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Original article

Synthesis, biological evaluation and SAR studies of novel bicyclic antitumor platinum(IV) complexes

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ABSTRACT

The present study describes the synthesis, anticancer activity and SAR studies of novel platinum(IV) complexes having 1,2-bis(aminomethyl)carbobicyclic or oxabicyclic carrier ligands, bearing chlorido and/or hydroxido ligands in axial position and chlorido or malonato ligands in equatorial position (labile ligands). These complexes were synthetized with the aim of obtaining new anticancer principles more soluble in water and therefore more bioavailable. Several substitution patterns on the platinum atom have been designed in order to evaluate their antiproliferative activity and to establish structure–activity relationship rules. The synthesis of platinum(IV) complexes with axial hydroxyl ligands on the platinum(IV) were carried out by reaction of $K_2Pt(OH)_2Cl_4$ with the corresponding diamines. The complexes with axial chlorido ligands on the platinum(IV) atom were synthesized by direct reaction of diamines with K_2PtCl_6. Carboxylated complexes were synthesized by the substitution reaction of equatorial chlorido ligands by silver dicarboxylates. The most actives complexes were those having malonate as a labile ligand, no matter of the structure of the carrier ligand. Regarding the influence of the structure of the non-labile 1,4-diamine carrier ligand on the cytotoxicity, it was found that the complexes having the more lipophilic and symmetrical bicyclo[2.2.2]octane framework were much more active than those having an oxygen or methylene bridge.

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1. Introduction

The history of platinum-based anticancer drugs began with cisplatin (Fig. 1). This compound was first described by M. Peyrone [1] in 1844 and its anticancer activity was unveiled in 1964 by Barnet Rosenberg [2]. Cisplatin is one of the most effective antitumor agents in the actual oncological therapeutics.

Since the discovery of the important anticancer activity of cisplatin more platinum compounds have been synthesized. In the search for new platinum anticancer drugs, great efforts were devoted to the design of complexes more efficient and less toxic than drugs already in clinical use. Moreover, in some cases, after the initial treatment, tumors become resistant [3,4], so an important objective, which is involving great research efforts, is the development of new drugs without acquired resistance [5].

The mechanism of antitumor action of platinum drugs is not unique, because apart from DNA that is believed to be the main target of this kind of compounds by forming bifunctional adducts [6], epigenetic proteins are proven to be also biological targets for these compounds. The major adduct formed by platinum based-complexes is an intra-strand d(GpG) adduct with platinum cross-linking N7 atoms of neighbor guanine residues of DNA [7]. Thus, DNA undergoes an unstacking of the bases that leads to a pronounced bend in DNA at the site of platination [8–10]. It has also been shown that carrier amine ligands of cisplatin analogues appear to modulate the antitumor properties of this class of drugs. When designing a new molecule the structure and the stereoelectronic properties should be taken into account and modified and modulated accordingly.

Platinum(II) complexes having a diamino ligand with the two *N*-donors attached to a six-membered carbocycle appear to have an interesting anticancer activity [11,12]. In a previous research work [13] we synthesized and biologically evaluated *cis*-platinum(II)

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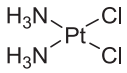


Fig. 1. Cisplatin.

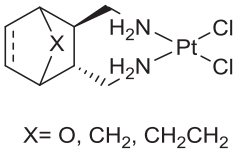


Fig. 2. Basic structure of the platinum compounds studied in previous work.

complexes with ligands containing a carbobicyclic framework and two methylamino substituents in 1,2 positions in order to have a 1,4-diaminobutane-like substructure (Fig. 2) with moderated steric hindrance and interesting stereo-differentiating properties. This type of molecules was designed to modulate the flexibility, the steric hindrance and the lipophilicity of the diamine carrier ligand, by inserting several types of bridges into the cyclohexane template [14] But a drawback problem of this type of complexes was their low solubility in water, whose consequence was a poor pharmacokinetic profile, which would make difficult their clinical use.

Platinum(IV) complexes could have several advantages in comparison to their platinum(II) congeners, from different points of view: chemical synthetic possibilities, their potential improvement of clinical practice, by using several administration routes, due to they act as prodrugs, as well as their better toxicological profile. Among these advantages could be highlighted: (a) possibility of oral administration (intestinal absorption), (b) reduced reactivity and thus reduced side-effects and increase of stability in plasma (c), more structural versatility and more possibility of structural variations at the level of axial and equatorial labile ligands, which affords a higher molecular diversity and so more potential for drug optimization; and finally, (d) capability to act as a prodrug and to be reduced *in vivo* to more reactive platinum(II) analogues.

As a part of our research project we are working in the design of new ligands and complexes in order to get platinum-based drugs more soluble in water and also we are seeking to establish structure–activity relationship rules for these compounds in order to increase their efficiency, safety and applicability to the clinical treatment. Our aim is to improve the expectancy and quality of life of patients, making these studies as translational as possible. In the present work, we have studied the synthesis of new platinum(IV) complexes having 1,2-bis(aminomethyl)carbobicyclic ligands and the dihydroxylated derivatives in C4 and C5 positions. Also we have worked on the preparation of platinum(IV) complexes having dicarboxylates as labile ligands (Fig. 3). Moreover, we have studied the antiproliferative activity of these complexes and we have studied the difference of their stereo-electronic properties by computational calculation, in order to establish a structure–activity relationship for this family of compounds.

2. Results and discussion

2.1. Synthesis and characterization of 1,2-diamine ligands

The synthesis of ligands **1**–**3** was performed by following a synthetic pathway developed by our research group [13,14] and other authors [15] (Fig. 4). The Diels–Alder reaction between fumaronitrile and dienes such as furan, cyclopentadiene or cyclohexadiene afforded cycloadduct **7**, **8** and **9** in moderate to good yields. The double bond of these cycloadducts was hydrogenated with H_2 in the presence of Pd/C catalyst with complete conversion and high yield. Finally, nitriles were reduced with $LiAlH_4$ in diethyl ether to yield the corresponding diamines in a moderate to good yields. All intermediates and finals products were purified and spectroscopically characterized.

In order to increase the hydrophilicity of compounds, two hydroxyl groups were introduced in the ligands to obtain derivatives **19**–**21**. The synthesis of these ligands [13] was accomplished following the synthetic route illustrated in Fig. 5. As in the synthesis of ligands **10**–**12**, the first step was a Diels–Alder reaction. Then the double bond of cycloadducts was dihydroxylated with catalytic amounts of OsO_4, using H_2O_2 to oxidize Os^{VI} to Os^{VIII} and to

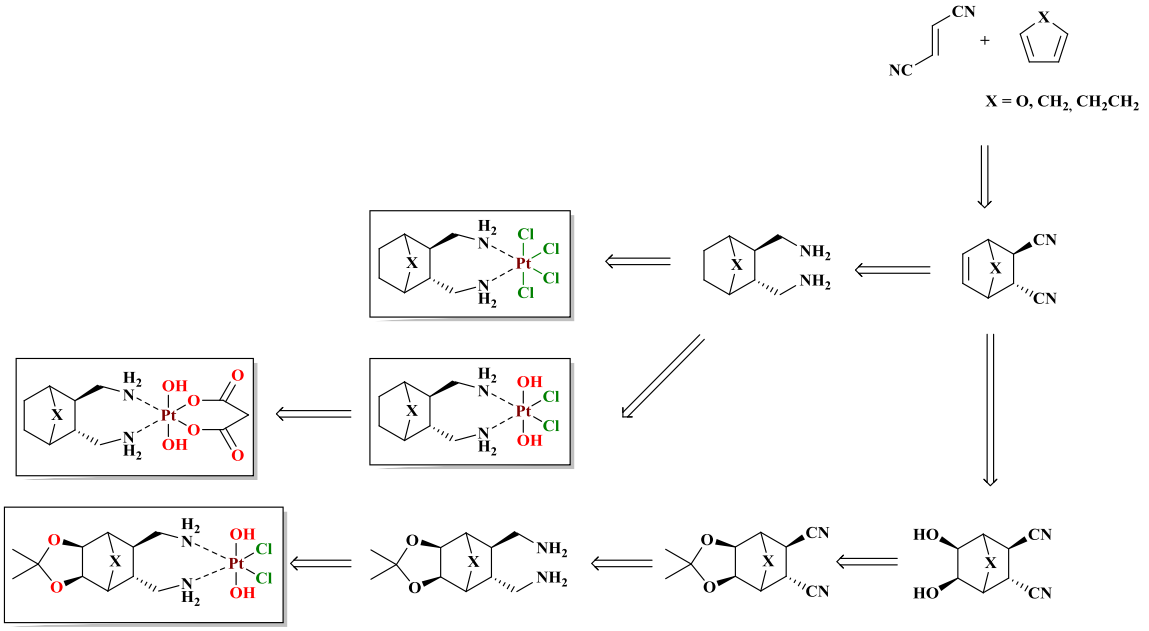
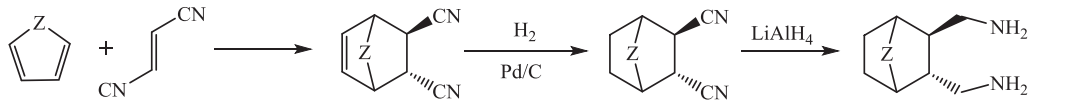


Fig. 3. Basic retro-synthetic scheme for the compounds studied in this work.



1, Furan Z = O
2, Cyclopentadiene Z = CH_2
3, 1,3-Cyclohexadiene Z = CH_2-CH_2

4, Z = O (82 %)
5, Z = CH_2 (73 %)
6, Z = CH_2CH_2 (35%)

7, Z = O (96 %)
8, Z = CH_2 (84 %)
9, Z = CH_2CH_2 (72%)

10, Z = O (41 %)
11, Z = CH_2 (83 %)
12, Z = CH_2CH_2 (97%)

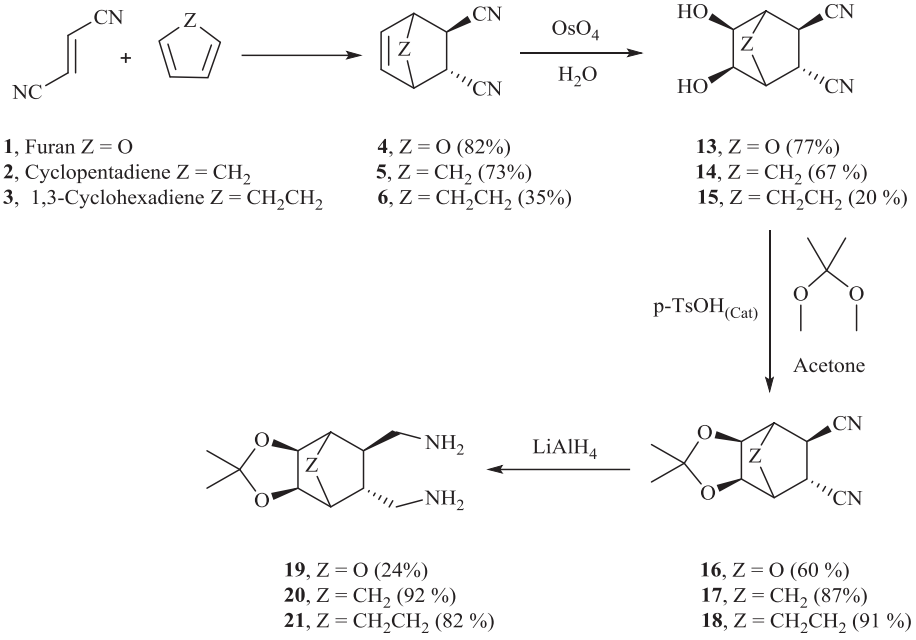
Fig. 4. Synthetic pathway for diamines **10**–**12**.

