Synthesis of novel allyl palladium complexes bearing purine-based NHC ligands with antiproliferative and proapoptotic activity against human ovarian cancer cell lines

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Introduction

In the last decades many efforts have been devoted to develop new strategies against neoplastic pathologies. Even inorganic chemists have taken part in this challenge and from the epochal discovery of the anti-cancer properties of *cis*platin, many researchers have employed their best energies for planning and preparing new metal-based drugs, unfortunately without obtaining the exciting results of the early years.[2] The general problem of the pharmacologic approach to cancer is connected to the difficulty of predicting every possible interaction of the drug with the huge number of biomolecules occurring in cellular and extra-cellular systems. The specificity of a metal-based anticancer drug mainly depends on the possibility of the metal centre of directly binding to the biological target and thus altering its functionality. DNA is the most recurring genomic target and the ability of platinum to coordinate strongly to nucleic acid, forming intrastrand crosslinks, is the reason of the effectiveness of *cis*platin and its second and third generation analogues.[3,4] A lot of studies have subsequently proved that many potential metal-based anticancer drugs have non-genomic targets (usually proteins) regulating the cell cycle or inhibiting over-expressed enzymes present in the cancer cells.[2d,5]

Unfortunately, the possible reactivity with biomolecules different from the specific diseased sites may induce a general toxicity, which often represents the companion side effect of chemotherapeutic drugs.

In principle a better selectivity could be obtained when the metal has a structural role i.e. it is functional to achieve the appropriate shape of the complex. In these cases binding to the molecular target occurs through non-covalent interactions in a manner similar to most organic drugs. Within this context, the problem is the actual difficulty of precisely defining the shape and the size of the target site and consequently synthesizing the appropriately tailored compounds.[2g]

These preliminary remarks highlight that the rational design of a metal-based anticancer drug is an elegant but often impracticable strategy. Therefore an extensive screening of anti-tumour properties of different metal compounds can in some way compensate for our inability to control their almost unpredictable reactivity in the bloodstream and cellular environment. In our choice to contribute to this systematic search we have preliminarily set some guidelines suggested by previous literature contributions. Firstly, we have opted for palladium-based compounds. Palladium complexes have structures and reactivity strictly comparable to Platinum analogues and in several cases have shown better anti-cancer activity than *cis*platin[6]. However the fast dissociation pattern of palladium complexes compared to platinum[7] represents a problem since the speciation, which heavily affects the biological activity and the pharmacokinetic properties, could be increased. To remedy this contraindication the most direct option is the introduction of ligands firmly anchored to the metal and hence we planned to employ N-Heterocylic carbenes (NHCs) which are known to give strong σ-bonds with most of the transition metal[8]. Moreover, several NHC-palladium complexes have already exhibited an interesting cytotoxic activity[9,6a,e] and tumour growth suppression even in vivo.[6e]

A potential improvement introduced in our work consists in using some innovative NHC ligands with a purinic framework[10] in hope that the natural imprint of the moiety could make our palladium complexes more compatible with the biological matrix. (Chart 1). This kind of synergy has already given interesting outcomes and in the most favourable cases the ligand has become a real targeting vector of the metal compound.[11]

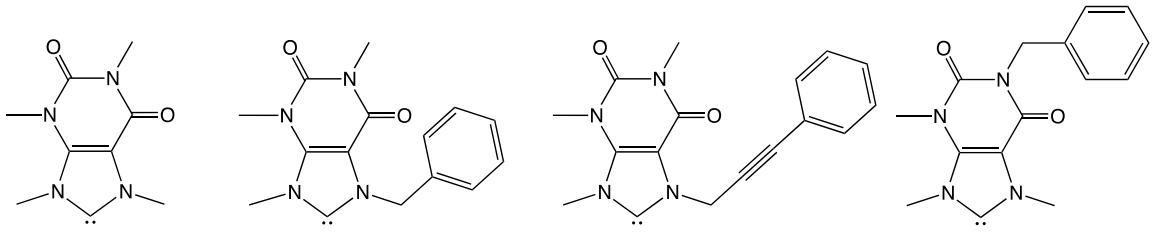


Chart 1 : Purine-based NHCs ligands used in this work

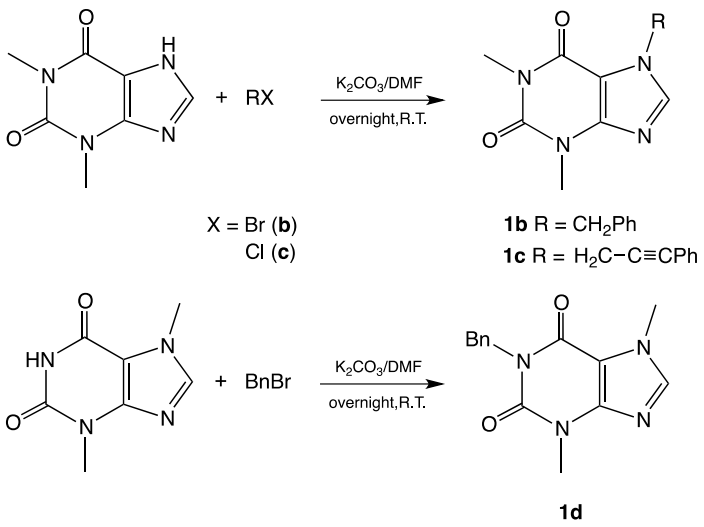
Furthermore, with the aim at enhancing the versatility of our palladium complexes sometimes isocyanides or phosphines have also been introduced into the coordination sphere. Among the latter we tested PTA (1,3,5-triaza-7-phosphadamantane) and TPPTS (3,3',3''-phosphinetribenzenesulfonate), which should increase the water-solubility of the metal compounds, an often very profitable feature for the pharmacologic use of a product.[12]

However the most original contribution of this paper is represented by the first attempt to utilize the palladium-η^3-allyl organometallic function for preparing compounds with cytotoxic properties. This organometallic fragment is well known for its involvement in numerous catalytic processes (the most famous is the Tsuij-Trost reaction) [13] but its behaviour in biological systems remains an almost unexplored field. In previous works some of us have prepared numerous compounds based on this functional group and bearing a large number of different spectator ligands and studied their properties and reactivity.[14] This experience suggests that the allyl residue could: a) remain bound to the metal centre, conferring a specific shape to the complex and thereby allowing it to be hosted in an active biological site; b) be released in the cellular environment and thus directly interact with some potential bio-target. The issue is surely difficult to tackle, but the analysis of its practical consequences remains the main objective of this paper.

Results and Discussion

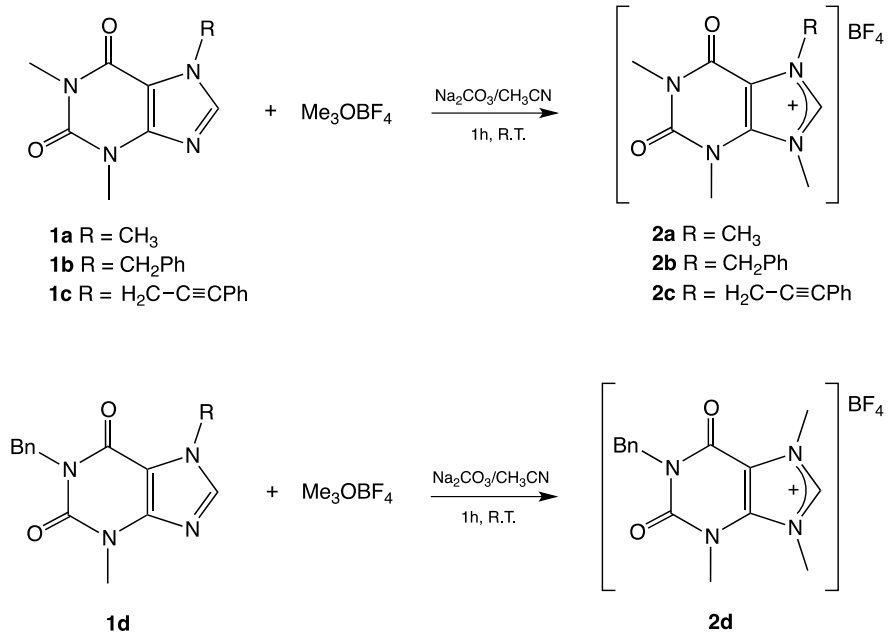
Synthesis of Imidazolium Salts from Functionalized Xanthines

Compounds **1b**,[15] **1c**[16]and **1d**[17] were re-synthesized adopting a slightly modified literature procedure (Scheme1). We have chosen not particularly severe conditions and the reaction of theophylline or theobromine with two equivalents of benzyl-bromide (for **1b** and **1d**) or 1-(phenyl)propargyl-3-chloride (for **1c**), carried out in DMF at R.T. overnight, gave the functionalized bases in good yields (93□□95%).



Scheme 1 Synthesis of functionalyzed xanthines

Despite the low reactivity of the sp^2 N9 atom, commercial caffeine (**1a**), the alkylated theophyllines **1b**, **1c** and theobromine **1d** react with a small excess of Meerwein's salt in CH_3CN at R.T. in the presence of Na_2CO_3 under not controlled atmosphere to give the corresponding N9 methylated imidazolium salts in one hour's time. (Scheme 2). The acetonitrile is probably more suitable for promoting the nucleophilic substitution than chlorinated solvents thanks to its high dielectric constant, whereas the presence of Na_2CO_3 in heterogeneous phase is necessary since the strong HBF_4 acid that is partially formed from the hygroscopic Me_3OBF_4 would protonate the N9 nitrogen of the xanthine so that the ensuing derivative would be no longer available for methylation.



Scheme 2 Synthesis of imidazolium salts from functionalyzed xantines

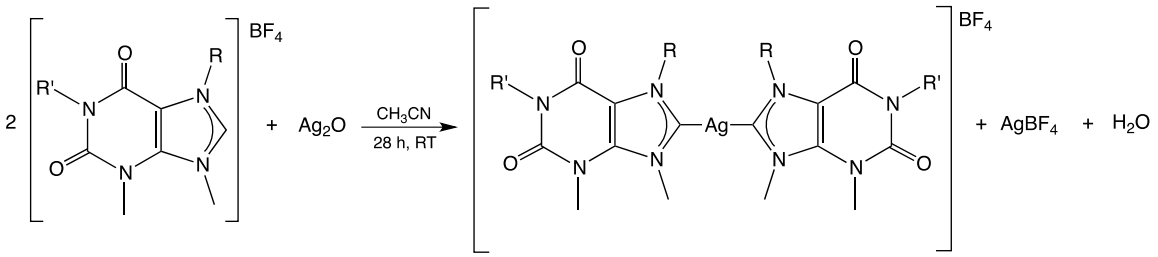
As an example we report in Figure S1 (see ESI) the ^{1}H NMR spectra related to the methylation of caffeine (**1a**) which show that in the absence of Na_2CO_3 the methyl caffeine **2a** coexists with its protonated counterpart, whereas only the pure **2a** derivative was detected in solution when the base was added to the reaction mixture.

