Available online at www.sciencedirect.com
ScienceDirect
Journal of Organometallic Chemistry 692 (2007) 1358–1362
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Iodination of cisplatin adduct of Vitamin B_{12} $[\{B_{12}\}\text{-CN-}\{cis\text{-}PtCl(NH_3)_2\}]^+$

Pilar Ruiz-Sánchez, Stefan Mundwiler, Alfredo Medina-Molner, Bernhard Spingler, Roger Alberto *

Institute of Inorganic Chemistry, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

Received 13 September 2006; received in revised form 3 November 2006; accepted 3 November 2006
Available online 11 November 2006

Abstract

The cisplatin adduct of vitamin B_{12}, $[\{B_{12}\}\text{-CN-}\{cis\text{-}PtCl(NH_3)_2\}]^+$ (**1**), reacts with iodide in aqueous solution to form $[\{B_{12}\}\text{-CN-}\{trans\text{-}PtI_2(NH_3)\}]$ (**3**) in good yield. Mono-substitution of chloride was not observed since a subsequent replacement of one NH_3 by a second iodide is very fast as compared to the $Cl^- \rightarrow I^-$ exchange. The same reaction conditions allowed to introduce radioiodide ^{131}I. Vitamin B_{12} can therefore be labeled with radionuclides via binding to the Pt(II) center.

Keywords: Vitamin B_{12}; Cisplatin; Radiopharmacy; Iodination

1. Introduction

Cyanocobalamin or vitamin B_{12} (abbreviated as "B_{12}") is an essential water soluble vitamin that is commonly found in a variety of food such as fish, meats, and dairy products. Insufficient B_{12} supply is responsible for a number of diseases such as pernicious anemia. This disease was fatal until the 1920s when Whipple et al. discovered that ingesting large amounts of liver seemed to cure the disease. Inspired by this result, Minot and Murphy set about to identify the curative substance and in 1948 finally Folkers et al. isolated red crystalline B_{12} from liver [1–3]. The chemical structure of the molecule was determined by Hodgkin et al. in 1956 by crystallography and awarded the 1964 Nobel Prize in chemistry [4]. Finally Woodward and Eschenmoser were able in a cooperative effort to chemically synthesize B_{12} in 1971 after 10 years of intense research [5,6]. In 1996, the entire biosynthetic pathway of the corrin ring in the aerobic Pseudomonas denitrificans and in the anaerobic Propionibacterium shermanii was elucidated [7,8].

Humans require B_{12} as a cofactor for two essential enzymatic processes in cytoplasm and in mitochondria. The bioavailability of vitamin B_{12} is very low and, as a result, mammals developed a very complex but highly efficient biochemical uptake mechanism, involving three different transport systems (haptocorrin, intrinsic factor and transcobalamin II) [9]. In contrast to healthy body cells, the demand for vitamin B_{12} at places of enhanced proliferation, such as tumour tissues or bacterial infections, is elevated. Its high need makes B_{12} therefore very attractive as an agent to target cancer cells or bacterial infections [10,11].

B_{12} is taken up by TC II receptor mediated endocytosis into cells, released from TC II and converted into its active forms methylcobalamin or adenosylcobalamin by several enzymatic processes. During these processes, the cobalt center is enzymatically reduced from Co(III) to Co(II) and Co(I) and subsequently alkylated [12]. During this coenzyme synthesis, the axial ligands (cyanide or water) in B_{12} are released. Thus, the upper axial position represents a promising site to introduce therapeutic molecules or analytical probes since they are cleaved from the targeting vector B_{12} during the conversion processes [13].

* Corresponding author. Tel.: +41 1 635 46 54/31; fax: +41 1 635 68 03.
E-mail address: ariel@aci.unizh.ch (R. Alberto).