regenerate the active species of osmium, according to the well-known catalytic cycle [16]. The product formed had the *cis* configuration due to the formation of a cyclic osmium diester as an intermediate, which adopts this preferred stereochemical configuration due to its lower annular tension. On the other hand, the configuration of the major product was *exo*, generated by the preferential attack of OsO_4 by the less hindered convex face of the C=C double bond. In the next step diols **13**–**15** were protected as an acetonide by reaction with 2,2-dimethoxypropane, in the presence of TsOH in catalytic amounts [17]. This protection was carried out to facilitate the synthetic work and the isolation of products in the following steps. Finally nitriles were reduced with $LiAlH_4$, in diethyl ether, to diamines **19**–**21**. All intermediates and finals products were purified and physically and spectroscopically characterized. The structure of carbon framework of bicyclic carrier ligands was confirmed by ^1H and ^{13}C NMR, 1D and 2D correlations, as exemplified by spectra illustrated in the Supplementary information Fig. S1(a)–(d) for compound **13**. All signals were unequivocally assigned by COSY ^1H–^1H and HSQC ^{13}C–^1H and a careful comparison was performed among the signals of the different ligands in order to establish the carbon connectivity and the relative stereochemistry. Thus, in the case of compound **13** that presents six stereocenters, the relative stereochemistry between the OH groups and between the CN groups, may be theoretically deduced from the well-established mechanisms of *cis-exo*-dihydroxylation by OsO_4 and the Diels–Alder reaction, respectively. This assignment is consistent with the relative configuration *cis-exo* (C5–OH, C6–OH) and *trans* (C2–CN, C3–CN) that can be established by the Karplus equation [18] on the basis of J_{H-H} coupling constants, observed in high-field ^1H NMR spectra, and 3D molecular modeling.

2.2. Synthesis of platinum(IV) complexes

In the synthesis of platinum(IV) complexes **22**–**24**, diamines **10**–**12** were directly coordinated to commercial platinum(IV) salt, K_2PtCl_6 (Fig. 6) at 70 °C for 24–30 h, until complete conversion [19]. The products precipitated in the reaction medium and they were separated by filtration. The low yields of isolated platinum(IV) complexes are due to the fact that products were carefully washed with water and ethanol to discard unchanged substrate and inorganic salts and part of the product was in the mother liquor because of their solubility in that media. The yields are not optimized because our aim was to obtain very pure samples of complexes for their characterization and biological evaluation. The optimization of yields could be accomplished by modification of the reaction conditions and the work-up and it is underway in our laboratory.

The synthesis of platinum(IV) complexes **25**–**30** was carried out by reaction of the corresponding ligands **10**–**12** and **19**–**21** with $K_2PtCl_4(OH)_2$ (Fig. 7) [20]. The platinum(IV) salt, $K_2PtCl_4(OH)_2$, was obtained by oxidation of K_2PtCl_4 with H_2O_2. This potassium salt was spectroscopically characterized by IR, mass spectrometry and ^{195}Pt NMR, which confirmed the obtention of *trans*-$K_2PtCl_4(OH)_2$ [21]. The complexation was accomplished by the reaction of ligands with this platinum(IV) salt at room temperature for 2 days and heating for 1 h at 80 °C. In general, the product precipitated in the reaction



1, Furan Z = O
2, Cyclopentadiene Z = CH_2
3, 1,3-Cyclohexadiene Z = CH_2CH_2

4, Z = O (82%)
5, Z = CH_2 (73%)
6, Z = CH_2CH_2 (35%)

13, Z = O (77%)
14, Z = CH_2 (67 %)
15, Z = CH_2CH_2 (20 %)

16, Z = O (60 %)
17, Z = CH_2 (87%)
18, Z = CH_2CH_2 (91 %)

19, Z = O (24%)
20, Z = CH_2 (92 %)
21, Z = CH_2CH_2 (82 %)

Fig. 5. Synthetic pathway for diamines **19**–**21**.

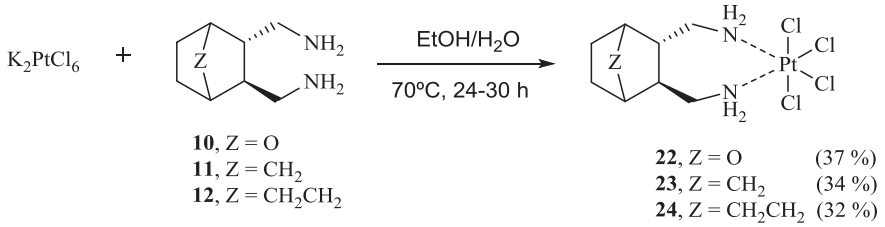


Fig. 6. Synthesis of complexes **22**–**24**.

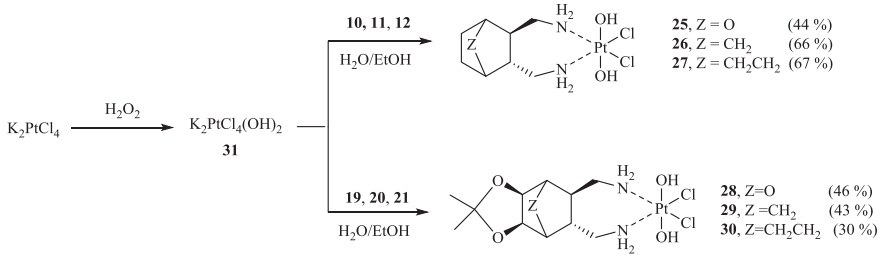


Fig. 7. Synthetic route for complexes **25**–**30**.

medium, except for the case of complex **28** in which the solid did not precipitated, so, the solvent was evaporated under vacuum, and the resulting solid residue was lixiviated with methanol to extract the excess of unconverted ligand, and, finally, the solid was separated by filtration. The obtained yellow-orange solid was studied in solid state by mass spectrometry, FT–IR spectroscopy and elemental analysis (EA), and, in solution, by ^{1}H NMR spectroscopy. The structure of complexes was confirmed by both, a careful study of fragmentation in HRMS (Electrospray) and C, H and N determination by EA. Several trials of crystallization were carried out in different solvents and for different complexes, in order to get single crystals for X-ray diffraction analysis, but they were unsuccessful. Also attempts of recording ^{13}C NMR spectra of complexes were performed in DMSO-d_6 and/or DMF-d_7, but we got complicated spectra, difficult to interpret, corresponding to a mixture in equilibrium of solvolitic species formed along several hours of NMR data acquisition.

The infrared spectra of the complexes are quite similar to each other in several regions. Thus, the spectra contain bands assigned to ν(Pt–N) stretching in the range 590–540 cm^{-1}, which in the case of *trans* dihydroxo-complexes get overlapped with ν(Pt–O) bands. On the other hand, N–H stretching bands appear between 3300 and 3020 cm^{-1}. The *trans* configuration for hydroxido ligands is consistent with the presence of strong and wide ν(O–H) stretching bands in the range 3550–3450 cm^{-1} and δPt(O–H) bands at 1000–980 cm^{-1} [22]. Moreover, in compounds having an oxygen bridge in their carbon framework (**22**–**28**) strong bands corresponding to ν(C–O) stretching are observed in the range 1100–1000 cm^{-1}. The complexes having the acetalic function (**28**, **29** or **30**) have even more intense bands in this region. All complexes bearing chlorido ligands present also bands corresponding to ν(Pt–Cl) in the range 350–330 cm^{-1} [23].

2.3. Synthesis and characterization of malonatoplatinum(IV) complexes

The malonato complexes were synthesized [24] by direct ligand shift of the corresponding dichlorido complexes with silver malonate disalt, prepared from malonic acid and silver nitrate, suspended in water and stirred at 45 °C, away from light, for 3 days(Fig. 8) [25]. After separation of the solid (AgCl) by filtration, the solvent was evaporated to obtain the final products, that were spectroscopically characterized.

In malonate bearing complexes (**33**, **34** and **35**) the IR spectra displayed typical patterns expected for carboxylate ligands coordinated to the Pt atom in a bidentated mode. The carbonyl vibration bands ν(C=O) appear in the region of 1657–1717 cm^{-1}. Additionally, the C–O single-bond vibrations ν_s(COO$^-$) of carboxylate functions appear in the range 1390–1370 cm^{-1}, consistent with similar structures published in the scientific literature [26, 22b,c].

3. Biological testing

3.1. Cell growth inhibitory activity

HL-60 cells were treated for 24 and 72 h with a range of concentrations of evaluated platinum complexes and of cisplatin, as a reference or standard, and cell viability was measured by XTT assay (Table 1). Compounds reduced cell viability with different efficacy in human leukemia (HL-60) cell line, depending on their structure, which indicates a clear structure–activity relationship as commented below in the SAR discussion. Thus, compound **40** showed to be the most active (5 times more active than CDDP at 72 h and 10 time more active at 24 h), followed by complexes **24**, **39** (1.4 and 1.1 times more active than cisplatin at 72 h, respectively, and 2.5 time more active, in both cases, at 24 h) and complex **34** with similar activity than CDDP (at 72 h) but twice as active at 24 h. In these four cases the activity at 24 h was always much higher than that of cisplatin (twofold), which may indicate that the cytotoxicity process is more rapid for these complexes than for cisplatin, until they reach the steady-state phase. In complexes **22**, **23**, **25**, **26**, **27**, **28** and **33** the cell growth inhibitory activity is lower than that of cisplatin. Finally complexes **29** and **30** showed IC_{50} values higher than 100 μM at 72 h, which indicate a very low cytotoxic activity.