In this respect, we think that the low yield obtained by other authors in the Meerwein's salt mediated methylation of the modified xanthines is probably due to this side reaction, which makes the workup of the process difficult.[15,18]

Synthesis of the Silver NHC complexes

We recently reported that the reaction between the imidazolium salts **2a**, **2b**, **2d** and Ag_2O carried out in acetonitrile produces a 1:1 mixture of the corresponding silver bis-NHC complexes **3** and $AgBF_4$.[19] This result was confirmed by a combination of spectroscopic and analytical data such as microanalysis, NMR, ESI-MS spectra and in one case (**3b**) even X-ray diffraction analysis. In this paper we have applied this protocol

for the synthesis of the novel complex **3c**. The disappearance of the imidazolic proton and the typical shift of the silver-coordinated carbon at ca. 187 ppm in the ^{1}H and ^{13}C NMR spectra respectively, represent simple tests to certify the predictable outcome of the reaction.



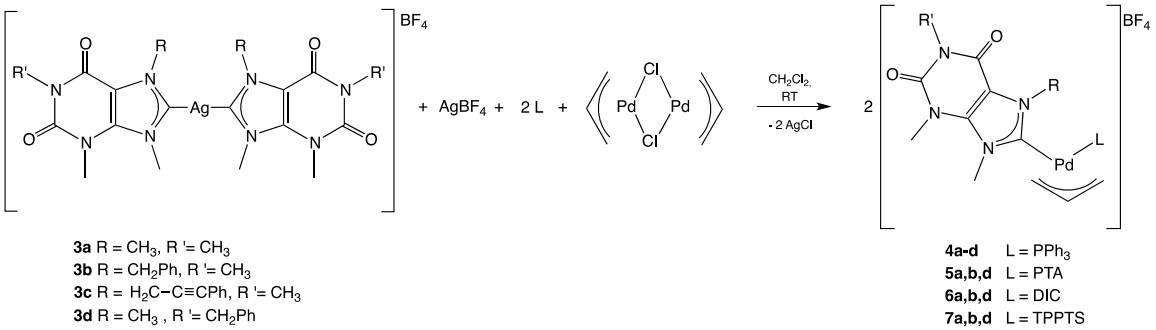
2a R = CH_3, R '= CH_3
2b R = CH_2Ph, R '= CH_3
2c R = $H_2C{-}C{\equiv}CPh$, R '= CH_3
2d R = CH_3 , R '= CH_2Ph

3a R = CH_3, R '= CH_3
3b R = CH_2Ph, R '= CH_3
3c R = $H_2C{-}C{\equiv}CPh$, R '= CH_3
3d R = CH_3 , R '= CH_2Ph

Scheme 3 Synthesis of silver NHC-purine based complexes

Synthesis of the Mixed NHC/Phosphine and NHC/Isocyanide Palladium η^3-allyl complexes

For this synthesis of the palladium complexes **4-7** we can take advantage of the presence of co-precipitated $AgBF_4$ as a stoichiometric by-product in the preparations of silver complexes **3**. As a matter of fact this condition allows to obtain the final complexes by one-pot reaction, by simply mixing the aforesaid mixture with a stoichiometric amount of palladium allyl dimer and phosphine (PPh_3, PTA, TPPTS) or isocyanide (DIC = 2,6-dimetilphenyl isocyanide) (Scheme 4). The precipitation of silver chloride is the driving force of the process and an indication of its progress. It is important to highlight that no formation of complexes bearing carbene/carbene and phopsphine-phosphine (or isocyanide-isocyanide) ligands has been observed and therefore a selective separation of the carbene/phosphine (or isocyanide) complexes is always possible from the reaction mixture.



Scheme 4 Synthesis of mixed NHC/phosphine and NHC/isocyanide palladium allyl complexes

The characterization of the twelve new complexes **4a,b,d**, **5a,b,d** and **6a,b,d** (compounds **7a,b,d** were reported in one of our previous works[19]) was performed by NMR, IR and elemental analysis. In particular an accurate examination of NMR spectra allows to elucidate some specific features of these derivatives that we can summarize on the following points:

a) In every NMR spectrum of the complexes containing a coordinated phosphine (**4a-d** and **5a,b,d**) the presence of two complete sets of signals is manifest. This is a consequence of the coexistence of a couple of atropoisomers due to the hindered rotation of the asymmetric carbene ligands around the Pd-C bond. In the mixtures the two isomers are always present in practically equal amounts.

   For the less sterically crowded isocyanide-complexes **6a,b,d** this restriction is not operative and thus only one set of signals is observable in solution at room temperature.

b) The presence in the ^{1}H NMR spectra of five distinct signals (for each atropoisomer) ascribable to allyl protons can be explained by the presence of two different spectator ligands and by the absence of any rearrangement (η^3-η^1-η^3 or *syn-syn/anti-anti*) sometimes observed even at room temperature.[20]

c) The coordination of the PPh_3 and PTA (respectively for complexes **4a-d** and **5a,b,d**) is proved by the marked downfield shift of the two peaks (one for each atropoisomer) observed in $^{31}P\{^{1}H\}$ NMR spectra when compared to those of the

free phoshines. ($\Delta\delta$ = 30 ÷ 45 ppm).

d) The coordination of DIC in complexes **6a,b,d** is certified by the weak signal of coordinated isocyanide carbon at ~150 ppm in the $^{13}C\{^1H\}^1H$ NMR spectra and the significant highfield shift of the *ortho* methyl protons with respect to their original position in the ^1H NMR spectra of the free ligand. This conclusion is also supported by the IR spectra which show an intense band at 2170 cm^{-1} attributable to the CN stretching of the coordinated isocyanide.

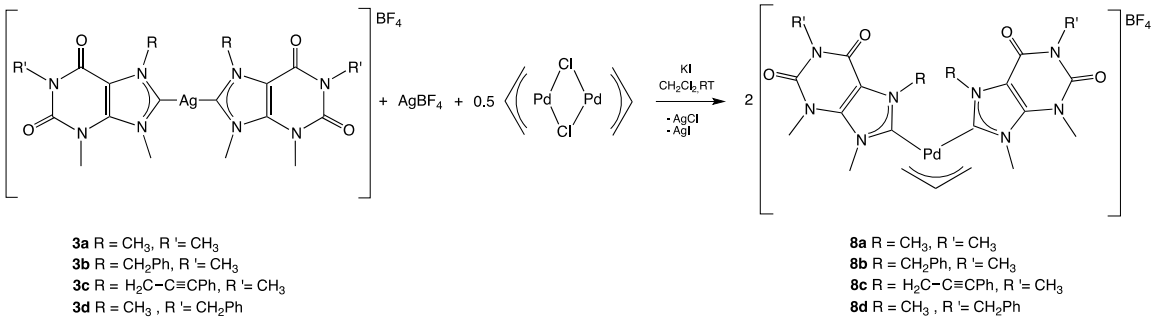
e) The resonances of carbene carbons are always found in a narrow range between 180-188 ppm, confirming the coordination of the NHC purine-based ligand at the palladium(II) centre. Moreover in the spectra of the compounds **4a-d** and **5a,b,d** these signals resonate as doublets due to the J^2 coupling with the *cis*-phosphine.

The cationic nature of synthesized derivatives is indirectly confirmed by the presence in all IR spectra of an intense absorption around 1050 cm^{-1}, ascribable to the BF_4^- anion.

Synthesis of the Bis(NHC) Palladium η^3-allyl Complexes

This class of complexes were synthesized by reacting the stoichiometric mixture of silver precursors **3** and $AgBF_4$ with palladium allyl dimer in the presence of KI. (Scheme 5). The addition of potassium iodide is necessary to remove all the silver from the reaction mixture.

Transmetallation proceeds in good yield and the resulting cationic complexes were all stable in solution and in air.



Scheme 5 Synthesis of bis(NHC) palladium allyl complexes

The most significant conclusion that can be immediately inferred from the NMR spectra of species **8** is the presence of only one set of signals. This observation indicates that there is a free rotation of the two *N*-heterocyclic carbene ligands around the Pd-C bond and thus the three hypothetical atropoisomers deriving from hindered rotation are not distinguishable at room temperature.

The symmetric structure of these complexes is demonstrated by the presence of only three signals ascribable to the allylic fragment in the ^{1}H NMR spectra (two doublets with different coupling constants for the *syn* and *anti* terminal protons and a multiplet for the central proton) and two in the $^{13}C\{^{1}H\}^{1}H$ NMR spectra.

Finally the $^{13}C_{NHC}$-Pd(II) resonances at about 185 ppm represent a compelling evidence of the coordination of carbene ligands to the palladium centre.

X-Ray Crystal Structure Determination of 4a e 8d

Crystalline forms of **4a** and **8d** contain one crystallographically independent palladium complex (Figure1 and 2). The complexes bear a positive charge that is balanced by a BF_4^- counterion, located close to the allyl ligand, which represents the area where the metal is more exposed (shortest F···Pd contacts are 3.50(1) Å in **4a** and 3.489(8) Å in **8d**). Palladium adopts square planar coordination spheres with bond lengths and angles (Table 2SI) in agreement with literature structural data of complexes with similar ligands. A query on CSD (version 5.38), using the Allyl-Pd-Imidazole fragment produces 29 hits with an average bite angle of 68.3(6)° and 2.04(1) Å Pd···$C_{carbenic}$ bond lengths (2.32(1) Å for Pd···P bonds). The allyl ligand has poor steric bulk constraints and can adopt alternative conformations, specular wih respect to the palladium coordination plane, as can be seen in more than half of similar structures already published. Models show that the xanthines minimize steric repulsions in the solid state, adopting a roughly perpendicular orientation with respect to the palladium coordination plane (85.68° in **4a** and 69.19°-75.98° in **8d**, in agreement with the average 79(9)° extracted from CSD). The molecular model of **4a** is well superimposable with the related triphenylphosphine-(η^3-allyl)-(tetramethylimidazolin-2-ylidene)-palladium tetrafluoroborate complex[21] (CCDC Number: 714135); the comparison highlights a degree of phosphine ligand

conformational freedom (Figure S27), which can be related to different crystal packing contacts. Crystal packing of **4a** and **8d** shows hydrophobic contacts among neighbour molecules, involving several CH···π and minor π···π interactions. Furthermore, structure **4a** has cavities (258 $Å^3$, estimated with PLATON[22] 'CALC VOID' routine) filled with disordered solvent molecules (one CH_2Cl_2 molecule for each cavity).