0022-328X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2006.11.005

We recently reported the coordination of activated cisplatin, *cis*-$[PtCl(NH_3)_2(H_2O)]^+$, directly to the cyanide in vitamin B_{12}, to yield $[\{B_{12}\}\text{-CN-}\{cis\text{-}PtCl(NH_3)_2\}]^+$ **1** in which the cyanide forms a linear bridge between Pt(II) and Co(III) [14]. The Pt(II) center can mediate the binding of further pharmacophores or radioactive compounds. Our interest is focused on radiopharmaceuticals. Since Pt(II) binds strongly to iodide we aimed at introducing ^{131}I into Pt(II) to receive the {Co–C≡N–Pt–^{131}I} moiety, which represents a convenient pathway to radio-iodinated vitamin B_{12} derivatives. We present in this study exchange reactions with inactive iodide and **1**, which serves as a mechanistic model for the later introduction of ^{131}I. Expectedly, the chloride of the platinum center is readily exchanged by I^- to yield the highly stable complex $[\{B_{12}\}\text{-CN-}\{trans\text{-}PtI_2(NH_3)\}]$ (**3**) in which the chloride and one NH_3 ligand have been replaced by iodide [15]. The concept of our study involved the exchange of the chloride ligand in **1** with KI in order to assess the possibility to label the complex with ^{131}I or ^{123}I.

2. Experimental

All reactions were performed under nitrogen atmosphere. HPLC analyses were performed on a Merck-Hitachi L-7000 system equipped with a diode array UV/Vis spectrometer. The following HPLC column, solvent system, and gradient was used: Column: Macherey Nagel C-18ec RP columns (5 μm particle size, 100 Å pore size, 250 × 3 mm); solvent system: 0.1% trifluoroacetic acid in water (A); MeOH (B); gradient: 0–5 min (75% A), 5–30 min (75% A → 0% A). IR spectra were recorded in KBr on a Bio-Rad FTS-45 spectrometer. NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer. The chemical shifts of ^{195}Pt NMR spectra are relative to K_2PtCl_4 at −1626.4 ppm. NaI^{131} injection solution was obtained from Mallinckrodt Med. BV, The Netherlands. For stabilization of iodide against oxidation to iodine, the solution contains thiosulfate at a concentration of about 10 mM. The radionuclide ^{131}I is present without any inactive iodide (no carrier added), therefore the concentration is very low and about 10 nM.

2.1. Reactivity of $[\{B_{12}\}\text{-CN-}\{cis\text{-}PtCl(NH_3)_2\}]^+$ 1 with I^- in aqueous solution

Complex **1** (10 mg, 6.17 μmol) was dissolved in 0.1 ml of bidistillated water and treated with 50 equiv of KI (51 mg, 0.31 mmol). The reaction was allowed to stir 1 h at room temperature and the progress was monitored by HPLC at 360 nm. After this time period, the starting material had converted quantitatively into **3**. The product solution was desalted with a C_{18} SepPak cartridge and dried under vacuum to yield $[\{B_{12}\}\text{-CN-}\{trans\text{-}PtI_2(NH_3)\}]$ (**3**) as red-brownish powder. ^{195}Pt NMR (107 MHz, CD_3OD, 293 K) δ: −3326 ppm. IR (KBr) ν_{CN}: 2218 cm^{-1}. ESI-MS, 1844.3 ($M+Na^+$) 1821.4 (M^+). Crystals could be grown from the crude solid dissolved in water by vapor diffusion with acetone. Experimental details for data collection and refinement of the structure are given in the Supplementary material.

2.2. Reactivity with $^{131}I^-$

To a solution of **1** (5 mg, 3.08 μmol) in 100 μl water, 200 μl of a NaI^{131} solution was added. The reaction was followed by HPLC with β^- detection and allowed to proceed at room temperature for 6 h.