3.2. Apoptosis determination

Apoptosis was assessed by annexin V–PI staining in HL-60 cells treated with platinum complexes and cisplatin for 24 h. With all types of drugs, apoptosis was observed and the percentages are

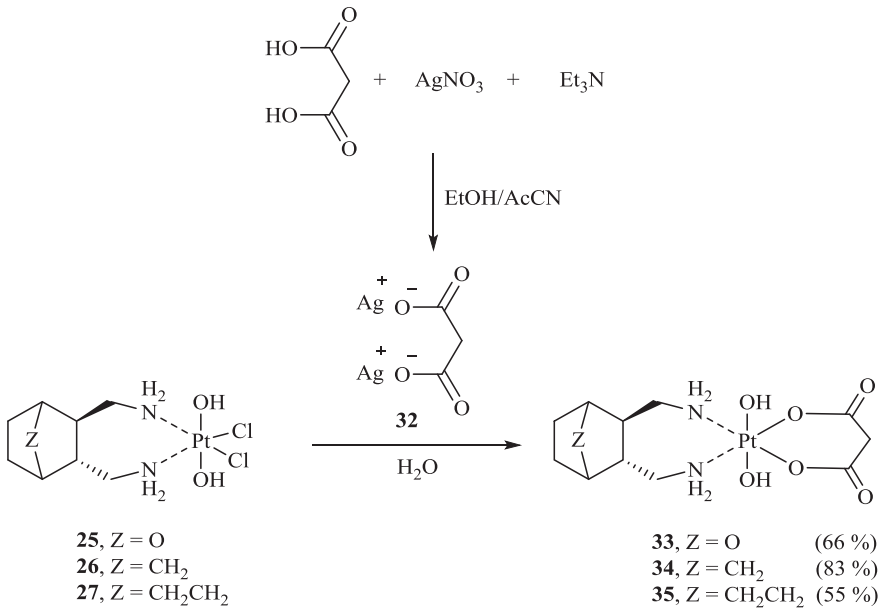


Fig. 8. Synthesis of malonatoplatinum(IV) complexes **33**–**35**.

presented in Table 2. A significant induction of apoptosis (~30% of apoptotic cells) was observed in compounds **39**, **34**, **26** and **23**, at the same level tan CDDP. In the case of complexes **40** and **25** the percentage of apoptosis was slightly lower (~24%). The remaining evaluated platinum complexes showed higher % of cells in a late apoptosis and/or necrosis state and in a lesser extent as necrotic cells (R4). In this last sense **27** and **33** induce higher damage and/or death on cells. The viability of cells observed by cytometry at 24 h (IC_{50}, μM) decreases, with respect to cisplatin, for all evaluated compounds except for **24**, **26** and **33** in which it slightly increases. The lowest cell viability was observed for **27** and **35** in which it decreases down to 12% and 15%, respectively, in relation to CDDP.

The study of apoptosis by flow cytometry was repeated for complexes **27** and **35** but at 14 h. In this new assay we observed a high increase of % of apoptotic cells (R2 values), that gave positive to annexin and negative to propidium iodide. This behavior indicates that at lower time the cells undergo an early apoptosis, in the presence of **27** and **35**, which could be due to the more rapid cytotoxic action of these compounds.

4. Structure–activity relationship

4.1. SAR studies about the drug likeness and bioavailability of Pt(IV) complexes

According to the fragment based drug design for Pt(IV) complexes, the non-labile ligands were designed for having a medium size (<500 Da) and not very high steric hindrance (with Van der Waals volumes and Connolly solvent excluded volumes ≤ 300 $Å^3$) and a non-steric-demanding geometrical morphology, with an ovality value around 1.2–1.3. Moreover, a limited number of hydrogen bond donors and acceptors were considered in order to fulfill the requirements of Lipinski's and Veber's rules for good bioavailability.

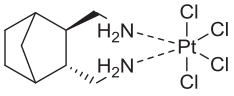
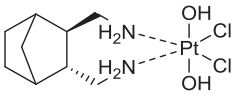
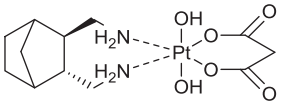
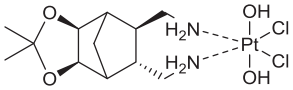
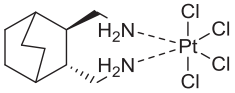
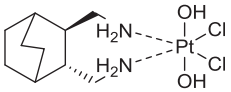
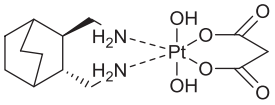
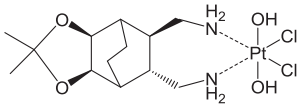
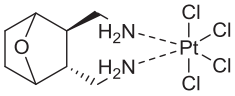
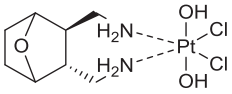
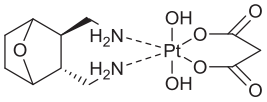
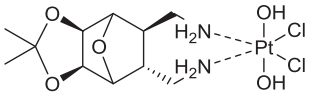
The oxygen, methylene and ethylene bridges in the non-labile ligands (carrier ligands) were considered in order to compare the different lipophilicity (logP and logD) of the three types of scaffolds and their influence in the cytotoxic activity (IC_{50}). The introduction in the carrier ligand of the acetalic function (1,3-dioxolane subunit) (complexes **28**, **29**, **30**) was envisioned as way of getting a possible prodrug [27] that in the cytosol medium of cancer cell (with around 0.3 pH units lower than in normal cells, which intracellular pH value falls within the range 6.0–7.4) [28] should undergo a chemical and/or enzymic hydrolysis to afford a dihydroxylated ligand, more prone to be soluble in water due to solvation effects. That is the case of heptaplatin, a water soluble platinum complex, bearing an isobutyl acetalic subunit liable to be hydrolyzed at low pH values, which is used for gastric cancer in some countries [27b, 29]. This was the aim due to one of the major problems of actual platinum-based drugs, *i.e.* their very low water solubility. As a matter of fact, clinically platinum drug usually are administered as a suspension in a physiologic saline solution [30].

To improve this water solubility we decided to act especially at the level of the labile ligands, which are the ones to be first exchanged during the absorption and distribution process of drug. Thus, we exchanged in the tetrachloroplatinated subunit ($PtCl_4$), of complexes **22**–**24**, two of the chlorido ligands by two hydroxide ligands (complexes **25**–**27**) and also by the dimalonate bidentated ligand (complexes **33**–**35**).

With this aim the chemical synthesis of carrier ligands and their corresponding Pt(IV) complexes was carried out and their cytotoxic activity evaluated on HL-60 cancer cells, as described previously. A careful analysis and comparison of data allowed us to establish the following structure–activity relationships.

In first place, regarding the change of the bridge nature it is possible to observe very clearly how the introduction of an oxygen bridge decreases considerable the activity (compare the IC_{50} values either at 24 h or at 72 h of complexes **22** to **24** and **33** to **35**; and also **39** and **40**). The most active complexes no matter of the labile ligands are in general the ones having an ethylene bridge. The structural basis for this empirical observation was carefully studied in order to find a rational SAR. Thus, when going to the oxygen bridged to the ethylene bridged complexes cLogP and LogD increase, with slight raise of lipophilicity, LogS decreases, producing a tiny reduction of water solubility, the dipolar moment (μ) roughly maintains its value, so that the polarity of molecules does not change very much. Molar refractivity (cMR) and molecular polarizability (MP) increase and the topological polar surface area (tPSA) maintain its value (see Tables S2 and S3 from the Supplementary information). These three parameters indicate that the passive

Table 1
IC_{50} values of platinum compounds, and cisplatin against HL-60 cells.

Structure	Complex	IC_{50} (μM)		IC_{50} (μM), relative to CDDP	
		72 h	24 h	72 h	24 h
	**23**	12.28 ± 1.29	45.51 ± 3.24	5.7	2.9
	**26**	14.74 ± 3.23	112.5 ± 13.1	6.8	7.2
	**34**	2.35 ± 0.61	7.97 ± 1.89	1.1	0.5
	**29**	>100	>200	>46.5	>12.8
	**24**	1.61 ± 0.13	5.95 ± 1.4	0.7	0.4
	**27**	32.77 ± 5.33	>100	15.2	>6.4
	**35**	4.99 ± 1.21	21.67 ± 2.34	2.3	1.4
	**30**	>100	>200	>46.5	>12.8
	**22**	62.59 ± 5.86	>200	29.1	12.8
	**25**	28.44 ± 2.52	57.92 ± 4.18	13.2	3.7
	**33**	56.63 ± 3.75	112.44 ± 6.37	26.3	7.2
	**28**	81.47 ± 6.48	>200	37.9	12.8
	CDDP	2.15 ± 0.1	15.61 ± 1.15	1	1

membrane transport could be favored for the complexes with the carrier ligand having an ethylene bridge. There is an increase of steric hindrance, observing the Van der Waals volumes of complexes an also the diammino ligand contribution to this volume (maintaining constant the PtL_4 subunit contribution). The calculated Pt electron charge and its Hückel orbital electronegativity indicate that changing the nature of the carrier ligand does not change the reacting capability of platinum(IV) to undergo ligand exchange. The change observed in topological parameters (Wiener index and projection areas) indicates an obvious modification of morphology and geometry of the complex. All three types of complexes fulfill the Lipinski's and Veber's rules, but this is not a differentiating property and it only may indicate a potential good bioavailability for the three types of complexes (see Tables S2 and S3 from the Supplementary information). As a conclusion we may state that the introduction of an oxygen bridge in the carrier ligand makes the cytotoxicity of Pt(IV) complex to decrease considerably, maintaining their potential bioavailability.

Table 2
Percentages of cells in the diverse states from the flux cytometry studies at 24 h.

Treatment (IC_{50}, 24 h, μM)	% vital cells (R1)	% apoptotic cells (R2)	% dead cells (R3)	% damaged cells (R4)
Control	92.44	4.78	2.59	0.19
CDDP	60.93	33.06	4.94	1.06
23	52.75	29.63	15.40	2.22
26	63.59	29.10	6.83	0.47
34	49.57	28.33	17.89	4.21
29	–	–	–	–
24	70.51	19.69	8.92	0.88
27*	48.52 (*66.16*)	9.35 (*27.05*)	28.50 (*6.11*)	15.64 (*0.58*)
35*	45.65 (*44.45*)	13.08 (*35.16*)	38.02 (*20.11*)	3.28 (*0.28*)
30	–	–	–	–
22	–	–	–	–
25	58.97	24.33	14.72	1.98
33	62.48	15.36	12.11	10.05
28	–	–	–	–

(*) Values between parentheses correspond to flux cytometry studies at 14 h.
R1: Cells that keep alive (give negative staining values for annexin and propidium iodide).
R2: Cells under apoptotic process (give positive respect to annexin and negative with propidium iodide).
R3: Cells with advanced processes of apoptosis and/or necrosis (give positive to both dyes).
R4: Cells under necrosis (give positive to the propidium iodide test).

Continuing with the evaluation of carrier ligand structural modifications, we evaluated the influence of introducing the acetalic function on the bicyclic scaffold, with the intention to add a masked diol to the molecule, expecting an increase of water solubility, once unprotected by enzymic cleavage *in vivo*. However, the experimental observation of cytotoxicity on HL-60 cancer cells for complexes **28**–**30** was disappointing in the sense that it considerably decreased respect to referenced complexes **19**–**24**. The analysis of structural parameters indicated a decrease of lipophilicity (LogP and LogD), an increase of hydrophilicity (LogS) and slight increase of cMR and molecular polarizability. Additionally, the Lipinski's rule is not fulfilled by these three complexes due to their molecular weight is higher than 500 Da. However in our opinion, the variation of these parameters does not justify the change of activity of complexes **28**–**30** in relation with **22**–**24**. A more feasible explanation could be found in the increase of steric and dipolar interactions of **28**–**30** in their approach to DNA bases. Thus, a considerable increase of tPSA is observed and also a great rise of all steric hindrance measuring parameters (Connolly SASA, Connolly SES, VdW volume and Connolly SEV) and an important variation of topological parameters (Wiener index, projection areas and ovality), evidence of a high modification of the molecular geometry (see Tables S2 and S3 from the Supplementary information). This makes us to consider that steric hindrance and dipolar destabilizing interactions are the factors responsible of this low activity and that the acetalic subunit represents a steric demanding group and a source of undesired polar interactions with the target molecule [31].