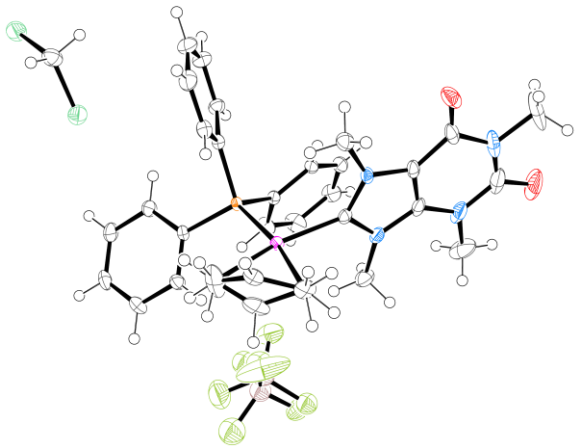


Figure 1 Ellipsoid representation of **4a** (B) crystal ASU contents (50% probability).

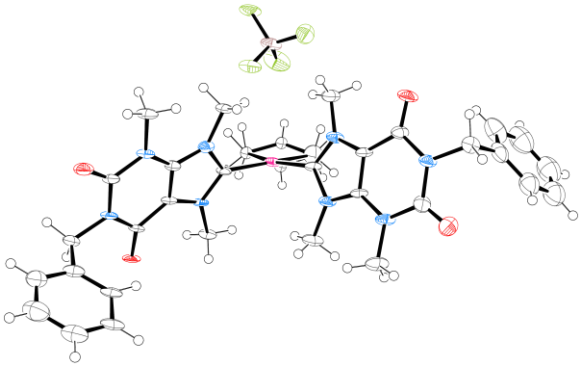


Figure 2 Ellipsoid representation of **8d** (B) crystal ASU contents (50% probability).

Antiproliferative Activity on Human A2780 and SKOV-3 Cell Line

The antiproliferative activity was assayed on two human cancer cell lines: A2780 and SKOV-3. Stock solutions (25-50 mM) of each complex were prepared in DMSO, H_2O, or DMSO/H_2O (1:1); the working solutions (5 mM, 500 μM, 50 μM and 5μM) were obtained using H_2O only.

All the complexes **4a-d**, **5a,b,d**, **6a,b,d, 7a,b,d**, **8a-d**, and the synthetic precursor $[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$ have been tested on cisplatin-sensitive A2780 cell line and on cisplatin-resistant SKOV-3 cell line in comparison to cisplatin (positive control), in order to evaluate the relative IC_{50} values expressed in μM concentration. All data obtained are reported in **Table 1** for both the analysed cell lines, showing that all the complexes, except for **7a**, displayed good anti-proliferative effects on the cisplatin-sensitive A2780 cells, but mainly derivatives **4c**, **4d** and **5d**, exhibited greater activity than the positive control cisplatin (IC_{50}: 1.46±0.22 μM), showing 0.09±0.02, 0.81±0.08 and 0.85±0.22 μM IC_{50} values, respectively. On the cisplatin-resistant SKOV-3 cells, the observed IC_{50} values generally were similar to that found in the treatment with our reference cisplatin (IC_{50}: 5.94±0.08 μM) or higher, such as in the case of the complexes **5d**, **7a**, **7b**, **7d**, **8b**, **8c** and **8d**, showing IC_{50} between 38.49±6.51 and 66.31±13.16 μM.

In particular the complex **4d** displayed a very interesting activity also on the SKOV-3 cells, exhibiting an IC50 value (1.71±0.99 μM) lower than that of cisplatin. The nude ligands **2a** and **2b**, also tested on the same cell models, were completely inactive (data not shown) at the concentrations used on both cell lines (IC_{50}>100 μM).

Table 1. Effects of the Pd-complexes on the proliferation of A2780 and SKOV-3 cells. The inhibition of cell growth is represented as IC_{50}.

	IC_{50} (μM)	
Complex	**A2780 (IC_{50})**	**SKOV-3 (IC_{50})**
Cisplatin	1.46±0.22	5.94±0.08
$[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$	7.78±0.21	9.67±4.26
4a	4.60±2.10	5.20±1.06
4b	4.00±2.16	3.40±1.91
4c	0.09±0.02	4.02±0.09
4d	0.81±0.08	1.71±0.99
5a	6.60±2.19	5.20±0.08
5b	7.60±0.07	6.50±0.71
5d	0.85±0.22	50.54±0.47
6a	5.00±2.22	4.03±0.71
6b	3.72±0.06	5.20±0.99

Pro-apoptotic Effects on Human A2780 and SKOV-3 Cell Lines

In order to asses whether the antiproliferative activity of the analyzed Pd-complexes is associated with induction of apoptosis, both A2780 and SKOV-3 cells were also evaluated with a Muse cytometer (Merck Millipore, Billerica, MA, USA), after treatment with two concentrations close to the IC_{50} values previously determined. The found pro-apoptotic effects are reported in Table 2, Figure 3 (representative examples) and in Figures 1A-E and 2A-E in the supplementary materials (all results). In Table 2, the percentage of total apoptosis was reported. In the reported plots (figures) we also describe the percentage of live and death cells and cells in early or in late apoptosis. Each data was compared to the analysis of untreated cells (negative control, C-) in which the observed total apoptosis was ≤ 5%.

On the A2780 cell line, the complexes that showed the highest pro-apoptotic activity were **4c**, **4d**, **6b**, **6d**, **8b**, **8c**, and **8d**, with total pro-apoptotic effects between 21.00% (**4d**) and 98.35% (**4c**); in addition, complex **4c** showed the greatest total pro-apoptotic activity even with the lowest concentration tested (54.04%). The remaining compounds were found to be moderately or poorly active in inducing apoptosis on this cisplatin-sensitive cell line, and the synthetic precursor $[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$ determined a pro-apoptotic effect on 29.40% of cells.

On the cisplatin-resistant SKOV-3 cell line, derivatives **4b**, **4c**, **4d**, **5d**, **6a**, **6b**, **6d**, **7a**, **7b**, **7d**, **8a**, **8b**, **8c**, **8d** were found to be particularly active, with total pro-apoptotic effects between 27.49% (**5d**) and 84.04% (**4c**). In particular, complex **4c** showed the highest activity even at the lowest concentration used (1 μM) with 43.78% of apoptotic cells.

Table 2. Pro-apoptotic effects of the Pd-complexes on A2780 and SKOV-3 cell lines detected at two different concentrations. (C-: untreated cells)

Complex	**Total apoptosis (%)**	
	A2780	**SKOV-3**
C-	2.61	5.30
Cisplatin	38.25 (2.5 μM)	56.04 (2.5 μM)
$[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$	8.15 (1 μM) 29.40 (10 μM)	6.55 (1 μM) 7.10 (10 μM)
4a	3.80 (1 μM) 12.35 (10 μM)	6.35 (1 μM) 12.71 (10 μM)
4b	8.35 (1 μM) 10.66 (10 μM)	7.25 (1 μM) 44.31 (10 μM)
4c	54.04 (0.1 μM) 98.35 (1 μM)	43.78 (1 μM) 84.04 (10 μM)
4d	2.25 (1 μM) 21.00 (10 μM)	6.50 (0.5 μM) 52.35 (5 μM)
5a	3.00 (1 μM) 15.25 (10 μM)	2.45 (1 μM) 3.10 (10 μM)
5b	4.25 (1 μM) 7.05 (10 μM)	3.00 (1 μM) 3.65 (10 μM)
5d	5.49 (1 μM) 5.76 (10 μM)	9.82 (50 μM) 27.49 (100 μM)
6a	9.50 (1 μM) 9.20 (10 μM)	15.05 (1 μM) 34.55 (10 μM)
6b	26.52 (1 μM) 61.18 (10 μM)	6.26 (1 μM) 69.10 (10 μM)
6d	1.40 (1 μM) 79.45 (10 μM)	3.45 (1 μM) 52.95 (10 μM)
7a	3.20 (50 μM) 2.21 (100 μM)	12.55 (50 μM) 44.96 (100 μM)
7b	2.66 (1 μM) 1.60 (10 μM)	11.25 (25 μM) 53.01 (50 μM)
7d	2.97 (1 μM) 5.65 (10 μM)	8.73 (50 μM) 36.37 (100 μM)
8a	2.65 (1 μM) 3.50 (10 μM)	3.65 (1 μM) 30.89 (10 μM)
8b	5.69 (1 μM) 43.03 (10 μM)	6.32 (50 μM) 56.60 (100 μM)
8c	1.39 (1 μM) 23.66 (10 μM)	7.84 (50 μM) 52.01 (100 μM)
8d	5.90 (1 μM) 61.17 (10 μM)	12.74 (25 μM) 79.32 (50 μM)

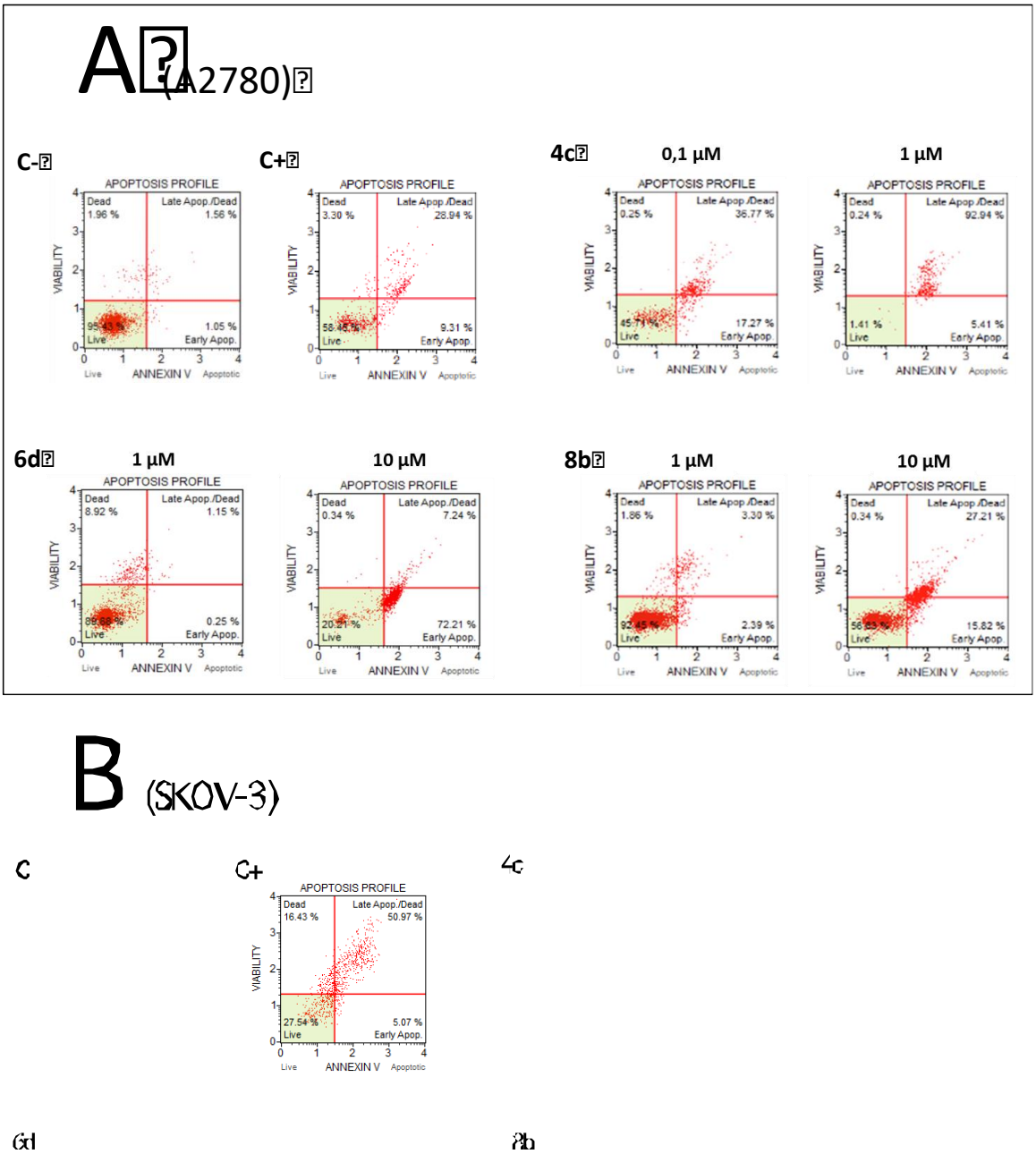


Figure 3 Representative apoptosis profile of A2780 (panel A) and SKOV-3 (panel B) cells untreated (**C-**), treated with cisplatin (**C+**) and with complexes **4c**, **6d** and **8b** (1-10 µM) for 72 h.