3. Results and discussion

The cisplatin-B_{12} conjugate **1** reported by Mundwiler et al. represents a water soluble and water stable starting material for the metal mediated introduction of further molecules or ions. Due to the leaving group properties of halides bound to Pt(II), it is expected that the Cl^- is replaced by iodide. Similar observations have been made with model Pt(II) complexes of the form $[PtCl_3(NH_3)]^-$ [15,16]. Since a coordinated iodide exerts a strong trans effect, one ammonia trans to iodide could further be replaced by a second iodide. The progress of the substitution reaction can nicely be monitored by HPLC at 360 nm. At a ratio $\mathbf{1}/I^- = 1$ we found almost exclusive formation of one new single peak with a retention time of about 16.3 min (Fig. 1).

Close to 50% of the starting material persisted which pointed toward the formation of a doubly instead of singly substituted product, thus, the coordination of two iodides to Pt(II) in **1** instead of only 1. We could isolate the new peak from the reaction mixture by preparative HPLC. Spectroscopic data and finally an X-ray structure analysis confirmed the formation of the complex $[\{B_{12}\}\text{-CN-}\{trans\text{-}PtI_2(NH_3)\}]$ (**3**).

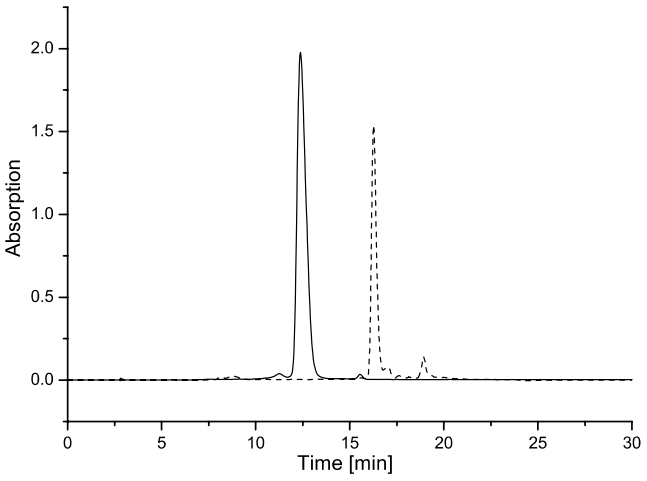


Fig. 1. HPLC traces monitored at 360 nm of $[\{B_{12}\}\text{-CN-}\{cis\text{-}PtCl(NH_3)_2\}]$ (—) and of the product $[\{B_{12}\}\text{-CN-}\{trans\text{-}PtI_2(NH_3)\}]$ **3** (---) after 1 h with 50 equiv of KI.

The CoC≡NPt stretching vibration is very indicative for any changes occurring on Pt(II) or on Co(III). The IR spectrum shows for **3** the ν_{CN} stretching band at 2218 cm^{-1}, which is 19 cm^{-1} blue shifted as compared to **1** or 81 cm^{-1} blue shifted as compared to B_{12}. Furthermore, the ^{195}Pt NMR spectrum gives a broad peak at −3326 ppm (Fig. 2). In this region, signals of cisplatin or transplatin complexes bound to two iodide ligands are usually observed. No signal was found in the region around −2800 ppm where the resonance of $[PtI(NH_3)_3]^+$ should appear. NMR data is therefore clearly consistent with the formation of **3** [15]. An X-ray structure analysis confirmed the trans position of the two iodides and an ORTEP presentation of compound **3** is given in Fig. 3.

At stoichiometric conditions, HPLC analysis gave no evidence for the formation of a mono-substituted intermediate complex [{B_{12}}-CN-{*cis*-$PtI(NH_3)_2$}] (**2**) (Scheme 1). Compound **2** would be the major product after ^{131}I labeling process since $^{131}I^-$ is present at extremely low concentration (nanomolar range) in comparison to **1** which excludes the formation of **3**. On the macroscopic level, the substitution of chloride by the first iodide seems to be the slow step. The strong trans effect of iodide labilizes