The analysis of structural parameters of labile ligands and their correlation with the experimentally observed cytotoxicity data affords also very interesting results. As above mentioned, the substitution of two chlorido ligands by two hydroxido ligands was carried out in order to increase the water solubility of the resulting complexes. This aim was afforded as observed empirically (such as it was predicted by the increase of logS values and decrease of logP and logD). On the other hand, this ligand exchange improved the cytotoxicity of complex **25** *versus* **22**, but decreased the activity of **26** *versus* **23** and also considerably reduced the activity of **27** *versus* **24**. The comparative study of the structural parameters showed us how when introducing OH ligands several trends could be observed: dipolar moment μ maintains its value, cMR decreases, tPSA considerably increases, the steric factors (ASA, VdWSA, VdWV, etc.) do not change very much, electronic properties like molecular polarizability and Hückel orbital electronegativity decrease slightly, Mulliken Pt charge increases and topology parameters remain almost unaltered. Lipinski's and Veber's rules are fulfilled by compounds **25**–**27** and overall fragment-based drug-likeness and drug score does not change in a significant manner (See Tables S2 and S3 from the Supplementary Information). We may conclude that the exchange of Cl ligands by OH ligands, even though it improved certain bioavailability parameters, however, did not improve the cytotoxicity (except for the case of the pair **22**/**25**) due to the change of the reduction potential of Pt(IV) to Pt(II). This factor is very important because it is considered that Pt(IV) complexes act as prodrugs, which are reduced *in vivo* to Pt(II) species that are the active ones (Fig. 9). According to reduction potentials, in a platinum(IV) complex with the same carrier ligands and leaving groups, the diaxial dichlorido derivative will undergo the platinum(IV) reduction easier than a derivative with the axial dihydroxido complex. Experiments *in vitro* showed that the easier the reduction occurs, the compound showed the better activity [32].

We were interested in evaluating the influence on cytotoxicity of the simultaneous exchange of both chlorido labile ligands (as leaving groups) by a malonate bidentated ligand, maintaining the hydroxido axial ligands, comparing the **25**–**27** series to the **33**–**35** series of complexes.

Analyzing the structural data it is possible to observe how in **33**–**35** complexes LogP decreases but LogD, LogS, μ, cMr and especially tPSA increase compared to **25**–**27** series. The introduced polar functional groups increase MR, but decrease LogP due to MR is a measure of non-lipophilic interactions, while LogP is a measure of lipophilic interactions. These changes are consistent with the introduction of a molecular subunit with four new additional oxygen atoms, which affords an increase of water solubility of these complexes. However, the raise of LogD could only be interpreted on the basis of the enolization at the level of C2 atom in malonate ligand. This is possible due to the pK_a values of hydrogen atoms on C2 (5.57; 5.68 and 5.73 for **33**, **34** and **35**, respectively) [33], which make possible the complete ionization at this position at physiologic pH = 7.40, that is the pH value adjusted for the cell culture medium. Steric hindrance evaluation parameters (SASA, Connolly SES, VdWSA, VdWV) increase as also do the topological and geometric parameters (Wiener index and projection surfaces), as a consequence of introducing a bulkier group (see Tables S2 and S3 from the Supplementary information). However, this fact may not represent a serious problem for the access of complexes **33**–**35** to their target (N7 of guanine in DNA molecule) due to they probably undergo an exchange with aqua ligands in the intracellular media prior to

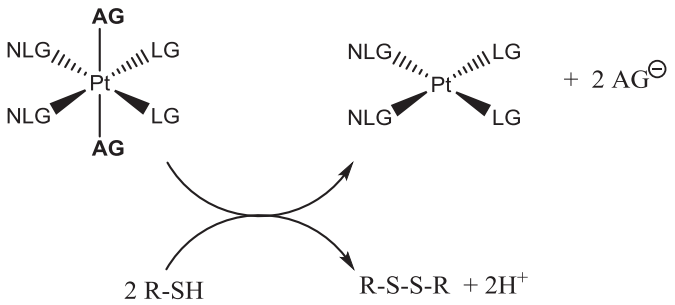


Fig. 9. Reduction of platinum(IV) complexes by thiol-containing biomolecules (RSH) like glutathione (among others), resulting in the corresponding platinum(II) analogues (NLG = Non-leaving group; LG = leaving group; AG = axial group).

interact with the final target. The biological activity (IC_{50} values, both at 24 and 72 h) got worse for **33** in relation with **25,** but improve considerably for **34** and **35,** compared to **26** and **27**, respectively. This finding prompted us to use in the future non-enolizable succinate and cyclobutanedicarboxylate ligands, instead of the malonate labile ligand in order to avoid undesired side reactions and to improve drug stability in plasma.

We may conclude that the insertion of an oxygen bridge and/or an acetalic function of the non-labile carrier ligand produces a considerably decrease in the cytotoxic activity due to stereo-electronic factors as envisioned from the structural parameters analysis. On the other hand, when we actuate on the labile leaving ligands, the exchange of chlorido by hydroxido ligands induces an important reduction of cytotoxic activity, probably due to the modification of the reduction potential of Pt atom. However, the exchange by bidentated malonate ligands improve considerably the desired activity as a probable consequence of the improvement of water solubility and bioavailability, never the less the influence of stereo-electronic effects are not discarded.

4.2. Parameters calculation

The structural parameters related to bioavailability, steric hindrance, drug likeness, electronic properties, molecular topology and fragment-based drug score were calculated by using ChemAxon's software [34]. Alternatively, for the determination of lipophilicity other methods were evaluated for comparison [34]. Connolly solvent accessible surface area, Connolly solvent excluded surface, Connolly solvent excluded volume, logS and several topological parameters like ovality or Wiener index were calculated by using ChemBioOffice-13 Chem3D software (MM2, MMFF94) and GAMESS, MOPAC-2012 and GAUSSIAN-03 software packages. All calculations were performed on the minimum energy conformation and for the major species at physiological pH (7.4). Thus, geometry and energy calculations were preoptimized by molecular mechanics MM2 followed by semiempirical quantum mechanical PM6 algorithm [35], implemented in the MOPAC software. Density functional theory (DFT) based methods at the B3LYP functional level [36,37] were used for subsequent full refinements, within the Gaussian-03W (Revision E.01, version 6.1) software package [38]. For carbon, hydrogen, chlorine, nitrogen and oxygen atoms, the 6–31++G(d, p) basis set was used [39] and for platinum atom, the LANL2DZ basis set and effective core potential was used [40]. All calculations were performed on the isolated molecules (gas phase), as consideration of solvation by the molecules of the solvent by a polarizable continuum model (PCM) [41] produced a loss in computational performance (increase of CPU calculation time and change of convergence behavior), but did not result in significant changes of the calculated energies.

5. Experimental section

5.1. Materials and methods

The reactions that required an inert atmosphere were conducted under dry nitrogen or argon and the glassware was oven dried (100 °C). Tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone prior to use. Methanol and acetonitrile were dried by refluxing them over CaH_2 under nitrogen. *Mass spectra* were obtained on a Voyager-DE-RP (Applied Biosystems in the 1300–300 *m/z* range), and on a LC/MSD TOF (Agilent Technologies) by the electrospray (ESP-MS) technique. *HRMS* results are quoted by indicating the calculated *m/z* values for monoisotopic spectral lines and the observed *m/z* ratios. The Δ variations in milliDaltons (mDa) between observed and calculated *m/z* ratios are also indicated. *Infrared spectra* were recorded on a FT–IR NICOLETE 6700 spectrophotometer in a 4500–300 cm^{-1} range in the form of film, KBr plates or by the ATR system (Attenuated Total Reflectance). *NMR spectra* were obtained on a Varian Unity-300 Plus, a Mercury-400 or a Varian Inova-500 (the solvent is specified in each case). *^1H NMR* spectra were obtained at 300, 400 or 500 MHz frequencies and the chemical shifts are given in ppm relative to tetramethylsilane (TMS). The assignment of the multiplicity of signals was as follows: s (singlet), d (doublet), t (triplet) and m (multiplet). *^{13}C NMR* experiments for organic ligands were recorded at 50, 75 or 100 MHz and were referenced depending on the solvent. In the case of platinum complexes ^{13}C NMR spectra were not recorded because during acquisition time (several hours) an exchange of one or more chlorido ligands by solvent molecules (D_2O, DMF, DMSO) takes place, generating a mixture of complexes that afford very complicated spectra, difficult to interpret. For *^{195}Pt NMR* spectra, solutions of K_2PtCl_4 and K_2PtCl_6 in water and/or other solvents were used as standards. *Thin layer chromatography (TLC)* was carried out by using silica gel or alumina as stationary phases on aluminum plates (solvents and developing reagents are specified in each case). *Column chromatography* was performed on silica gel (SiO_2 60 AC.C 35–70 μm) (eluents were specified in each case). *Gas chromatography* was carried out in a Hewlet-Packard 5890A apparatus with a FID detector (T = 250 °C, hydrogen pressure = 4.2 psi, air pressure = 2.1 psi), using a Hewlett-Packard-Crosslinked Ph–Me-Silicone capillary column (25 m, 0.2 mm, 2.5 μm). *Melting points* were measured on a Galenkamp apparatus. Melting points of platinum complexes are not indicated because they decompose when heating. *Elemental analyses* (C, H, N, S) were obtained with a Carlo Erba EA1180 apparatus.