Conclusions

In this contribution we have proposed a complete and optimised protocol for the synthesis of novel cationic allyl palladium complexes bearing NHC ligands with purinic framework. The combination of the palladium-allyl organometallic function with the natural imprint of the spectator ligand seems to play a crucial role in promoting the cytotoxic properties of these compounds. Their antiproliferative activity has been tested *in vitro* on *cis*platin-sensitive A2780 and *cis*platin-resistant SKOV-3 cell lines and compared with cisplatin as etalon drug. The complexes **4c**, **4d**, and **5d** (for A2780) and **4d** for SKOV-3) are significantly more cytotoxic than cisplaltin, showing that the simultaneous coordination of one phosphine and one N-hetercocyclic carbene on the palladium allyl fragment represents the most promising configuration. Moreover it was proved that the anti-proliferative activity of many of the synthesized complexes is associated with induction of apoptosis.

A future extension of this encouraging first study on anti-tumoral properties of palladium allyl compounds will have as the primary aim the identification the true cellular target of these complexes and possibly the assessment of a more precise correlation between structure and activity.

Experimental

All syntheses of complexes were carried out using standard Schlenk techniques under an atmosphere of dry nitrogen. Solvents were dried and distilled according to standard methods: CH_2Cl_2 was firstly treated with 3Å molecular sieves and then distilled over P_2O_5 ; CH_3CN was distilled over CaH_2 and stored over 3Å molecular sieves. All the other chemicals were commercially available grade products and were used as purchased.

Complexes **3a**, **3b**, **3d** and **7a**, **7b**, **7d** were synthesized according to the procedure described in a previous work.[19]

The IR, ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer and on a Bruker 300 or 400 Avance spectrometer, respectively.

Elemental analysis was carried out using an Elemental CHN "CUBO Micro Vario" analyzer.

ESI-MS analyses were performed using a LCQ-Duo (Thermo-Finnigan) operating in positive ion mode. Instrumental parameters: capillary voltage 10 V, spray voltage 4.5 kV, capillary temperature 200 °C, mass scan range from 150 to 2000 amu, N_2 was used as sheath gas and the He pressure inside the trap was kept constant.

The pressure directly read by an ion gauge (in the absence of N_2 stream) was 1.33×10^{-5} Torr. Sample solutions were prepared by dissolving the compounds in acetonitrile and directly infused into the ESI source with a syringe pump at 8 μL min^{-1} flow rate.

General procedure for the synthesis of alkyl□functionalized caffeine, theophylline and theobromine (1)

5.55 mmol (ca. 1g) of the starting xanthine (theophylline or theobromine) and 1.15 g (8.33 mmol) of K_2CO_3, were suspended in 20 mL of DMF. Two equivalents (11.1 mmol) of the appropriate alkyl halide were added to the suspension and the resulting mixture was vigorously stirred overnight at R.T. Finally the compounds were precipitated by addition of H_2O (ca. 100 mL).

7-benzyl-1,3-dimethylxanthine (1b)

White solid, yield 93 %.

^{1}H-NMR (400 MHz, $CDCl_3$, T=298K, ppm) δ: 3.43 (s, 3H, N-CH_3), 3.60 (s, 3H, N-CH_3), 5.52 (s, 2H, N-CH_2), 7.30-7.40 (m, 5H, Ph), 7.58 (s, 1H, NCHN).^{13}C{^{1}H}-NMR ($CDCl_3$, T=298K, ppm) δ: 28.0 (N-CH_3), 29.8 (N-CH_3), 50.3 (N-CH_2), 107.0 (C^5), 128.0, 128.7, 129.1, 135.4 (C_{Ph}), 140.9 (N-CH-N), 148.9 (C^4), 151.7 (C=O), 155.3 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_{14}H_{15}N_4O_2$ $[M+H]^+$ 271.12; found: 271.06.

Anal. Calcd. for $C_{14}H_{14}N_4O_2$: C 62.21, H 5.22, N 20.73. Found: C 62.32, H 5.14, N 20.64.

7-phenylpropargyl-1,3-dimethylxanthine (1c)

Pink solid, yield 95 %.

^{1}H-NMR (400 MHz, $CDCl_3$, T=298K, ppm) δ: 3.45 (s, 3H, N-CH_3), 3.63 (s, 3H, N-CH_3), 5.42 (s, 2H, N-CH_2), 7.32-7.50 (m, 5H, Ph), 7.94 (s, 1H, NCHN). ≡

^{13}C{^{1}H}-NMR ($CDCl_3$, T=298K, ppm) δ: 28.0 (N-CH_3), 29.8 (N-CH_3), 37.4 (N-CH_2), 80.5 (≡C-CH_2), 87.6 (≡C-Ph), 106.8 (C^5), 121.5, 128.5, 129.2, 131.9 (C_{Ph}), 140.6 (N-CH-N), 148.9 (C^4), 151.7 (C=O), 155.3 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_{16}H_{15}N_4O_2$ $[M+H]^+$ 295.12; found: 294.98.

Anal. Calcd. for $C_{16}H_{14}N_4O_2$: C 65.30, H 4.79, N 19.04. Found: C 65.49, H 4.83, N 18.89.

1-benzyl-3,7-dimethylxanthine (1d)

White solid, yield 95 %.

^{1}H-NMR (400 MHz, $CDCl_3$, T=298K, ppm) δ: 3.59 (s, 3H, N-CH_3), 4.00 (s, 3H, N-CH_3), 5.22 (s, 2H, N-CH_2), 7.25-7.55 (m, 5H, Ph), 7.51 (s, 1H, NCHN). ^{13}C{^{1}H}-NMR ($CDCl_3$, T=298K, ppm) δ: 29.8 (N-CH_3), 33.6 (N-CH_3), 44.5 (N-CH_2), 107.7 (C^5), 127.5, 128.4, 128.8, 137.3 (C_{Ph}), 141.5 (N-CH-N), 148.9 (C^4), 151.6 (C=O), 155.3 (C=O).

ESI ESI-MS (CH_3CN): m/z Calcd. for $C_{14}H_{15}N_4O_2$ $[M+H]^+$ 271.12; found: 271.14.

Anal. Calcd. for $C_{14}H_{14}N_4O_2$: C 62.21, H 5.22, N 20.73 . Found: C 62.45, H 5.07, N 20.99.

General procedure for the synthesis of the imidazolium salts from functionalized theophylline and theobromine (2)

Into a 50 mL flask, 1.8 mmol of functionalized xanthine (**1a□d** with **1a** = commercial caffeine) were dissolved in ca. 25 mL of CH_3CN. After the addition of one equivalent (1.8 mmol) of Me_3OBF_4 the resulting solution was vigorously stirred for 5 min and then 100 mg of Na_2CO_3 were added to the mixture which was stirred for 45 min. Further addition of 0.6 equivalents (1.1 mmol) of Me_3OBF_4 and 10 min of additional stirring led to virtual completion. The excess of base Na_2CO_3 and the $NaBF_4$ formed were filtered off and the solvent completely removed under reduced pressure. The solid was washed with three aliquots of a 2:1 mixture of Et_2O/ CH_2Cl_2 on a sintered glass filter, dried under vacuum and characterized.

1,3,7,9-tetramethylxanthinium tetrafluoroborate (2a)

White solid, yield 97 %, m. p. = 132-133 °C.

^{1}H-NMR (400 MHz, CD_3CN, T=298K, ppm) δ: 3.35 (s, 3H, N-CH_3), 3.74 (s, 3H, N-CH_3), 4.08 (s, 6H, 2N-CH_3), 8.47 (s, 1H, NCHN). ^{13}C{^{1}H}-NMR (CD_3CN, T=298K, ppm) δ: 28.7 (N-CH_3), 31.8 (N-CH_3), 36.3 (N-CH_3), 37.6 (N-CH_3), 109.1 (C^5), 139.3(N-CH-N), 140.2 (C^4), 151.1 (C=O), 154.2 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_9H_{13}N_4O_2$ $[M]^+$ 209.10; found: 209.07.

Anal. Calcd. for $C_9H_{13}BF_4N_4O_2$: C 36.52, H 4.43, N 18.93. Found: C 36.74, H 4.61, N 18.88

7-benzyl-1,3,9-trimethylxanthinium tetrafluoroborate (2b)

White solid, yield 91 %, m. p. = 251-253 °C

^{1}H-NMR (400 MHz, CD_3CN, T=298K, ppm) δ□□3.33 (s, 3H, N-CH_3), 3.73 (s, 3H, N-CH_3), 4.06 (s, 3H, N-CH_3), 5.70 (s, 2H, N-CH_2), 7.45-7.48 (m, 5H, Ph), 8.56 (s, 1H, NCHN). ^{13}C{^{1}H}-NMR (CD_3CN, T=298K, ppm) δ□□28.8 (N-CH_3), 31.8 (N-CH_3), 37.9 (N-CH_3), 52.7 (N-CH_2), 108.4 (C^5), 129.2, 129.7, 129.8, 133.9 (C_{Ph}), 138.9(N-CH-N), 140.6 (C^4), 151.0 (C=O), 154.0 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_{15}H_{17}N_4O_2$ $[M]^+$ 285.13; found: 285.04.

Anal. Calcd. for $C_{15}H_{17}BF_4N_4O_2$: C 48.41, H 4.60, N 15.06. Found: C 48.39, H 4.64, N 15.18.