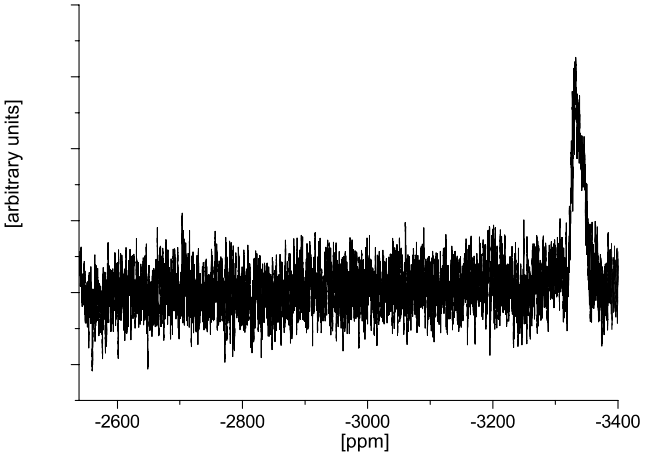


Fig. 2. ^{195}Pt NMR of [{B_{12}}-CN-{*trans*-$PtI_2(NH_3)$}] (CD_3OD, 293 K).

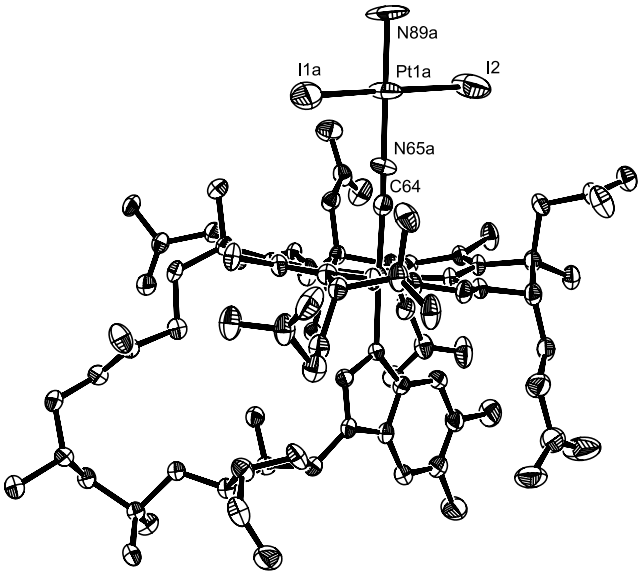
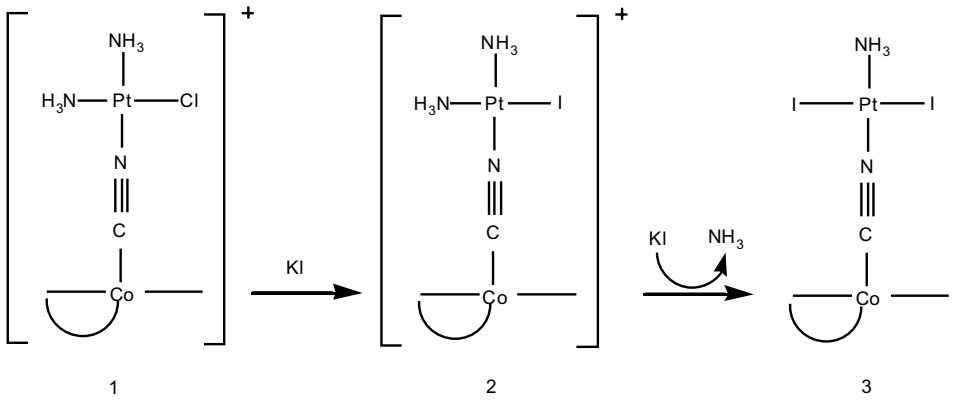


Fig. 3. ORTEP presentation of [{B_{12}}-CN-{*trans*-$PtI_2(NH_3)$}]. Important bond lengths are: Co–C(64) 1.900(8), C(64)–N(65a) 1.057(12), N(65a)–Pt(1a) 2.008(9), Pt(1a)–I(1a) 2.6273(19), Pt(1a)–I(2) 2.6985(16) Å.

the *trans*-NH_3 making it a leaving group. Subsequently and relatively more rapid than the first substitution, a second iodide replaces this *trans*-NH_3 to form the finally isolated product **3** (Scheme 1).