5.2. Synthesis of 7-oxabicyclo[2.2.1]hept-5-en-2,3-dicarbonitrile, **4**

Fumaronitrile (1007 mg, 12.9 mmol) was dissolved in furan (20 mL, 279 mmol), under anhydrous conditions. The reaction mixture was stirred at r.t. for 68 h, and the mixture filtrated to isolate compound **4**. The mother liquor was refluxed again for 19 h and an additional amount of compound **4**, was isolated by filtration (1546 mg, overall yield = 82%). White solid, **Mp**: 113–114 °C (furan). **IR** (KBr): υ_{max} 3100, 2961, 2925, 2244, 1559. **^1H NMR** (300 MHz, $CDCl_3$, 25 °C) δ 2.75 (d, 1H; J = 4 Hz, H2), 3.27 (t, 1H; J = 4 Hz, H3), 5.35–5.5 (m, 2H; H1, H4), 6.62 (dd, 1H, dd; J_1 = 1.9 Hz, J_2 = 5.9 Hz, H5 or H6), 6.68 (dd, 1H; J_1 = 1.6 Hz, J_2 = 5.8 Hz, H5 or H6). **^{13}C NMR** (75 MHz, $CDCl_3$, 25 °C) δ 33.7 (C2 or C3), 35.1 (C2 or C3), 79.5 (C1 or C4), 82.7 (C1 or C4), 117.0 (C1′ or C1″), 118.1 (C1′ or C1″), 135.0 (C5 or C6), 136.2 (C5 or C6). **MS** (CI, NH_3): *m/z* (%) 102 (100, M−2(CH−CN)+$N_2H_7^+$), 103 (33, M−2(CH−CN)+$N_2H_7^+$+H^+), 164 (9, M+NH_4^++H^+), 181 (56, M+$N_2H_7^+$). **Anal.** Calcd for $C_8H_6N_2O$: C, 65.75; H, 4.14, N, 19.17. Found: C, 65.81; H, 4.12, N, 19.22. **TLC** (alumina, hexane/ethyl acetate 60:40 and developed with potassium permanganate as a white spot on a purple background). R_F: 0.48.

5.3. Preparation of bicyclo[2.2.1]hept-5-en-2,3-dicarbonitrile, **5**

Fumaronitrile (1.0 g, 13.1 mmol) was dissolved in absolute ethanol (15 mL) and cyclopentadiene was added dropwise (1.2 mL, 13.9 mmol), under anhydrous conditions. After the addition was completed, the reaction mixture was cooled in an ice-water bath and stirred for 30 min. The volume of ethanol was reduced to the half, under vacuum and the obtained solution was kept at 4 °C in the dark for 3 days, obtaining a white solid that was filtrated off (1.70 g, yield = 73%). **Mp**: 61–62 °C. **IR** (KBr) 3080, 2990, 2950, 2244. **^1H NMR** (200 MHz, $CDCl_3$): δ 1.75–1.78

(1H, m, H7a), 1.80–1.83 (1H, m, H7b), 2.51 (1H, dd, J_1 = 2 Hz, J_2 = 4.2 Hz, H2), 3.17 (1H, dd, J_1 = 3.4 Hz, J_2 = 4.2 Hz, H3), 3.40–3.46 (2H, m, H1, H4), 6.35–6.40 (2H, m, H5, H6). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 34.6 (C2 or C3), 34.7 (C2 or C3), 46.3 (C1 or C4), 47.3 (C7), 48.3 (C1 or C4), 119.4 (C1′ or C1″), 119.9 (C1′ or C1″), 135.6 (C5 or C6), 137.1 (C5 or C6). **MS** (CI, NH_3): *m/z* (%) 145 (1, $M+H^+$), 162 (88, $M+NH_4^+$), 163 (10, $M+NH_4^++H^+$). **Anal.** Calcd for $C_9H_8N_2$: C, 74.98; H, 5.59, N, 19.43. Found: C, 75.01; H, 5.55, N, 19.38. **TLC** (silica gel, ethyl acetate/methanol 80:20, developed with anisaldehyde): R_F = 0.73.

5.4. Synthesis of bicyclo[2.2.2]oct-5-ene-2,3-dicarbonitrile, **6**

In a 100 mL flask fitted with magnetic stirring and nitrogen atmosphere, a solution of fumaronitrile (4.12 g, 53 mmol) in absolute ethanol (48 mL) was placed. Cyclohexadiene (2.5 mL, 26.2 mmol) was added dropwise. BF_3–OEt_2 in catalytic amounts (5 μL) was then added at once by a microsyringe, and the reaction mixture was first stirred at r.t. for 43 h, and afterward irradiated in a microwave reactor for 4 h at 70 °C. The solvent was evaporated under vacuum and the resulting solid was purified by sublimation of the excess of fumaronitrile (0.4 torr, bath temperature 80 °C, 2 h) to obtain compost **6** as a white solid (sublimation residue) (1.472 g, yield = 35%). **Mp**: 75–76 °C. **IR** (KBr): υ_{max} 3060, 2952, 2876, 2244. **^{1}H NMR** (300 MHz, $CDCl_3$, 25 °C): δ 1.31–1.51 (m, 2H; H7A or H8A), 1.67–1.76 (m, 1H, H7B), 1.97–2.06 (m, 1H; H7B), 2.72 (m, 1H; H2), 2.93–3.06 (m, 3H; H1, H3, H4), 6.46 (m, 2H, H5, H6). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C): δ 19.4 (C7 or C8), 23.7 (C7 or C8), 31.8 (C1 or C2 or C3 or C4), 32.6 (C1 or C2 or C3 or C4), 33.1 (C1 or C2 or C3 or C4), 33.2 (C1 or C2 or C3 or C4), 119.5 (C1′ or C1″), 120.1 (C1′ or C1″), 132.2 (C5 or C6), 134.2 (C5 or C6). **MS** (CI, NH_3): *m/z* (%) 176 (92, $M+NH_4^+$), 193 (100, $M+N_2H_7^+$). **Anal.** Calcd for $C_{10}H_{10}N_2$: C, 75.92; H, 6.37, N, 17.71. Found: C, 75.85; H, 6.41, N, 17.78. **CCF** (silica gel, hexane/ethyl acetate 50:50, developed with anisaldehyde): R_F = 0.79. **GC** (35 °C, 1 min; 5 °C/min; 250 °C, 20 min): t_R = 19.62 min.

5.5. Synthesis of ligands **7**–**12**

The ligands **7**–**9** and **10**–**12** were synthesized following the synthetic pathway shown in the discussion section (Fig. 4), from fumaronitrile and the corresponding diene, according to the procedures previously described in the literature [13–15]. Their structures were confirmed by spectroscopic methods (FT–IR, ^{1}H and ^{13}C NMR and MS).

5.6. Synthesis of (1S*, 2R*,3R*,4R*,5S*,6R*)-5,6-dihydroxy-7-oxabicyclo[2.2.1]octane-2,3-dicarbonitrile, **13**

To a solution of cycloadduct **4** (1.20 g, 8.20 mmol) in acetone/diethyl ether 8:2 (23 mL), OsO_4 (79.1 mg, 0.31 mmol) suspended in water (4 mL) and H_2O_2 35% (v/v) was added dropwise at 0 °C. The resulting mixture was stirred in the dark for 6 h. The solvent was evaporated under vacuum and the product was purified by a flash column chromatography on silica gel eluting with ethyl acetate/acetone, 8:2, to obtain diol **13** (1317 mg, yield = 77%) as a white solid. **Mp**: 230–231 °C. **IR** (ATR): υ_{max} 3354–3444 (O–H, st), 2966–2927 (Csp^3-H, st), 2252 (C≡N, st), 1457, 1212, 1181, 1092, 1050, 1009, 918. **^{1}H NMR** (400 MHz, D_2O): δ 91: 3.33 (d, 1H, J = 5.2 Hz, H2), 3.44 (dd, 1H, J_1 = 5.6 Hz, J_2 = 5.2 Hz, H3), 4.07 (d, 1H, J = 6.4 Hz, H1), 4.28 (d, 1H, J = 6.4 Hz, H4), 4.68–4.74 (m, 2H; H5, H6). **^{1}H NMR** (500 MHz, acetone-d_6): δ 3.39(br d, 1H,, J = 5 Hz, H3), 3.44(br t, 1H, J = 5 Hz, H2), 4.20(br t, 1H, J = 6 Hz, H6), 4.32(br t, 1H, J = 6 Hz, H5), 4.57–4.60 (m, 1H, OH-6), 4.65–4.66 (m, 1H, OH-5), 4.66 (br s, 1H; H4), 4.75 (dd, 1H, J_1 = 1.51 Hz, J_2 = 5.5 Hz, H1). **^{13}C NMR** (125 MHz, acetone-d_6): δ 33.66 (C2), 33.75(C3), 70.28 (C5), 72.34 (C6), 82.51 (C1), 86.15 (C4), 118.60 (C1′), 119.15 (C1″). **COSY ^{1}H–^{1}H** (500 MHz, acetone-d_6): H1–H2, H1–H4 (W coupling), H2–H3, H5–H6, H5–OH, H6–OH. **HSQC ^{13}C–^{1}H** (500 MHz, acetone-d_6): C1–H1, C2–H2, C3–H3, C4–H4, C5–H5, C6–H6. **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%) 146 (100, M–2OH), 162 (73, M–H_2O), 197 (12, M+NH_4). **Anal.** Calcd for $C_8H_8N_2O_3$: C, 53.33; H, 4.48, N, 15.55. Found: C, 53.37; H, 4.43, N, 15.60. **CCF** (silica gel, ethyl acetate, developed with anisaldehyde): R_F = 0.52.

5.7. Preparation of (1S*, 2S*, 3S*, 4R*, 5R*, 6S*)- 5,6-dihydroxybicyclo[2.2.1]heptane-2,3-dicarbonitrile, **14**

Substrate **5** (250 mg, 1.7 mmol), was dissolved in a mixture acetone/ether 8/2 (6 mL). To this solution, OsO_4 (16 mg) in water (1 mL) was added and afterward 35% H_2O_2 (0.8 mL) was also added at 0 °C. The resulting mixture was stirred in the dark for 6 h. The reaction mixture was concentrated to dryness under vacuum to obtain a brown solid which was submitted to column chromatography, using mixtures of hexane/ethyl acetate of increasing polarity. Diol **14** (215 mg, 67% yield) was eluted with hexane/ethyl acetate 3/7. White solid, **Mp**: 137–138 °C (hexane/ethyl acetate 7/3). **IR** (ATR): υ_{max} 3423 (O–H, st), 2983–2948 (Csp^3-H, st), 2248 (C≡N, st), 1268, 1083, 1026. **^{1}H NMR** (400 MHz, acetone-d_6): δ 1.54 (ddd, 1H, J_{7A-7B} = 11.2 Hz, J_2 = J_3 = 1.6 Hz, $H7_A$), 2.08 (ddd, 1H, J_{7A-7B} = 11.4 Hz, J_2 = 3.3 Hz, J_1 = 2.0 Hz, $H7_B$), 2.51 (s, 1H; H-1), 2.56 (1H, dd, J_2 = 4.4 Hz, J_1 = 1.6 Hz, H4), 2.87 (dd, 1H, J_{2-3} = 5.4 Hz, J_{2-7} = 1.8 Hz, H2), 3.28 (dd, 1H, J_{2-3} = 5.6 Hz, J_{3-4} = 1.4 Hz, H3), 3.92 (dd, 1H, J_{6-OH} = 4.8 Hz, J_{5-6} = 5.2 Hz, H6), 4.09 (ddd, 1H, J_{5-OH} = 5.6 Hz, J_{5-6} = 5.2 Hz, J_{5-7} = 1.6 Hz, H5), 4.53 (d, 1H, J_{6-OH} = 4.8 Hz, OH-6), 4.58 (d, 1H, J_{5-OH} = 5.2 Hz, OH-5). **^{13}C NMR** (100 MHz, acetone-d_6): δ 32.3 (C-7), 33.6 (C-2), 33.9 (C-3), 47.6 (C-4), 50.0 (C-1), 70.8 (C-5), 73.2 (C-6), 119.5 (C-1′ or C-1″), 121.1 (C-1′ or C-1″). **MS** (CI, NH_3): *m/z* (%) 109 (23, M–2CN–OH), 178 (100, M). **Anal.** Calcd for $C_9H_{10}N_2O_2$: C, 60.66; H, 5.66, N, 15.72. Found: C, 60.71; H, 5.53, N, 15.78. **CCF** (silica gel, hexane/ethyl acetate 7:3, developed with anisaldehyde): R_F = 0.43.