7-phenylpropargyl-1,3,9-trimethylxanthinium tetrafluoroborate (2c)

White solid, yield 92 % (612 mg), m.p. = 231 dec. °C.

^{1}H-NMR (400 MHz, CD_3CN, T=298K, ppm) δ: 3.37 (s, 3H, N-CH_3), 3.76 (s, 3H, N-CH_3), 4.14 (s, 3H, N-CH_3), 5.57 (s, 2H, N-CH_2), 7.42-7.60 (m, 5H, Ph), 8.85 (s, 1H, NCHN). ^{13}C{^{1}H}-NMR (CD_3CN, T=298K, ppm) δ: 28.8 (N-CH_3), 31.9 (N-CH_3), 37.9 (N-CH_3), 40.7 (N-CH_2), 79.2 (≡<u>C</u>-CH_2), 89.4 (≡<u>C</u>-Ph), 108.3 (C^5), 121.6, 129.4, 130.4, 132.5 (C_{Ph}), 138.8 (N-CH-N), 140.6 (C^4), 151.0 (C=O), 154.0 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_{17}H_{17}N_4O_2$ $[M]^+$ 309.13; found: 309.02.

Anal. Calcd. for $C_{17}H_{17}BF_4N_4O_2$: C 51.54, H 4.33, N 14.14. Found: C 51.73, H 4.28, N 14.31.

1-benzyl-3,7,9-trimethylxanthinium tetrafluoroborate (2d)

White solid, yield 94 % (621 mg), m. p. = 171-172 °C.

^{1}H-NMR (400 MHz, CD_3CN, T=298K, ppm) δ: 3.74 (s, 3H, N-CH_3), 4.08 (s, 3H, N-CH_3), 4.09 (s, 3H, N-CH_3), 5.17 (s, 2H, N-CH_2), 7.28-7.42 (m, 5H, Ph), 8.48 (s, 1H, NCHN). ^{13}C{^{1}H}-NMR (CD_3CN, T=298K, ppm) δ: 31.9 (N-CH_3), 36.4 (N-CH_3), 37.6 (N-CH_3), 45.6 (N-CH_2), 109.2 (C^5), 128.3, 128.6, 129.1, 137.1 (C_{Ph}), 139.4 (N-CH-N), 140.5 (C^4), 151.0 (C=O), 154.1 (C=O).

ESI-MS (CH_3CN): m/z Calcd. for $C_{15}H_{17}N_4O_2$ $[M]^+$ 285.13; found: 285.11.

Anal. Calcd. for $C_{15}H_{17}BF_4N_4O_2$: C 48.41, H 4.60, N 15.06. Found: C 48.65, H 4.72, N 15.14.

Preparation of a 1:1 mixture of 3c and $AgBF_4$

0.1130 g (0.2852 mmol) of the imidazolium salt **2c** was dissolved in 30 mL of anhydrous CH_3CN into a 100 mL two necked flask and 0.09364 g (0.1571 mmol) of Ag_2O was added under inert atmosphere (Ar).

The mixture was stirred for 24 h at R.T. in the dark. The solution was filtered on a zmillipore membrane filter in order to remove the Ag_2O in excess. The resulting clear solution was concentrated under vacuum and the title complex precipitated by addition of diethylether. The white complex was separated by filtration and repeatedly washed with diethylether and *n*-pentane and finally dried under vacuum.

0.1305 g (yield 91%) of 1:1 mixture of **3c** and $AgBF_4$ was obtained.

^{1}H-NMR (400 MHz, CD_3CN, T=298K, ppm) δ: 3.35 (s, 6H, 2NCH_3), 3.70 (s, 6H, 2NCH_3), 4.03 (s, 6H, 2NCH_3), 5.40 (s, 4H, 2NCH_2), 7.27-7.43 (m, 10H, 2Ph). ^{13}C{^{1}H}-NMR (CD_3CN, T=298K, ppm) δ: 27.9 (CH_3, NCH_3), 31.4 (CH_3, NCH_3), 40.1 (CH_2, NCH_2), 40.2 (CH_3, NCH_3), 82.5 (C, CH_2-<u>C</u>≡), 88.7 (C, Ph-<u>C</u>≡), 108.7 (C, C^5), 121.3-131.8 (Ph), 140.4 (C, C^4), 150.7 (C, C=O), 153.5 (C, C=O), 187.1 (C, carbene).

IR (KBr): $\nu_{C\equiv C}$= 2218 cm^{-1}, ν_{CO}= 1710, 1668 cm^{-1}, ν_{BF}=1054 cm^{-1}.

Anal. Calcd. for $C_{34}H_{32}Ag_2B_2F_8N_8O_4$: C 40.59, H 3.21, N 11.14. Found: C 40.82, H 3.04, N 11.35.

Synthesis of complex 4a

0.0195 g (0.053 mmol) of the dimer $[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$ and 0.0279 g (0.106 mmol) of PPh_3 were dissolved in ca. 20 mL of anhydrous CH_3CN into a 50 mL two necked flask under inert atmosphere (Ar). The resulting mixture was treated with 0.0388 g (0.048 mmol) of **3a**/$AgBF_4$ and stirred at RT for ca. 15 min.

The precipitated AgCl was removed by filtration on a millipore membrane filter.

Addition of diethylether to the concentrated solution yielded the precipitation of the complex **4a** as a yellow solid which was filtered off on a gooch and washed with *n*-pentane.

0.0576 g of **4a** was obtained (yield 85%).

^{1}H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 3.16 (d, 1H, J= 13.4 Hz, *anti* allyl-H *trans* C), 3.26 (d, 1H, J= 13.7 Hz, *anti* allyl-H *trans* C), 3.27 (m, 1H, *anti* allyl-H *trans* P), 3.38 (s, 6H, $2NCH_3$), 3.59 (s, 6H, $2NCH_3$), 3.61 (s, 6H, $2NCH_3$), 3.68 (m, 1H, *anti* allyl-H *trans* P), 3.76 (s, 3H, NCH_3), 3.78 (s, 3H, NCH_3), 4.11 (d, 1H, J= 8.4 Hz, *syn* allyl-H *trans* C), 4.19 (d, 1H, J= 7.6 Hz, *syn* allyl-H *trans* C), 4.59 (dd, 1H, $J_{H\text{-}H}=J_{H\text{-}P}$= 5.9 Hz, *syn* allyl-H *trans* P), 4.80 (dt, 1H, $J_{H\text{-}H}=J_{H\text{-}P}$= 6.6 Hz, *syn* allyl-H *trans* P), 5.71 (m, 1H, *central* allyl-H), 5.99 (m, 1H, *central* allyl-H), 7.25-7.53 (m, 30H, 6Ph). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 28.6 (CH_3, NCH_3), 31.7 (CH_3, NCH_3), 36.9 (CH_3, NCH_3), 37.1 (CH_3, NCH_3), 38.7 (CH_3, NCH_3), 38.9 (CH_3, NCH_3), 68.5 (d, CH_2, $J_{C\text{-}P}$= 28.5 Hz, allyl *trans P*), 69.3 (d, CH_2, $J_{C\text{-}P}$= 27.6 Hz, allyl *trans P*), 69.4 (d, CH_2, $J_{C\text{-}P}$= 1.8 Hz, allyl *trans C*), 69.4 (d, CH_2, $J_{C\text{-}P}$= 1.7 Hz, allyl *trans C*), 110.4 (C, C^5), 110.6 (C, C^5), 121.4 (d, CH, $J_{C\text{-}P}$= 5.4 Hz, *central* allyl), 122.8 (d, CH, $J_{C\text{-}P}$= 5.1 Hz, *central* allyl), 129.2-133.2 (Ph), 141.0 (C, C^4), 141.1 (C, C^4), 150.4 (C, C=O), 153.0 (C, C=O), 185.8 (d, C, $J_{C\text{-}P}$= 19.7 Hz, carbene), 186.2 (d, C, $J_{C\text{-}P}$= 19.3 Hz, carbene). $^{31}P\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 25.9, 25.8.

IR (KBr): ν_{CO}= 1709, 1668 cm^{-1}, ν_{BF}=1059 cm^{-1}.

Anal. Calcd. for $C_{30}H_{32}BF_4N_4O_2PPd$: C 51.12, H 4.58, N 7.95. Found: C 51.42, H 4.32, N 8.10.

Synthesis of complex 4b

Complex **4b** was prepared in an analogous manner to that described for **4a** starting from 0.0144 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0358 g of **3b**/$AgBF_4$ and 0.0196 g of PPh_3.

0.0511 g (yield 88%) of **4b** was obtained.

^{1}H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 2.38 (m, 1H, *anti* allyl-H *trans* P), 2.93 (d, 1H, J= 13.6 Hz, *anti* allyl-H *trans* C), 3.17 (d, 1H, J= 13.4 Hz, *anti* allyl-H *trans* C), 3.40 (s, 3H, NCH_3), 3.42 (s, 3H, NCH_3), 3.60 (m, 1H, *anti* allyl-H *trans* P), 3.62 (s, 3H, NCH_3), 3.63 (s, 3H, NCH_3), 3.86 (s, 3H, NCH_3), 3.96 (dd, 1H, J_{H-H} = 6.8 Hz, J_{H-P} = 6.8 Hz, *syn* allyl-H *trans* P), 4.09 (d, 1H, J= 6.4 Hz, *syn* allyl-H *trans* C), 4.19 (d, 1H, J= 6.1 Hz, *syn* allyl-H *trans* C), 4.70 (dd, 1H, J_{H-H} = 7.7 Hz, J_{H-P} = 7.7 Hz, *syn* allyl-H *trans* P), 4.80 and 5.57 (AB system, 2H, J=14.8 Hz, NCH_2), 5.03 and 5.63 (AB system, 2H, J=15.0 Hz, NCH_2), 5.22 (m, 1H, *central* allyl-H), 5.97 (m, 1H, *central* allyl-H), 7.00-7.60 (m, 40H, 8Ph).

^{13}C{^{1}H}-NMR (T=298K, $CDCl_3$, ppm) δ: 28.7 (CH_3, NCH_3), 28.8 (CH_3, NCH_3), 31.9 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 39.5 (CH_3, NCH_3), 52.4 (CH_2, NCH_2), 52.7 (CH_2, NCH_2), 68.6 (d, CH_2, J_{C-P}= 1.9 Hz, allyl *trans*-C), 68.7 (d, CH_2, J_{C-P}= 1.8 Hz, allyl *trans*-C), 70.1 (d, CH_2, J_{C-P}= 27.7 Hz, allyl *trans*-P), 70.8 (d, CH_2, J_{C-P}= 27.8 Hz, allyl *trans*-P), 110.1 (C, C^5), 110.3 (C, C^5), 121.0 (CH, *central* allyl), 122.8 (CH, *central* allyl), 127.2-135.3 (Ph), 141.1 (C^4), 141.3 (C^4), 143.7 (Ph), 150.3 (C, C=O), 150.4 (C, C=O), 152.8 (C, C=O), 152.9 (C, C=O), 187.5 (d, C, J_{C-P}= 18.5 Hz, carbene), 187.8 (d, C, J_{C-P}= 18.4 Hz, carbene). ^{31}P{^{1}H}-NMR (T=298K, $CDCl_3$, ppm) δ: 25.6, 26.3

IR (KBr): ν_{CO}= 1709, 1668 cm^{-1}, ν_{BF}=1056 cm^{-1}

Anal. Calcd. for $C_{36}H_{36}BF_4N_4O_2PPd$: C 55.37, H 4.65, N 7.17. Found: C 55.12, H 4.80, N 7.38.