To confirm this hypothesis, we applied conditions with excess of **1** over iodide. Yet, it is impossible to mimic the exact ratios of radioactive labeling since the products could simply not be detected anymore by spectroscopical methods at this concentration. Applying a ratio of $I^-/\mathbf{1} = 0.05$ peaks of **2** and **3** could still be detected. From stoichiometry one would expect the formation of about 5% and at $I^-/\mathbf{1} = 1$ 100% of mono-substituted product **2**. However, the traces recorded at ratios $I^-/\mathbf{1} > 0.5$ always showed the formation of essentially one single new species with a retention time of 16.3 min, identical with **3** and in quantitative yield relative to the equivalents of KI added. For example, at a ratio $I^-/\mathbf{1} = 0.5$, 50% of the mono-substituted compound **2** should form. In fact, we observed about 25% of



Scheme 1. Proposed mechanism for the reaction of [{B_{12}}-CN-{*cis*-$PtCl(NH_3)_2$}] with KI.

the di-substituted product **3** only. Only at I^-/**1** of 0.2, a small additional peak with a retention time of 14.0 min corresponding to compound **2** could be detected in about 4% yield and a ratio **3**/**2** ratio of 3. Lowering I^-/**1** to 0.02 showed the two peaks of **2** and **3** in a ratio of 1/1 and in about 5% total yield (Table 1). Confirming the hypothesis that the second substitution must occur faster than the first one.

The possibility of substituting one chloride by iodide represents the base for labeling studies. Since the concentration of ^{131}I is about 10 nM only compound **2**(^{131}I) should form. First attempts to label **1** with iodide I^{131} did not succeed, since the commercially available solution contained thiosulfate $[S_2O_3]^{2-}$ in high concentrations (10 mM). Thiosulfate is, of course, a very strong ligand and "deactivates" the platinum center by irreversible coordination. The reaction of the commercial ^{131}I solution with **1** immediately gave one new peak at a retention time of 5.7 min. To efficiently remove thiosulfate and not changing the system we used an unconventional method. A large excess of **1** was added which trapped $[S_2O_3]^{2-}$ as mentioned before. The remaining "free" compound **1** could then nicely be labeled with ^{131}I showing one new peak with a retention time of 14.1 min. This labeled compound represents **2**(^{131}I) since its behavior is comparable to the observation made with inactive iodide and **1** in large excess (see above). An HPLC trace with radioactive detection is shown in Fig. 4. Over 24 h, this HPLC trace did not change, confirming the stability of the labeled product. It is, thus, possible to label B_{12} through the cisplatin complex bound to it. To our knowledge, it is the first time that iodination through platinum binding has been performed, a method that could potentially also be applied to other biomolecules.

Table 1
Yield of final products **2** and **3** vs. ratio I^-/**1** detected from HPLC traces after 1 h

Ratio I^-/**1**	**2** (%)	**3** (%)
0.02	≈2.5	≈2.5
0.2	≈4.0	12.4
0.5	–	24.5
1	–	50.6
2	–	90.3
10	–	93

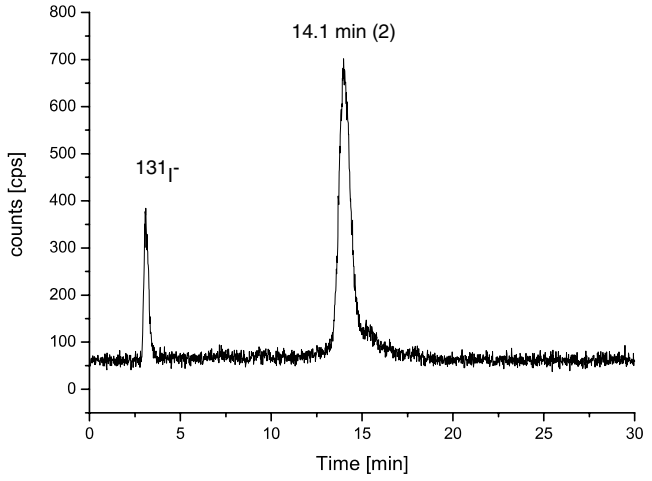


Fig. 4. Labeling of [{B_{12}}-CN-{*cis*-PtCl(NH_3)$_2$}] with $Na^{131}I$. The peak with a retention time of 14.1 min represents complex **2**(^{131}I) [{B_{12}}-CN-{*cis*-$Pt^{131}I(NH_3)_2$}].