5.8. Synthesis of (1S*, 2S*, 3S*, 4R*, 5R*, 6S*)-5,6-dihydroxybicyclo[2.2.2]octane-2,3-dicarbonitrile, **15**

To a solution of cycloadduct **6** (423.7 mg, 2.68 mmol) in acetone/diethyl ether 8:2 (6.5 mL), OsO_4 (20.1 mg, 0.08 mmol) suspended in water (1.4 mL) and H_2O_2 35% (v/v) additions were added dropwise at 0 °C. The reaction mixture was stirred for 6 h at room temperature. The solvent was evaporated under vacuum and the product was purified by flash column chromatography on silica gel to obtain **15** (103.6 mg, yield = 20%), by elution with hexane/ethyl acetate, 50:50. Diol **15** was obtained as a white solid. **Mp**: 205–207 °C. **IR** (ATR): υ_{max} 3430 (O–H, st), 2983–2948 (Csp^3-H, st), 2246 (C≡N, st), 1270, 1090, 1022. **^{1}H NMR** (400 MHz, CD_3OD, 25 °C): δ 1.51–1.57 (m, 2H; $H7_A$ or $H8_A$), 1.95–2.03 (m, 3H, H-7B, H-8B, H1 or H4), 2.15–2.16 (m, 1H; H1 or H4), 3.26–3.29 (m, 1H; H2 or H3), 3.36–3.39 (m, 1H; H2, H3), 4.04–4.11 (m, 2H; H5, H6), 4.47.4.51 (m, 2H, 2OH). **^{13}C NMR** (100 MHz, CD_3OD, 25 °C): δ 13.7 (C7 or C8), 16.5 (C7 or C8), 29.5 (C2 or C3), 30.1 (C2 or C3), 34.7 (C1 or C4), 35.1 (C1 or C4), 63.4 (C5 or C6), 65.5 (C5 or C6), 182.0 (C1′ or C1″), 183.9 (C1′ or C1″). **^{1}H–^{1}H COSY** (400 MHz, acetone-d_6): δ H1–H2, H1–H6, H4–H5, H2–H3, H1′–H1″. **^{13}C–^{1}H HETCOR** (gHSQC, 400 MHz, acetone-d_6): C1–H1, C2–H2, C3–H3, C4–H4, C5–H5, C6–H6, C7–H7, C8–H8. **MS** (DIP, CI, NH_3): *m/z* (%) 192 (100, M), 123 (45, M–2CN–OH), **Anal.** Calcd for $C_{10}H_{12}N_2O_2$: C, 62.49; H, 6.29, N, 14.57. Found: C, 62.31; H, 6.27, N, 14.62. **TLC** (SiO_2, ethyl acetate, developed by anisaldehyde): R_F = 0.44.

5.9. Synthesis of (1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-3,5,10-trioxatricyclo[5.2.1.0^{2,6}]decane-8,9-dicarbonitrile, **16**

To a solution of diol **13** (1173 mg, 6.52 mmol) in anhydrous acetone/dichloromethane 1:1 (250 mL), 2,2-dimethoxypropane (9.3 mL, 157 mmol) and a catalytic amount of *p*-toluenesulphonic acid (146.9 mg, 0.853 mmol) were added. The reaction mixture was stirred at 60 °C for 72 h. After that, the reaction mixture was evaporated to dryness, then dissolved in ethyl acetate (80 mL) and successively washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and with brine (50 mL). The organic solution was dried over anhydrous $MgSO_4$ filtered and concentrated to dryness under vacuum obtaining product **16** as a white solid (759.2 mg, yield = 84%). **Mp**: 240–242 °C. **IR** (ATR, neat): υ_{max} 2979, 2955, 3924 (υC–H), 2248 (δ_C≡N), 1460 (δC–H), 1377, 1269, 1205, 1140, 1077, 1044, 860. **^{1}H NMR** (400 MHz, acetone-d_6): δ 1.30 (s, 3H; H1′ or H1″), 1.39 (s, 3H; H1′ or H1″), 3.32–3.34 (d, 1H; J_1 = 5.28 Hz, H9 or H8), 3.51–3.54 (t, 1H; J_1 = 3.53 Hz, H9 or H8), 4.67–4.72 (m, 2H; H2, H6), 4.81 (m, 1H; H1 or H7), 4.88–4.90 (m, 1H; H1 or H7). **^{13}C NMR** (100 MHz, acetone-d_6): δ 24.28 (C1′ or C1″), 25.14 (C1′ or C1″), 32.78 (C8 or C9), 32.98 (C8 or C9), 78.89 (C6), 79.58 (C1 or C7), 80.54 (C2), 83.24 (C1 or C7), 111.81 (C4), 181.98 (C1‴ or C^{IV}), 183.00(C1‴ or C^{IV}). **gHSQC** (400 MHz, acetone-d_6): C1–H1, C2–H2, C6–H6, C7–H7, C8–H8, C9–H9, C1′-C1′, C1″-H1″. **^{1}H–^{1}H COSY** (400 MHz, acetone-d_6): δ H1–H2, H1–H6, H4–H5, H2–H3, H1′–H1″. **MS** (DIP, CI, NH_3): *m/z* (%) 220 (100, M), 194 (43, M–CN), 168 (23, M–2CN), 146 (34, M–Me_2CO_2), **Anal.** Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49, N, 12.72. Found: C, 60.01; H, 5.53, N, 12.58. **TLC** (SiO_2, hexane/ethyl acetate 1:1, developed by anisaldehyde as a brown spot on a pink background): R_F = 0.47.

5.10. Synthesis of (1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8,9-dicarbonitrile, 17

Diol **14** (60 mg, 0.34 mmol) dissolved in anhydrous acetone (6 mL) was placed in a 25 mL round bottomed flask and 2-methoxypropane (1 mL, 8.2 mmol) and *p*-toluenesulphonic acid (10 mg) were added at once. The mixture was stirred under reflux of solvent for 3 h and one additional hour at room temperature (control by TLC). The crude mixture was concentrated to dryness and the resulting thick oil was re-dissolved in ethyl acetate (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 2 mL) and with brine (2 × 2 mL). The organic phase was dried over anh. $MgSO_4$, filtered and concentrated in vacuo, obtaining 64 mg (87% yield) of pure product as a brown solid. **Mp**: 162–163 °C (ethyl acetate). **IR** (ATR): υ_{max} 2991–2952, 2937–2913 (Csp^3-H, st), 2246 (C≡N, st), 1465, 1382, 1272, 1208, 1169, 1158, 1075, 1050, 864. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.31 (3H, s, H1′ or H1″), 1.45 (3H, s, H1′ or H1″), 1.53 (1H, dd, $J_{10A-10B}$ = 11.8 Hz, J_2 = 1.4 Hz, $H10_A$), 2.05 (1H, dd, $J_{10A-10B}$ = 11.8 Hz, J_2 = 1.8 Hz, $H10_B$), 2.46 (1H, dd, J_1 = 5.2, J_2 = 2.0 Hz, H1 or H7), 2.74 (2H, s, H8 and H9), 3.02 (1H, dd, J_1 = J_2 = 4.8 Hz, H1 or H7), 4.12 (1H, dd, J_2 = 5.6 Hz, J_2 = 1.2 Hz, H6 or H2), 4.50 (1H, dd, J_1 = 5.2 Hz, J_2 = 1.2, H6 or H2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 24.6 (C1′ or C1″), 25.4 (C1′ or C1″), 31.4 (C10), 32.4 (C7 or C1), 32.4 (C7 or C1), 43.1 (C8 or C9), 45.8 (C8 or C9), 77.8 (C2 or C6), 79.7 (C2 or C6), 110.8 (C4), 117.6 (CN), 119.2 (CN). **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%) 190 (9, M–CH_2–CH_3+H^+), 203 (100, M–CH_3), 219 (6, M+H^+), 236 (9, M+NH_4^+). **Anal.** Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47, N, 12.84. Found: C, 66.07; H, 6.53, N, 12.77. **TLC** (SiO_2, ethyl acetate, developed by anisaldehyde as a brown spot on a pink background): R_F = 0.70.

5.11. Synthesis of (1S*, 2S*, 3S*, 4R*, 5R*, 6S*)-4,4-dimethyl-3,5-dioxatricyclo[5.2.2.0^{2,6}]undecane-8,9-dicarbonitrile, **18**

2,2-Dimethoxypropane (1.5 mL) and a catalytic amount of *p*-toluenesulphonic acid (18.8 mg) were added to a suspension of diol **15** (83.7 mg, 0.436 mmol) in anhydrous acetone (8 mL). The reaction mixture was stirred at 60 °C for 4 h. The solution was evaporated under vacuum and the residue was re-dissolved in ethyl acetate. The organic solution was washed with saturated solution of sodium bicarbonate (6 mL) and brine (6 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under vacuum, obtaining product **18** as a thick oil (92 mg, yield = 91%). **IR** (Film): υ_{max} 2989–2960, 2933–2915 (Csp^3-H, st), 2248 (C≡N, st), 1470, 1390, 1285, 1210, 1150, 1162, 1079, 1052, 868. **^{1}H NMR** (400 MHz, acetone-d_6, 25 °C): δ 1.37 (s, 3H; H1′ or H1″), 1.49 (s, 3H; H1′ or H1″), 1.50–1.59 (m, 2H; H10A, H11A), 1.87–1.97 (m, 2H, H10A, H11A), 2.24–2.76 (m, 1H; H6 or H9), 2.32–2.35 (m, 1H; H6 or H9), 4.36–4.43 (m, 2H; H1, H5), 3.22–3.24 (m, 1H; H7, H8), 3.49–3.51 (m, 1H; H7, H8). **^{13}C NMR** (100 MHz, acetone-d_6, 25 °C): δ 13.1 (C10 or C11), 16.29 (C10 or C11), 23.9 (C1′ or C1″), 25.0 (C1′ or C1″), 32.1 (C6 or C9), 32.2 (C6 or C9), 71.3 (C1 or C5), 73.0 (C1 or C5), 109.0 (C3), 119.2 (C7 or C8), 119.5 (C7 or C8). **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%) 204 (12, M–CH_2–CH_3+H^+), 217 (100, M–CH_3), 233 (6, M+H^+), 247 (9, M+NH_4^+). **Anal.** Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94, N, 12.06. Found: C, 67.31; H, 6.88, N, 11.98. **TLC** (SiO_2, ethyl acetate, developed by anisaldehyde as a brown spot on a pink background): R_F = 0.54.