Synthesis of complex 4c

Complex **4c** was prepared in an analogous manner to that described for **4a** starting from 0.0160 g of [Pd(μ-Cl)(η^3-allyl)]$_2$, 0.0418 g of **3c**/$AgBF_4$ and 0.0218 g of PPh_3.

0.0559 g (yield 84%) of **4c** was obtained.

^{1}H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 2.97 (d, 1H, J= 13.3 Hz, *anti* allyl-H *trans* C), 3.20 (d, 1H, J= 12.8 Hz, *anti* allyl-H *trans* C), 3.36 (m, 1H, *anti* allyl-H *trans* P), 3.42 (s, 6H, 2NCH_3), 3.61 (s, 3H, NCH_3), 3.62 (s, 3H, NCH_3), 3.68 (s, 3H, NCH_3), 3.76 (m, 1H, *anti* allyl-H *trans* P), 3.82 (s, 3H, NCH_3), 4.04 (d, 1H, J= 6.1 Hz, *syn* allyl-H *trans* C), 4.22 (d, 1H, J= 6.4 Hz, *syn* allyl-H *trans* C), 4.71 (dd, 1H, J_{H-H} = 6.0 Hz, J_{H-P} = 6.8

Hz,, *syn* allyl-H *trans* P), 4.82 and 5.28 (AB system, 2H, J=17.2 Hz, NCH_2), 4.88 and 5.47 (AB system, 2H, J=17.6 Hz, NCH_2), 4.89 (m, 1H, *syn* allyl-H *trans* P), 5.63 (m, 1H, *central* allyl-H), 6.03 (m, 2H, *central* allyl-H), 7.15-7.46 (m, 40H, 8Ph). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 28.7 (CH_3, NCH_3), 31.8 (CH_3, NCH_3), 39.4 (CH_3, NCH_3), 40.0 (CH_2, NCH_2), 68.9 (CH_2, allyl *trans*-C), 69.3 (CH_2, allyl *trans*-C), 69.9 (d, CH_2, J_{C-P}= 27.5 Hz, allyl *trans*-P), 70.4 (d, CH_2, J_{C-P}= 27.6 Hz, allyl *trans*-P), 81.6 (C, ≡C-CH_2), 81.9 (C, ≡C-CH_2), 86.5 (C, ≡C-Ph), 87.4 (C, ≡C-Ph), 109.6 (C, C^5), 109.7 (C, C^5), 121.4 (d, CH, J_{C-P}= 5.1 Hz, *central* allyl), 122.7 (d, CH, J_{C-P}= 5.2 Hz, *central* allyl), 128.4-134.0 (Ph), 141.2 (C, C^4), 150.4 (C, C=O), 152.6 (C, C=O), 152.7 (C, C=O), 187.3 (d, C, J_{C-P}= 18.8 Hz, carbene), 187.7 (d, C, J_{C-P}= 18.5 Hz, carbene). $^{31}P\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 25.4, 25.9.

IR (KBr): ν_{CO}= 1709, 1667 cm^{-1}, ν_{BF}=1058 cm^{-1}

Anal. Calcd. for $C_{38}H_{36}BF_4N_4O_2PPd$: C 56.70, H 4.51, N 6.96. Found: C 56.52, H 4.78, N 7.12.

Synthesis of the complex 4d

Complex **4d** was prepared in an analogous manner to that described for **4a** starting from 0.0154 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0383 g of **3d**/$AgBF_4$ and 0.0210 g of PPh_3.

0.0522 g (yield 84%) of **4d** was obtained.

^1H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 3.13 (d, 1H, J= 13.4 Hz, *anti* allyl-H *trans* C), 3.28 (d+m, 2H, J= 13.2 Hz, *anti* allyl-H *trans* C), 3.29 (m, 1H, *anti* allyl-H *trans* P), 3.58 (s, 3H, NCH_3), 3.59 (s, 3H, NCH_3), 3.60 (s, 3H, NCH_3), 3.62 (s, 3H, NCH_3), 3.68 (m, 1H, *anti* allyl-H *trans* P), 3.74 (s, 3H, NCH_3), 3.81 (s, 3H, NCH_3), 4.13 (d, 1H, J= 7.4 Hz, *syn* allyl-H *trans* C), 4.21 (d, 1H, J= 7.8 Hz, *syn* allyl-H *trans* C), 4.58 (dd, 1H, J_{H-H}=J_{H-P}= 5.2 Hz, *syn* allyl-H *trans* P), 4.79 (dd, 1H, J_{H-H} = 5.5 Hz, J_{H-P} = 5.5 Hz, *syn* allyl-H *trans* P), 5.15 (s, 4H, 2 NCH_2), 5.70 (m, 1H, *central* allyl-H), 5.99 (m, 1H, *central* allyl-H), 7.25-7.47 (m, 40H, 8Ph). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 31.8 (CH_3, NCH_3), 36.9 (CH_3, NCH_3), 37.0 (CH_3, NCH_3), 38.7 (CH_3, NCH_3), 38.9 (CH_3, NCH_3), 45.1 (CH_2, NCH_2), 68.3 (d, CH_2, J_{C-P}= 28.4 Hz, allyl *trans*-P), 69.1 (d, CH_2, J_{C-P}= 28.1 Hz, allyl *trans*-P), 69.2 (d, CH_2, J_{C-P}= 1.7 Hz, allyl *trans*-C), 69.4 (d, CH_2, J_{C-P}= 1.9 Hz, allyl *trans*-C), 110.7 (C, C^5), 121.3 (d, CH, J_{C-P}= 5.5 Hz, *central* allyl), 122.8 (d, CH, J_{C-}

$_P$= 5.3 Hz, *central* allyl), 127.9-136.4 (Ph), 141.0 (C, C^4), 141.1 (C, C^4), 150.2 (C, C=O), 152.7 (C, C=O), 186.1 (d, C, J_{C-P}= 19.3 Hz, carbene), 186.5 (d, C, J_{C-P}= 19.4 Hz, carbene). $^{31}P\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 25.9, 26.0.

IR (KBr): ν_{CO}= 1707, 1668 cm^{-1}, ν_{BF}=1056 cm^{-1}.

Anal. Calcd. for $C_{36}H_{36}BF_4N_4O_2PPd$: C 55.37, H 4.65, N 7.17. Found: C 55.22, H 4.58, N 7.42.

Synthesis of complex 5a

0.0411 g (0.112 mmol) of the dimer $[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$ and 0.0353 g (0.224 mmol) of PTA (1,3,5-triaza-7-phosphadamantane) were dissolved in ca. 30 mL of anhydrous CH_3CN in a 100 mL two necked flask under inert atmosphere (Ar). The resulting mixture was treated with 0.0787 g (0.0975 mmol) of **3a**/$AgBF_4$ and stirred at RT for ca. 1 hour.

The precipitated AgCl was removed by filtration on a millipore membrane filter.

The solution was dried under vacuum and the residue treated with 2 mL of CH_2Cl_2.

Addition of diethylether to the concentrated solution yielded the precipitation of the complex **5a** as a brownish solid which was filtered off on a gooch, washed with *n*-pentane and dried under vacuum.

0.0991 g of **5a** was obtained (yield 97%).

^1H-NMR (400 MHz, T=298K, D_2O, ppm) δ: 2.88 (d, 2H, J= 13.2 Hz, 2 *anti* allyl-H *trans*-C), 3.15 (m, 2H, 2 *anti* allyl-H *trans*-P), 3.32 (s, 3H, NCH_3), 3.33 (s, 3H, NCH_3), 3.74 (s, 3H, NCH_3), 3.77 (s, 3H, NCH_3), 3.80 (s, 3H, NCH_3), 3.87 (s, 3H, NCH_3), 3.92 (s, 3H, NCH_3), 4.05 (s, 3H, NCH_3), 4.21 (s, 12H, 6 NCH_2P_{PTA}), 4.38 (d, 2H, J= 7.4 Hz, 2 *syn* allyl-H *trans*-C), 4.47 (m, 2H, 2 *syn* allyl-H *trans*-P), 4.55 (m, 12H, 6 NCH_2N_{PTA}), 5.48 (m, 2H, 2 *central* allyl-H). $^{13}C\{^1H\}$-NMR (T=298K, D_2O, ppm) δ: 28.4 (CH_3, NCH_3), 31.8 (CH_3, NCH_3), 36.7 (CH_3, NCH_3), 36.9 (CH_3, NCH_3), 38.2 (CH_3, NCH_3), 38.4 (CH_3, NCH_3), 50.3 (CH_2, NCH_2P, J_{C-P} =13.6 Hz), 62.7 (CH_2, allyl *trans*-C), 62.8 (CH_2, allyl *trans*-C), 68.9 (d, CH_2, J_{C-P}= 4.8 Hz, allyl *trans*-P), 69.2 (d, CH_2, J_{C-P}= 5.2 Hz, allyl *trans*-P), 70.7 (CH_2, NCH_2N), 70.8 (CH_2, NCH_2N), 111.2 (C, C^5), 111.4 (C, C^5), 121.9 (CH, *central* allyl), 122.0 (CH, *central* allyl), 141.8 (C, C^4), 141.9 (C, C^4), 151.8 (C, C=O), 154.5 (C, C=O), 183.9 (C, J_{C-P}= 21.2 Hz, carbene). $^{31}P\{^1H\}$-NMR (T=298K, D_2O, ppm) δ: -52.4

IR (KBr): ν_{CO}= 1704, 1665 cm^{-1}, ν_{BF}=1031, 1084 cm^{-1}.

Anal. Calcd. for $C_{18}H_{29}BF_4N_7O_2PPd$: C 36.05, H 4.87, N 16.35. Found: C 36.34, H 4.97, N 16.14.

Synthesis of complex 5b

Complex **5b** was prepared in an analogous manner to that described for **5a** starting from 0.0353 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0804 g of **3b**/$AgBF_4$ and 0.0304 g of PTA.

0.0925 g (yield 82%) of **5b** was obtained.

