group is then cleaved from the diaminoalkane chain and a different derivative of isonicotinic acid is attached using another amide coupling reaction. Trinuclear platinum complexes could also potentially be made using any of these ligands via an adaptation of a method using pyrazolyl-based ligands to make trinuclear, BBR3464-like, complexes[22].

Disclosures

The authors have nothing to disclose.

References

1. Wheate, N. J., Walker, S., Craig, G. E., & Oun, R. The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Trans.* **39**, 8113-8127, doi:10.1039/c0dt00292e (2010).
2. Kiernan, M. C. The pain with platinum: oxaliplatin and neuropathy. *Eur. J. Cancer.* **43**, 2631-2633, doi:10.1016/j.ejca.2007.09.008 (2007).
3. Markman, M. *et al.* Clinical features of hypersensitivity reactions to carboplatin. *J Clin. Oncol.* **17**, 1141-1145 (1999).
4. Ding, D., Allman, B. L., & Salvi, R. Review: Ototoxic characteristics of platinum antitumor drugs. *Anatomical Rec.* **295**, 1851-1867, doi:10.1002/ar.22577 (2012).
5. Galluzzi, L. *et al.* Molecular mechanisms of cisplatin resistance. *Oncogene* **31**, 1869-1883 (2012).
6. Park, G. Y., Wilson, J. J., Song, Y., & Lippard, S. J. Phenanthriplatin, a monofunctional DNA-binding platinum anticancer drug candidate with unusual potency and cellular activity profile. *Proc. Natl. Acad. Sci. USA.* **109**, 11987-11992, doi:10.1073/pnas.1207670109 (2012).
7. Moghaddas, S. *et al.* Phosphaplatins, next generation platinum antitumor agents: A paradigm shift in designing and defining molecular targets. *Inorg. Chim. Acta* **393**, 173-181, doi:10.1016/j.ica.2012.05.040 (2012).
8. Farrell, N. P. Platinum formulations as anticancer drugs clinical and pre-clinical studies. *Curr. Top. Med. Chem.* **11**, 2623-2631, doi:10.2174/156802611798040714 (2011).
9. Wheate, N. J., & Collins, J. G. Multi-nuclear platinum complexes as anti-cancer drugs. *Coord. Chem. Rev.* **241**, 133-145, doi:10.1016/S0010-8545(03)00050-X (2003).
10. Gourley, C. *et al.* A phase I study of the trinuclear platinum compound, BBR3464, in combination with protracted venous infusional 5-fluorouracil in patients with advanced cancer. *Cancer Chemoth. Pharmacol.* **53**, 95-101, doi:10.1007/s00280-003-0721-x (2004).
11. Calvert, A. H. *et al.* Phase II clinical study of BBR3464, a novel, bifunctional platinum analogue, in patients with advanced ovarian cancer. *Eur. J. Cancer* **37**, S260 (2001).
12. Jodrell, D. I. *et al.* Phase II studies with BBR3464, a novel tri-nuclear platinum complex, in patients with gastric or gastro-oesophageal adenocarcinoma. *Eur. J. Cancer* **40**, 1872-1877, doi:10.1016/j.ejca.2004.04.032 (2004).
13. William, W. N., & Glisson, B. S. Novel strategies for the treatment of small-cell lung carcinoma. *Nat. Rev. Clin. Oncol.* **8**, 611-619, doi:10.1038/nrclinonc.2011.90 (2011).
14. Raynaud, F. *et al. cis*-Amminedichloro(2-methylpyridine) Platinum(II) (AMD473), a novel sterically hindered platinum complex: *In vivo* activity, toxicology, and pharmacokinetics in mice. *Clin. Cancer Res.* **3**, 2063-2074 (1997).
15. Holford, J. *et al.* Chemical, biochemical and pharmacological activity of the novel sterically hindered platinum co-ordination complex, *cis*-[amminedichloro(2-methylpyridine)] platinum(II) (AMD473). *Anti-Cancer Drug Des.* **13**, 1-18 (1998).
16. Munk, V. P. *et al.* Investigations into the interactions between DNA and conformationally constrained pyridylamine platinum(II) analogues of AMD473. *Inorg. Chem.* **42**, 3582-3590, doi:10.1021/ic0340231 (2003).
17. Wheate, N. J., & Collins, J. G. Multi-nuclear platinum drugs: A new paradigm in chemotherapy. *Curr. Med. Chem. - Anti-Cancer Agents.* **5**, 267-279, doi:10.2174/1568011053765994 (2005).
18. Beale, P. *et al.* A phase I clinical and pharmacological study of cis-diamminedichloro(2-methylpyridine) platinumm II (AMD473). *British journal of cancer* **88**, 1128-1134, doi:10.1038/sj.bjc.6600854 (2003).
19. Sessa, C. *et al.* Clinical and pharmacological phase I study with accelerated titration design of a daily times five schedule of BBR3464, a novel cationic triplatinum complex. *Ann. Oncol.* **11**, 977-983 (2000).
20. Brown, S. D. *et al.* Combining aspects of the platinum anticancer drugs picoplatin and BBR3464 to synthesize a new family of sterically hindered dinuclear complexes; their synthesis, binding kinetics and cytotoxicity. *Dalton Trans.* **41**, 11330-11339, doi:10.1039/c2dt31313h (2012).
21. Still, B. M., Anil Kumar, P. G., Aldrich-Wright, J. R., & Price, W. S. ^{195}Pt NMR - Theory and application. *Chem. Soc. Rev.* **36**, 665-686, doi:10.1039/b606190g (2007).
22. Wheate, N. J., Cullinane, C., Webster, L. K., & Collins, J. G. Synthesis, cytotoxicity, cell uptake and DNA interstrand cross-linking of 4,4'-dipyrazolylmethane-linked multinuclear platinum anti-cancer complexes. *Anti-Cancer Drug Des.* **16**, 91-98 (2001).