5.12. Synthesis of [(1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5,7-trioxatricyclo[5.2.1.0^{2,6}]dec-8-yl)methylamine], **19**

A solution of dinitrile **16** (691.9 mg, 3.14 mmol) in diethyl ether (150 mL) was slowly added *via* cannula to a suspension of $LiAlH_4$ (560 mg, 14.74 mmol) in anhydrous diethyl ether (50 mL). The reaction mixture was stirred for 90 min at r.t. When the reaction was complete a solution of NaOH (11 mL) was added to quench $LiAlH_4$ excess. The mixture was filtered and organic phase was concentrated to a dryness under vacuum, and the residue was submitted to a flash column chromatography on silica gel to obtain diamine **19** (169 mg, yield = 24%) by elution with 80:20 EtOH/NH_3 (35% w/w). Thick oil. **IR** (ATR, neat): υ_{max} 3367, 3303 (υN–H), 2985, 2934, 2914 (υC–H), 1458 (δC–H), 1380, 1269 (δC–N), 1056, 856. **^{1}H NMR** (400 MHz, CD_3OD): δ 1.28 (s, 3H; H1′ or H1″), 1.40 (s, 3H; H1′ or H1″), 1.51–1.56 (m, 1H; H9 or H8), 1.85–1.93 (m, 1H; H9 or H8), 3.19–3.27 (m, 2H; H1‴ or H1^{IV}), 3.25–3.40 (m, 2H; H1‴ or H1^{IV}), 4.06 (s, 1H; H2 or H6), 4.31–4.37 (m, 2H, H1, H7), 4.55–4.59 (m, 1H; H2 or H6). **^{13}C NMR** (100 MHz, CD_3OD): δ 23.64 (C1′ or C1″), 24.57 (C1′ or C1″), 43.28 (C8 or C9), 44.58 (C8 or C9), 50.31 (C1‴ or C1^{IV}), 53.93(C1‴ or C1^{IV}), 78.62 (C2 or C6), 81.10 (C1 or C7), 81.75 (C1 or C7), 82.24 (C2 or C6), 110.66 (C4). **^{1}H–^{1}H COSY** (400 MHz, CD_3OD): (H1 and H7)-(H2 or H6), (H1 and H7)-(H8 or H9), H8-(H1‴ or H1^{IV}), H9-(H1‴ or H1^{IV}). **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%) 246 (20, M+NH_4^+), 229 (15, M+H^+), 213 (100, M–CH_3). **Anal.** Calcd for $C_{11}H_{20}N_2O_3$: C, 57.87; H, 8.83, N, 12.27. Found: C, 57.75; H, 8.91, N, 12.15. **TLC** (SiO_2, ethanol/NH_3 35% (w/w) 70:30, developed with ninhydrin as a pink spot): R_F = 0.47.

5.13. Synthesis of [(1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]dec-8-yl)methylamine], **20**

A solution of dinitrile **17** (580.6 mg, 2.66 mmol) in dry diethyl ether (150 mL) was slowly added *via* cannula to a suspension of $LiAlH_4$ (545 mg, 14.36 mmol) in anhydrous diethyl ether (50 mL). The reaction mixture was stirred for 60 min at r.t. When the

reaction was complete a solution of NaOH (10 mL) was added to quench the $LiAlH_4$ excess. The mixture was filtered and the organic phase was concentrated to dryness under vacuum, and the residue was submitted to a flash column chromatography on silica gel to obtain diamine **20** (169 mg, yield = 24%), by elution with 80:20 EtOH/NH_3 (35% w/w), as a brownish thick oil. **IR** (Film): υ_{max} 3348–3274–3172 (N–H, st), 2979–2937 (Csp^3-H, st), 1654, 1561, 1382, 1374, 1316, 1270, 1208, 1164, 1044. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.93–1.01 (m, 1H; H8 or H9), 1.19–1.26 (m, 1H; $H10_A$), 1.29 (s, 3H; H1′ or H1″), 1.44 (s, 3H; H1′ or H1″), 1.53–1.63 (1H, m, H8 or H9), 1.71 (1H, d, $J_{10A-10B}$ = 10.4 Hz, H10B), 2.12 (1H, s, H1 or H7), 2.35 (s, 1H; H1 or H7), 2.54–2.66 (m, 2H; $H1'''_A$, $H1^{IV}{}_A$), 2.83–2.87 (m, 2H; $H1'''_B$, $H1^{IV}{}_B$), 4.04 (d, 1H, $J_{2\text{-}6}$ = 9.2 Hz, H2 or H6), 4.25 (d, 1H, $J_{2\text{-}6}$ = 9.2 Hz, H2 or H6). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 24.8 (C1′ or C1″), 26.1 (C1′ or C1″), 31.5 (C10), 42.7 (C1‴ or $C1^{IV}$), 43.9 (C1 or C7), 45.0 (C1 or C7), 46.1 (C1‴ or $C1^{IV}$), 46.2 (C8 or C9), 46.8 (C8 or C9), 78.0 (C2 or C6), 82.6 (C2 or C6), 109.7 (C4). **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%) 152 (5, M–$C(CH_3)_2$–CH_2NH_2), 183 (5, M–$C(CH_3)_2$), 227 (82, M+H$^+$). **Anal.** Calcd for $C_{12}H_{22}N_2O_2$: C, 63.69; H, 9.80, N, 12.38. Found: C, 63.71; H, 9.95, N, 12.27. **TLC** (SiO_2, ethanol/NH_3 35% (w/w) 70:30, developed with ninhydrin as a pink spot): R_F = 0.56.

5.14. Synthesis of [(1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5-dioxatricyclo[5.2.2.0^{2,6}]undec-8-yl)methylamine], **21**

A solution of dinitrile **18** (91 mg, 0.392) in dry diethyl ether (60 mL) was slowly added *via* cannula to a suspension of $LiAlH_4$ (108 mg, 2.85 mmol) in anhydrous diethyl ether (20 mL). The reaction mixture was stirred for 90 min at r.t. When the reaction was complete a solution of 30% w/v NaOH (6 mL) was added to quench the $LiAlH_4$ excess. The mixture was filtered and the organic phase was concentrated to dryness under vacuum, and the residue was submitted to a flash column chromatography on silica gel, to obtain diamine **21** (77.4 mg, yield = 82%), by elution with 80:20 EtOH/NH_3 (35% w/w), as a brown thick oil. **IR** (Film): υ_{max} 3355–3280–3175 (N–H, st), 2983–2932 (Csp^3-H, st), 1658, 1560, 1380, 1375, 1314, 1278, 1205, 1160. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.05–1.12 (m, 2H; $H10_A$ or $H11_A$, H9 or H8), 1.26–1.32 (m, 2H; $H10_A$ or $H11_A$, H9 or H8), 1.35 (s, 3H; H1′ or H1″), 1.53 (s, 3H; H1′ or H1″), 1.69–1.76 (m, 1H; $H10_B$ or $H11_B$), 1.88–1.97 (m, 3H; $H10_B$ or $H11_B$, H1, H7), 2.64–2.66 (m, 2H; H1‴ or $H1^{IV}$), 2.75–2.86 (m, 2H; H1‴ or $H1^{IV}$), 4.02–4.05 (dd, 1H; H2 or H6), 4.21–4.24 (m, 1H; H2 or H6). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 13.02 (C1‴ or $C1^{IV}$), 19.40 (C1‴ or $C1^{IV}$), 24.12 (C1′ or C1″), 25.72 (C1′ or C1″), 30.74 (C1 or C7), 31.25 (C1 or C7), 42.92 (C8 or C9), 44.67 (C8 or C9), 45.37 (C1‴ or $C1^{IV}$), 45.70 (C1‴ or $C1^{IV}$), 72.57 (C2 or C6), 76.15 (C2 or C6), 107.91 (C4). **MS** (DIP–CI–NH_3, 70 eV, 150 °C): *m/z* (%)259 (23, M+H$^+$), 258 (12, M+NH_4^+), 225 (100, M–CH_3), 198 (12, M–CMe_2). **Anal.** Calcd for $C_{13}H_{24}N_2O_2$: C, 64.97; H, 10.07, N, 11.66. Found: C, 64.83; H, 11.71, N, 11.57. **TLC** (SiO_2, ethanol/NH_3 35% (w/w) 80:20, developed with ninhydrin as a pink spot): R_F = 0.51.

5.15. Synthesis of cis-{[(3-aminomethyl-7-oxabicyclo[2.2.1]hept-2-yl)methylamine]tetrachlorido}platinum(IV), **22**

A solution of **10** (48.6 mg, 0.31 mmol) in ethanol (0.3 mL) was added to a solution of K_2PtCl_6 (99.1 mg, 0.31 mmol) in water (15 mL). The mixture was stirred for 30 h at 80 °C. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **22** (56.5 mg, yield = 37%) as a yellowish solid. **IR** (ATR, neat): υ_{max} 3408, 3187 (υN–H), 2960, 2850 (υC–H), 1454 (δC–H), 1035 (υC–O), 986 (δC–O–C), 560 (υPt–N), 330 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.45–1.70 (m, 4H; H5 and H6), 2.10–2.18 (m, 2H; H2 and H3), 2.85–3.20 (m, 2H; H1 and H4), 3.15–3.40 (m, 4H; H1′ and H1″), 5.80–6.50 (m, 4H; NH). **MS** [Electrospray (–)]: *m/z* 329 ($PtCl_3N_2$)$^-$, 336 ($PtCl_4$)$^-$, 371 (PCl_5)$^-$, 529 (M+Cl)$^-$. **HRMS** [Electrospray (–)] Calcd for $C_8H_{16}N_2OCl_5Pt^-$, (M+Cl)$^-$ (monoisotopic): *m/z* 524.9336, 525.9359, 526.9327, 527.9332, 528.9317, 529.9308, 530.9306. Observed: 524.9338 (50%) Δ(mDa) = 0.2; 525.9351 (53%) Δ(mDa) = 0.8; 526.9307 (100%) Δ(mDa) = 2; 527.9349 (82%) Δ(mDa) = 1.7; 528.9288 (100%) Δ(mDa) = 2.9; 529.9315 (50%) Δ(mDa) = 0.7; 530.9285 (50%) Δ(mDa) = 2.1. **Anal.** Calcd for $C_8H_{16}Cl_4N_2OPt$: C, 19.49; H, 3.27; N, 5.68. Found: C, 19.52; H, 3.31; N, 5.73.