^{1}H-NMR (400 MHz, T=298K, D_2O, ppm) δ: 2.76 (m, 2H, 2 *anti* allyl-H *trans*-C), 3.06 (m, 2H, 2 *anti* allyl-H *trans*-P), 3.28 (s, 3H, NCH_3), 3.29 (s, 3H, NCH_3), 3.72 (s, 6H, 3 NCH_2P_{PTA}), 3.75 (s, 6H, 3 NCH_2P_{PTA}), 3.77 (s, 3H, NCH_3), 3.80 (s, 3H, NCH_3), 3.88 (s, 3H, NCH_3), 4.06 (s, 3H, NCH_3), 4.27-4.70 (m, 16 H, 6 NCH_2N_{PTA} and 2 *syn* allyl-H *trans*-C and 2 *syn* allyl-H *trans*-P), 5.07 and 5.64 (AB system, 2H, J=15.4 Hz, NCH_2), 5.29 and 5.76 (AB system, 2H, J=15.7 Hz, NCH_2), 5.36 (m, 2H, 2 *central* allyl-H), 6.81-7.36 (m, 10H, 2Ph). $^{13}C\{^1H\}$-NMR (T=298K, D_2O, ppm) δ: 28.5 (CH_3, NCH_3), 31.9 (CH_3, NCH_3), 38.6 (CH_3, NCH_3), 38.9 (CH_3, NCH_3), 49.9 (d, CH_2, J= 13.5 Hz, NCH_2P), 50.0 (d, CH_2, J= 13.6 Hz, NCH_2P), 52.5 (CH_2, NCH_2), 52.7 (CH_2, NCH_2), 62.6 (CH_2, allyl *trans*-C), 63.0 (CH_2, allyl *trans*-C), 69.4 (d, CH_2, $J_{C\text{-}P}$= 26.7 Hz, allyl *trans*-P), 69.9 (d, CH_2, $J_{C\text{-}P}$= 27.8 Hz, allyl *trans*-P), 70.4 (CH_2, NCH_2N_{PTA}) 70.5 (CH_2, NCH_2N), 111.1 (C, C^5), 111.4 (C, C^5), 121.9 (CH, *central* allyl), 126.9-136.7 (Ph), 141.8 (C, C^4), 141.9 (C, C^4), 151.8 (C, C=O), 154.2 (C, C=O), 186.0 (d, C, $J_{C\text{-}P}$= 20.4, carbene), 186.1 (d, C, $J_{C\text{-}P}$= 20.5 Hz, carbene). $^{31}P\{^1H\}$-NMR (T=298K, D_2O, ppm) δ: -54.1, -54.0.

IR (KBr): ν_{CO}= 1705, 1664 cm^{-1}, ν_{BF}=1035, 1083 cm^{-1}

Anal. Calcd. for $C_{24}H_{33}BF_4N_7O_2PPd$: C 42.66, H 4.92, N 14.51. Found: C 42.82, H 4.78, N 14.22.

Synthesis of complex 5d

Complex **5d** was prepared in an analogous manner to that described for **5a** starting from 0.0352 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0800 g of **3d**/$AgBF_4$ and 0.0301 g of PTA.

0.1057 g (yield 94%) of **5d** was obtained.

^{1}H-NMR (400 MHz, T=298K, D_2O, ppm) δ: 2.83 (d, J= 13.8 Hz, 2H, 2 *anti* allyl-H *trans*-C), 3.07 (m, 2H, 2 *anti* allyl-H *trans*-P), 3.69 (s, 3H, NCH_3), 3.70 (s, 3H, NCH_3), 3.73 (s, 3H, NCH_3), 3.82 (s, 3H, NCH_3), 3.87 (s, 3H, NCH_3), 4.01 (s, 3H, NCH_3), 4.15 (s, 6H, 3 NCH_2P_{PTA}), 4.27 (s, 6H, 3 NCH_2P_{PTA}), 4.32 (d, J = 6.6 Hz, 2H, 2 *syn* allyl-H *trans*-C), 4.45 (m, 2H, 2 *syn* allyl-H *trans*-P), 4.50-4.70 (m, 12H, 6 NCH_2P_{PTA}), 5.10 (2s, 4H, 2 N-CH_2), 5.42 (m, 2H, 2 *central* allyl-H), 7.25-7.30 (m, 10H, 2Ph).

^{13}C{^{1}H}-NMR (T=298K, D_2O, ppm) δ: 31.9 (CH_3, NCH_3), 36.8 (CH_3, NCH_3), 37.1 (CH_3, NCH_3), 38.2 (CH_3, NCH_3), 38.5 (CH_3, NCH_3), 45.2 (CH_2, NCH_2), 50.3 (d, CH_2, J_{C-P}= 13.8 Hz, NCH_2P), 51.1 (d, CH_2, J_{C-P}= 15.4 Hz, NCH_2P), 62.8 (CH_2, allyl *trans*-C), 62.9 (CH_2, allyl *trans*-C), 69.2 (d, CH_2, J_{C-P}= 27.7 Hz, allyl *trans*-P), 70.8 (CH_2, NCH_2N), 71.3 (d, CH_2, J_{C-P}= 26.2 Hz, allyl *trans*-P), 111.4 (C^5), 122.0 (CH, *central* allyl), 123.0 (CH, *central* allyl), 127.2-136.2 (Ph), 142.3 (C, C^4), 151.6 (C, C=O), 154.2 (C, C=O), 184.2 (d, C, J_{C-P}= 19.0 Hz, carbene), 184.3 (d, C, J_{C-P}= 19.8 Hz, carbene). ^{31}P{^{1}H}-NMR (T=298K, D_2O, ppm) δ: -54.2, -53.1.

IR (KBr): ν_{CO}= 1708, 1668 cm^{-1}, ν_{BF}=1062, 1084 cm^{-1}

Anal. Calcd. for $C_{24}H_{33}BF_4N_7O_2PPd$: C 42.66, H 4.92, N 14.51. Found: C 42.52, H 4.99, N 14.32.

Synthesis of complex 6a

0.0173 g (0.047 mmol) of the dimer [Pd(μ-Cl)(η^3-C_3H_5)]$_2$ and 0.0124 g (0.094 mmol) of DIC (2,6-dimetilphenyl isocyanide) were dissolved in ca. 20 mL of anhydrous CH_3CN in a 50 mL two necked flask under inert atmosphere (Ar). The resulting mixture was treated with 0.0344 g (0.043 mmol) of **3a**/$AgBF_4$ and stirred at RT for ca. 15 min.

The precipitated AgCl was removed by filtration on a millipore membrane filter.

Addition of diethylether to the concentrated solution yielded the precipitation of the complex **6a** as a brownish solid which was filtered off on a gooch and washed with *n*-pentane.

0.0431 g of **6a** was obtained (yield 88%).

^{1}H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 2.41 (s, 6H, 2CH_3^{DIC}), 3.20 (d, 1H, J= 13.3 Hz, *anti* allyl-H), 3.40 (s, 3H, NCH_3), 3.41 (d, 1H, J= 12.7 Hz, *anti* allyl-H), 3.88 (s, 3H, NCH_3), 4.05 (s, 3H, NCH_3), 4.20 (s, 3H, NCH_3), 4.47 (d, 1H, J= 6.4 Hz, *syn* allyl-H),

4.79 (d, 1H, J= 7.5 Hz, *syn* allyl-H), 5.72 (m, 1H, *central* allyl-H), 7.17-7.31 (m, 3H, Ph^{DIC}). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 18.7 (CH_3, CH_3^{DIC}), 28.6 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 37.5 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 65.0 (CH_2, allyl-C), 69.3 (CH_2, allyl-C), 110.8 (C, C^5), 122.2 (*central* allyl), 128.4-135.6 (Ph), 141.4 (C, C^4), 150.6 (C, C=O), 150.7 (C, CN^{DIC}), 153.4 (C, C=O), 181.5 (C, carbene).

IR (KBr): ν_{CN}=2175 cm^{-1}, ν_{CO}= 1706, 1665 cm^{-1}, ν_{BF}=1056 cm^{-1}.

Anal. Calcd. for $C_{21}H_{26}BF_4N_5O_2Pd$: C 43.97, H 4.57, N 12.21. Found: C 44.24, H 4.77, N 12.04.

Synthesis of complex 6b

Complex **6b** was prepared in an analogous manner to that described for **6a** starting from 0.0180 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0428 g of **3b**/$AgBF_4$ and 0.128 g of DIC.

0.0491 g (yield 85%) of **6b** was obtained.

^1H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 2.36 (s, 6H, $2CH_3^{DIC}$), 3.42 (s, 3H, NCH_3), 3.48 (bd, 2H, 2*anti* allyl-H), 3.91 (s, 3H, NCH_3), 4.24 (bd, 1H, s*yn* allyl-H), 4.25 (s, 3H, NCH_3), 4.67 (d, 1H, J= 7.5 Hz, *syn* allyl-H), 5.65 (s, 2H, NCH_2), 5.73 (m, 1H, *central* allyl-H), 7.16-7.33 (m, 8H, Ph and Ph^{DIC}). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 18.8 (CH_3, CH_3^{DIC}), 28.7 (CH_3, NCH_3), 32.1 (CH_3, NCH_3), 39.9 (CH_3, NCH_3), 53.0 (CH_2, NCH_2), 65.5 (CH_2, allyl-C), 110.6 (C, C^5), 121.9 (CH, *central* allyl), 127.5-135.9 (Ph), 141.5 (C, C^4), 150.5 (C, C=O), 150.7 (C, CN^{DIC}), 153.2 (C, C=O), 183.5 (C, carbene).

IR (KBr): ν_{CN}=2175 cm^{-1}, ν_{CO}= 1709, 1670 cm^{-1}, ν_{BF}=1057 cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}BF_4N_5O_2Pd$: C 49.91, H 4.65, N 10.78. Found: C 49.84, H 4.83, N 10.90.

Synthesis of complex 6d

Complex **6d** was prepared in an analogous manner to that described for **6a** starting from 0.0184 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0437 g of **3d**/$AgBF_4$ and 0.0131 g of DIC.

0.0543 g (yield 92%) of **6d** was obtained.

^1H-NMR (400 MHz, T=298K, $CDCl_3$, ppm) δ: 2.41 (s, 6H, $2CH_3^{DIC}$), 3.19 (d, 1H, J= 13.5 Hz, *anti* allyl-H), 3.41 (d, 1H, J= 12.5 Hz, *anti* allyl-H), 3.86 (s, 3H, NCH_3), 4.05 (s,

3H, NCH_3), 4.19 (s, 3H, NCH_3), 4.47 (d, 1H, J= 7.1 Hz, *syn* allyl-H), 4.78 (d, 1H, J= 7.5 Hz, *syn* allyl-H), 5.18 (s, 2H, NCH_2), 5.72 (m, 1H, *central* allyl-H), 7.17-7.49 (m, 8H, Ph and Ph^{DIC}). $^{13}C\{^1H\}$-NMR (T=298K, $CDCl_3$, ppm) δ: 18.8 (CH_3, CH_3^{DIC}), 32.0 (CH_3, NCH_3), 37.5 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 45.2 (CH_2, NCH_2), 64.9 (CH_2, allyl-C), 110.9 (C, C^5), 122.2 (CH, *central* allyl), 127.8-136.3 (Ph), 141.5 (C, C^4), 150.5 (C, C=O), 150.6 (C, CN^{DIC}), 153.2 (C, C=O), 181.8 (C, carbene).