4. Conclusion

We have shown that Cl^- and, optionally, NH_3 in [{B_{12}}-CN-$PtCl(NH_3)_2]^+$ **1** can be substituted by good nucleophiles such as I^- without cleaving the Pt(II) complex from B_{12}. After substitution of Cl^-, the strong trans-effect of I^- induces a further substitution with corresponding release of the *trans*-NH_3 to yield the complex [{B_{12}}-CN-{*trans*-$PtI_2(NH_3)$}] in quantitative yield. Formation of the mono-substituted product [{B_{12}}-CN-{*cis*-$PtI(NH_3)_2$}] could not be observed on the macroscopic level unless the excess of B_{12} over iodide was very large. Labeling studies of **1** with radioiodide ^{131}I on the other hand gave the mono-substituted product since **1** is present in extremely large excess over ^{131}I. The reaction presented here can be applied to the conjugation of further molecules to B_{12} via binding to Pt(II).

Acknowledgments

P.R.-S. thanks to the scientific committee of ISBOMC'06 for the opportunity to participate as a speaker in the conference. This work is supported by the Swiss National Science Foundation.

Appendix A. Supplementary material

CCDC 626099 contains the supplementary crystallographic data for **3**. The data can be obtained free of charge via htpp://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2006.11.005.

References

[1] W.P. Murphy, G.R. Minot, Boston Med. Surg. J. 195 (1926) 410.
[2] R. West, Science 107 (1948) 398.
[3] E.L. Rickes, N.G. Brink, F.R. Koniuszy, T.R. Wood, K. Folkers, Science 107 (1948) 396.
[4] D.C. Hodgkin, Science 150 (1965) 979.
[5] R.B. Woodward, in: B. Zagalak, W. Friederich (Eds.), Vitamin B12, W. de Gruyter, Berlin, 1979, p. 37.
[6] A. Eschenmoser, in: B. Zagalak, W. Friederich (Eds.), Vitamin B12, W. de Gruyter, Berlin, 1979, p. 88.
[7] A.R. Battersby, F.J. Leeper, Top. Curr. Chem. 195 (1998) 143.
[8] A.I. Scott, Angew. Chem. Int. Ed. 32 (1993) 1223.

[9] R. Banerjee, Chemistry and Biochemistry of B_{12}, Wiley-Interscience, 1999.
[10] D.A. Collins, H.P.C. Hogenkamp, M.K. O'Connor, S. Naylor, L.M. Benson, T.J. Hardyman, L.M. Thorson, Mayo Clin. Proc. 75 (2000) 568.
[11] R.A. Giannella, S.A. Broitman, N. Zamcheck, J. Clin. Invest. 50 (1971) 1100.
[12] M.V. Fonseca, J.C. Escalante-Semerena, J. Biol. Chem. 276 (2001) 32101.
[13] S. Kunze, F. Zobi, P. Kurz, B. Spingler, R. Alberto, Angew. Chem. Int. Ed. 43 (2004) 5025.
[14] S. Mundwiler, B. Spingler, P. Kurz, S. Kunze, R. Alberto, Chem. Eur. J. 11 (2005) 4089.
[15] Y. Qu, N. Farrell, M. Valsecchi, L. de Greco, S. Spinelli, Magn. Reson. Chem. 31 (1993) 920.
[16] C.J. Ziegler, A.P. Silverman, S.J. Lippard, J. Biol. Inorg. Chem. 5 (2000) 774.