IR (KBr): ν_{CN}=2173 cm^{-1}, ν_{CO}= 1707, 1665 cm^{-1}, ν_{BF}=1056 cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}BF_4N_5O_2Pd$: C 49.91, H 4.65, N 10.78. Found: C 50.04, H 4.51, N 10.95.

Synthesis of complex 8a

0.0156 g (0.086 mmol) of the dimer $[Pd(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$ was dissolved in ca. 20 mL of anhydrous CH_3CN in a 50 mL two necked flask under inert atmosphere (Ar).

The resulting mixture was treated with 0.0690 g (0.171 mmol) of **3a**/$AgBF_4$, 0.0142 g (0.086 mmol) of KI and stirred at RT for ca. 15 min. The precipitated AgCl and AgI were removed by filtration on a millipore membrane filter. Addition of diethylether to the concentrated solution yielded the precipitation of the complex **8a** as a white solid which was filtered off on a gooch and washed with *n*-pentane.

0.0446 g of **8a** was obtained (yield 80%).

^1H-NMR (400 MHz, T=298K, CD_3CN, ppm) δ: 2.95 (d, 2H, J= 13.3 Hz, *anti* allyl-H), 3.30 (s, 6H, NCH_3), 3.73 (s, 6H, NCH_3), 3.93 (s, 6H, NCH_3), 4.02 (s, 6H, NCH_3), 4.12 (d, 2H, J= 7.4 Hz, *syn* allyl-H), 5.59 (m, 1H, *central* allyl-H). $^{13}C\{^1H\}$-NMR (T=298K, CD_3CN, ppm) δ: 28.4 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 37.7 (CH_3, NCH_3), 39.4 (CH_3, NCH_3), 61.6 (CH_2, allyl-C), 110.9 (C, C^5), 121.0 (CH, *central* allyl), 141.9 (C, C^4), 151.4 (C, C=O), 153.9 (C, C=O), 184.9 (C, carbene).

IR (KBr): ν_{CO}= 1706, 1668 cm^{-1}, ν_{BF}=1053 cm^{-1}.

Anal. Calcd. for $C_{21}H_{29}BF_4N_8O_4Pd$: C 38.76, H 4.49, N 17.22. Found: C 38.50, H 4.82, N 17.35.

Synthesis of complex 8b

Complex **8b** was prepared in an analogous manner to that described for **8a** starting from 0.0157 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0822 g of **3b**/$AgBF_4$ and 0.0142 g of KI.

0.0599 g (yield 87%) of **8b** was obtained.

^{1}H-NMR (400 MHz, T=298K, CD_3CN, ppm) δ: 2.79 (d, 2H, J= 13.2 Hz, *anti* allyl-H), 3.23 (s, 6H, NCH_3), 3.68 (s, 6H, NCH_3), 3.97 (s, 6H, NCH_3), 3.97 (bd, 2H, *syn* allyl-H), 5.39-5.61 (m, 5H, NCH_2 and *central* allyl-H), 6.94-7.24 (m, 10H, 2Ph).

$^{13}C\{^1H\}$-NMR (T=298K, CD_3CN, ppm) δ: 28.3 (CH_3, NCH_3), 31.9 (CH_3, NCH_3), 39.6 (CH_3, NCH_3), 53.0 (CH_2, NCH_2), 65.9 (CH_2, allyl-C), 110.4 (C, C^5), 121.2 (CH, *central* allyl), 125.7-137.2 (Ph), 142.0 (C, C^4), 151.2 (C, C=O), 153.2 (C, C=O), 185.3 (C, carbene).

IR (KBr): ν_{CO}= 1709, 1664 cm^{-1}, ν_{BF}=1058 cm^{-1}.

Anal. Calcd. for $C_{33}H_{37}BF_4N_8O_4Pd$: C 49.36, H 4.64, N 13.96. Found: C 49.57, H 4.12, N 14.22.

Synthesis of complex 8c

Complex **8c** was prepared in an analogous manner to that described for **8a** starting from 0.0157 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0863 g of **3c**/$AgBF_4$ and 0.0142 g of KI.

0.0592g (yield 81%) of **8c** was obtained.

^{1}H-NMR (400 MHz, T=298K, CD_3CN, ppm) δ: 3.02 (s, 6H, NCH_3), 3.03 (d, 2H, J= 13.4 Hz, *anti* allyl-H), 3.53 (s, 6H, NCH_3), 4.20 (m, 8H, NCH_3 and *syn* allyl-H), 5.44 (bs, 4H, NCH_2), 5.66 (m, 1H, *central* allyl-H), 7.16-7.48 (m, 10H, 2Ph). $^{13}C\{^1H\}$-NMR (T=298K, CD_3CN, ppm) δ: 28.4 (CH_3, NCH_3), 31.9 (CH_3, NCH_3), 39.8 (CH_3, NCH_3), 41.1 (CH_2, NCH_2), 65.9 (CH_2, allyl-C), 83.8 (CH_2-$\underline{C}$≡), 84.7 (C, Ph-$\underline{C}$≡), 110.4 (C, C^5), 121.6 (CH, *central* allyl), 121.9-131.0 (Ph), 141.7 (C, C^4), 150.5 (C, C=O), 153.4 (C, C=O), 185.5 (C, carbene).

IR (KBr): ν_{CO}= 1706, 1667 cm^{-1}, ν_{BF}=1056 cm^{-1}.

Anal. Calcd. for $C_{37}H_{37}BF_4N_8O_4Pd$: C 52.22, H 4.38, N 13.17. Found: C 52.37, H 4.10, N 13.55.

Synthesis of the complex 8d

Complex **8d** was prepared in an analogous manner to that described for **8a** starting from 0.0154 g of $[Pd(\mu\text{-}Cl)(\eta^3\text{-allyl})]_2$, 0.0806 g of **3d**/$AgBF_4$ and 0.0139 g of KI.

0.0595 g (yield 88%) of **8d** was obtained.

^{1}H-NMR (400 MHz, T=298K, CD_3CN, ppm) δ: 2.96 (d, 2H, J= 13.3 Hz, *anti* allyl-H), 3.73 (s, 6H, NCH_3), 3.93 (s, 6H, NCH_3), 4.02 (s, 6H, NCH_3), 4.13 (d, 2H, J= 7.4 Hz, 2 *syn* allyl-H), 5.13 (s, 4H, NCH_2), 5.59 (m, 1H, *central* allyl-H), 7.28-7.39 (m, 10H, 2Ph).

$^{13}C\{^1H\}$-NMR (T=298K, CD_3CN, ppm) δ: 31.5 (CH_3, NCH_3), 37.2 (CH_3, NCH_3), 38.8 (CH_3, NCH_3), 44.6 (CH_2, NCH_2), 61.1 (CH_2, allyl-C), 110.4 (C, C^5), 120.4 (CH, *central* allyl), 127.4-137.3 (Ph), 141.5 (C, C^4), 150.7 (C, C=O), 153.2 (C, C=O), 184.7 (C, carbene).

IR (KBr): ν_{CO}= 1708, 1667 cm^{-1}, ν_{BF}=1058 cm^{-1}.

Anal. Calcd. for $C_{33}H_{37}BF_4N_8O_4Pd$: C 49.36, H 4.64, N 13.96. Found: C 49.61, H 4.17, N 14.05.

Growth inhibition assays

Cell growth inhibition assays were carried out using two human ovarian cancer cell lines, A2780 and SKOV-3; A2780 cells are cisplatin-sensitive and SKOV-3 cells are cisplatin-resistant. Cell lines were obtained from ATCC (Manassas, VA) and maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL^{-1}), streptomycin (100 μg mL^{-1}) and glutamine (2 mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37°C in a 5% CO_2 atmosphere. Adherent cells were routinely used at 70% of confluence and passaged every 3 days by treatment with 0.05% trypsin-EDTA (Lonza).

Pure derivatives were added at serial dilutions and incubated for 3 days. After this time, cells were washed with PBS 1X and detached with trypsin. Cells were suspended in physiological solution and counted with a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/ml was determined as IC_{50} after 3 days of culture, when untreated cells are in log phase of cell growth [23,24]. All stock solutions were diluted in complete medium to give final concentrations. Cisplatin was employed as a control for the cisplatin-sensitive A2780, and for the cisplatin-resistant SKOV3.

Untreated cells were placed in every plate as negative control. The cells were exposed to the compounds in 1000 μL total volume for 72 hours.

Apoptosis assays

Annexin V and Dead Cell assays on IB3-1 cells, untreated and treated for 72 h with increasing doses of Palladium complexes, were performed with the Muse cell analyzer (Millipore, Billerica, MA, USA) method, according to the instructions supplied by the manufacturer. This procedure utilizes Annexin V to detect PS (PhosphatidylSerine) on the external membrane of apoptotic cells. A dead cell marker is also used as indicator of cell membrane structural integrity. Four populations of cells can be distinguished when using this assay: live, early apoptotic, late apoptotic and dead cells. Cells were washed with sterile PBS 1X, tripsinized, resuspended in the original medium and diluted (1:2) with the one step addition of the Muse Annexin V & Dead Cell reagent. After 20 min of incubation at room temperature, samples were analyzed, using Triton X 0.01%, as positive control [25]. Data from prepared samples were acquired and recorded utilizing the Annexin V and Dead Cell Software Module (Millipore, Billerica, MA, USA).

Crystal structure determinations

The crystal data of **4a** and **8d** were collected at 100K at the XRD1 beamline of the Elettra Synchrotron, Trieste (Italy)[26]. The data sets were integrated and corrected for Lorentz and polarization effects with the XDS package [27]. Data have been scaled using CCP4 Aimless code [28]. Crystals of **8d** showed significant radiation damage upon exposure to X-Rays, therefore data from three different crystals were merged to obtain a complete set of data. The structures were solved by direct methods using SHELXT program [29] and refined using full−matrix least−squares with all non−hydrogen atoms anisotropically and hydrogens included on calculated positions, riding on their carrier atoms. Geometric restrains on bond lengths and angles (DFIX, DANG) have been used in **4a** model for disordered fragments (i.e. CH_2Cl_2 solvent molecule and BF_4^- ion). Thermal parameters restrains (SIMU, ISOR and isotropic treatment of disordered allyl fragment)

have been introduced to successfully refine the structure of **8d**, impaired by electron density noise due to radiation damage effects and non-merohedral twinning (structure was refined as a 2-component twin, with domains related by twofold axis [1 0 0] and twin fraction refined to 7%). All calculations were performed using SHELXL−2017/1[30]. The Coot program was used for structure building [31]. The crystal data are given in Table S1. Pictures were prepared using Ortep3[32] software.

Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition numbers CCDC 1825947 (for **4a**) and 1825948 (for **8d**) These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures.

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