5.16. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.1]hept-2-yl)methylamine]tetrachlorido}platinum(IV), **23**

A solution of **11** (28.3 mg, 0.1834 mmol) in ethanol (0.1 mL) was added to a solution of K_2PtCl_6 (89.12 mg, 0.1834 mmol) in water (6.5 mL). The mixture was stirred for 24 h at 80 °C. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **23** (20.7 mg, yield = 34%) as a greenish solid. **IR** (ATR, neat): υ_{max} 3213, 3138 (υN–H), 2994, 2816 (υC–H), 1456 (δC–H), 580 (υPt–N), 325 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.15–1.49 (m, 10H; H1, H2, H3, H4, H5, H6, H7), 1.93–2.24 (m, 2H; H1′ or H1″), 2.48–2.87 (m, 2H; H1′ or H1″), 5.70–6.42 (m, 4H; NH). **MS** [Electrospray (–)]: *m/z* 329 (N_2Cl_3Pt)$^-$, 419 (M–Cl_2)$^-$, 455 (M–Cl)$^-$. **HRMS** [Electrospray (–)] Calcd for $C_9H_{18}N_2Cl_3Pt^-$, (monoisotopic) (M–Cl)$^-$: *m/z* (%) 453.0167, 454.0190, 455.0165, 456.0165, 457.0161. Observed: 453.0156 (50%) Δ(mDa) = 1.1; 454.0178 (58%) Δ(mDa) = 1.2; 455.0157 (100%) Δ(mDa) = 0.8; 456.0166 (58%) Δ(mDa) = 0.1; 457.0158 (67%) Δ(mDa) = 0.3. **Anal.** Calcd for $C_9H_{18}Cl_4N_2Pt$: C, 22.01; H, 3.69; N, 5.70. Found: C, 21.98; H, 3.72; N, 5.71.

5.17. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.2]oct-2-yl)methylamine]tetrachlorido}platinum(IV), **24**

A solution of **12** (31 mg, 0.194 mmol) in ethanol (0.2 mL) was added to K_2PtCl_6 (82.8 mg, 0.194 mmol) dissolved in water (9 mL). The mixture was stirred for 20 h at 80 °C. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **24** (17.6 mg, yield = 32%) as a brownish solid. **IR** (ATR, neat): υ_{max} 3525, 3217 (υN–H), 2959, 2883 (υC–H), 1460 (δC–H), 575 (υPt–N), 327 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.12–1.54 (m, 12H; H1, H2, H3, H4, H5, H6, H7, H8), 1.95–2.31 (m, 2H; H1′ or H1″), 2.51–2.90 (m, 2H; H1′ or H1″), 5.38–6.35 (m, 4H; NH). **MS** [Electrospray (–)]: *m/z* 469 (M–Cl)$^-$, 434 (M–Cl_2)$^-$, 329 (N_2Cl_3Pt)$^-$. **HRMS** [Electrospray (–), 175 V] Calcd for $C_{10}H_{20}N_2Cl_3Pt^-$, (monoisotopic) (M–Cl)$^-$: *m/z* (%) 468.0346, 469.0322, 471.0318, 472.0307. Observed: 468.0346 (63%) Δ(mDa) = 0; 469.0315 (100%) Δ(mDa) = 0.7; 471.0315 (74%) Δ(mDa) = 0.3; 472.0286 (28%) Δ(mDa) = 2.1. **Anal.** Calcd for $C_{10}H_{20}Cl_4N_2Pt$: C, 23.78; H, 3.99; N, 5.55. Found: C, 23.82; H, 4.01; N, 5.51.

5.18. Synthesis of dipotassium trans-dihydroxidotetrachloridoplatinate(IV) $K_2PtCl_4(OH)_2$, **31**

Compound $K_2PtCl_4(OH)_2$ was prepared by oxidation of K_2PtCl_4 according to the procedure described in the literature [20]. Thus, K_2PtCl_4 (51 mg, 123 mmol) was reacted with hydrogen peroxide 35% (0.5 mL) at 10 °C until effervescence ceased (20 min). The reaction mixture was cooled down to −10 °C by an ice-salt bath, then,

ice-cold ethanol (1.25 mL) was added at once. The pinkish precipitate was collected by filtration and washed with cold absolute ethanol, obtaining pure product **31** (43.1 mg, yield = 78%). **IR** (ATR, neat): υ_{max} 3575 (υO–H), 1001 (δO–H), 550 (υPt–O), 328 (υPt–Cl). **^{195}Pt NMR** (400 MHz, D_2O, 25 °C): δ 1250. **MS** [Electrospray (−)]: *m/z* (%) 185 $(M)^{2-}$, 317 $(M-OH_2Cl)^-$, 353 $(M-OH)^-$, 371 $(M+H)^-$. **HRMS** (Electrospray, 100 V, *m/z*): Calcd for $Cl_4H_2O_2Pt^{2-}$ (monoisotopic) $(M)^{2-}$: 184.4234, 184.9218, 185.4219, 185.9215, 186.4205, 186.9211. Found: 184.4236 (50%) Δ(mDa) = 0.2; 184.9220 (100%), Δ(mDa) = 0.2; 185.4220 (63%), Δ(mDa) = 0.1; 185.9217 (86%) Δ(mDa) = 0.2; 186.4208 (31%) Δ(mDa) = 0.3; 186.9212 (41%) Δ(mDa) = 0.1.

5.19. Synthesis of cis-{[(3-aminomethyl-7-oxabicyclo[2.2.1]hept-2-yl)methylamine]dichloridodihydroxido}platinum(IV), **25**

A solution of **10** (44.7 mg, 0.286 mmol) in ethanol (1 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (128.4 mg, 0.286 mmol) in water (9 mL). The reaction mixture was stirred in the dark for 48 h and then, heated up to 80° for 1 h. Finally it was keep at 4 °C for 48 h to induce precipitation. The precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **25** (57.4 mg, yield = 44%).

IR (ATR, neat): υ_{max} 3494 υ(O–H), 3197 υ(N–H), 2961, 2883 (υC–H), 1034 (υC–O), 1040 (δO–H), 986 (υC–O–C), 563 (υPt–O and υPt–N), 341 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.50–1.80 (m, 4H; H5, H6), 2.10–2.18 (m, 2H; H2, H3), 3.11–3.85 (m, 6H; H1, H4, H1′, H1″), 6.15–6.70 (m, 4H; NH). **MS** [Electrospray (−)]: *m/z* (%) 421 $(M-O_2H_3)^-$, 457 $(M-O_2H_3+Cl)^-$. **HRMS** (Electrospray, 175 V). Calcd for $C_8H_{16}Cl_3N_2O_1Pt^-$ (monoisotopic) $(M-O_2H_3)^-$: *m/z* (%):454.9953, 455.9976, 456.99466, 457.9943, 458.9938. Observed: 454.9925 (59%) Δ(mDa) = 2.8; 455.9968 (60%) Δ(mDa) = 0.8; 456.9940 (100%) Δ(mDa) = 2.8; 457.9926 (53%) Δ(mDa) = 1.7; 458.9930 (75%) Δ(mDa) = 0.8. **Anal.** Calcd for $C_8H_{18}Cl_2N_2O_3Pt$: C, 21.06; H, 3.98; N, 6.14. Found: C, 21.09; H, 3.94; N, 6.17.

5.20. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.1]hept-2-yl) methylamine]dichloridodihydroxido}platinum(IV) **26**

A solution of **11** (17.7 mg, 0.105 mmol) ethanol (0.3 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (47.15 mg, 0.105 mmol) in water (3 mL). The reaction mixture was stirred in the dark for 48 h and then, heated up to 80° for 1 h. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **26** (44 mg, yield = 66%). **IR** (ATR, neat): υ_{max} 3572 (υO–H), 3215 (υN–H), 2951, 2874 (υC–H), 1604 (δN–H), 1458 (δC–H), 1045 (δO–H), 1000 (δO–H), 565 (υPt–O and υPt–N), 332 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 0.90–1.70 (m, 6H; H5, H6, H7), 1.72–2.50 (m, 4H; H1, H2, H3, H4), 2.60–3.70 (m, 4H; H1′ and H1″), 5.20–6.05 (m, 4H; NH). **^{195}Pt NMR** (400 MHz, DMSO-d_6, 25 °C): δ 247.50 ppm. **MS** [Electrospray (−)]: *m/z* (%) 419 $(M-O_2H_3)^-$, 455 $(M-O_2H_3+Cl)^-$. **Anal.** Calcd for $C_9H_{20}Cl_2N_2O_2Pt$: C, 23.80; H, 4.44; N, 6.17. Found: C, 23.78; H, 4.46; N, 6.13.

5.21. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.2]oct-2-yl) methylamine]dichloridodihydroxido}platinum(IV), **27**

A solution of **12** (16.1 mg, 0.096 mmol) in ethanol (0.4 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (43.1 mg, 0.096 mmol) in water (3 mL). The reaction mixture was stirred in the dark for 48 h and then, heated up to 80° for 1 h. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The product was washed with water and ethanol and dried under vacuum to obtain product **27** (29.3 mg, yield = 67%). **IR** (ATR, neat): υ_{max} 3400 υ(O–H), 3182 υ(N–H), 3920, 2864 (υC–H), 1457 (δC–H), 1040 (δO–H), 563 (υPt–O and υPt–N), 337 (υPt–Cl). MS [Electrospray (−)]: *m/z* (%) 433 $(M-O_2H_3)^-$, 469 $(M-O_2H_3+Cl)^-$. **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 1.10–1.75 (m, 10H; H5, H6, H7 and H8), 1.82–1.90 (m, 2H; H2 and H4), 2.80–3.55 (m, 4H; H1′ and H1″), 5.85–7.05 (m, 4H; NH). **MS** [Electrospray (+)]: *m/z* (%) 477 $(M-O_2H_2+CH_3CN)^+$, 469 $(M+H)^+$, 456 $(M-O_2H_2+Na)^+$, 452 $(M-O_2H_2+NH_4)^+$. **Anal.** Calcd for $C_{10}H_{22}Cl_2N_2O_2Pt$: C, 25.65; H, 4.74; N, 5.98. Found: C, 25.68; H, 4.72; N, 6.03.

5.22. Synthesis of cis-{[(1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5,7-trioxatricyclo[5.2.1.$0^{2,6}$]dec-8-yl) methylamine]dichloridodihydroxido}platinum(IV), **28**

A solution of **19** (54.7 mg, 0.24 mmol) in ethanol (0.7 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (107.9 mg, 0.24 mmol) in water (10 mL). The reaction mixture was stirred in the dark for 48 h, and then heated to 80° for 1 h. To obtain the solvent was evaporated under vacuum and precipitate was lixiviated with methanol, to extract excess of ligand. The product was washed with methanol and dried under vacuum to obtain product **28** (57.7 mg, yield = 46%). **IR** (ATR, neat): υ_{max} 3464 υ(O–H), 3192 υ(N–H), 2982, 2900 (υC–H), 1420 (δC–H), 1158 (υC–O), 1064 (υC–O–C), 1042 (δO–H), 560 (υPt–O and υPt–N), 340 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 1.31 (s, 3H, Me), 1.42 (s, 3H, Me), 1.95–2.20 (m, 2H; H8 and H9), 3.05–3.70 (m, 8H; H1, H2, H6, H7, H1′ and H1″), 5.61–6.65 (m, 4H; NH). **MS** [Electrospray (−)]: *m/z* (%) 329 $(PtCl_3N_2)^-$, 336 $(PtCl_4)^-$, 529 $(M-O_2H_2+Cl)^-$. **HRMS** (Electrospray, 175 V). Calcd for $C_{11}H_{20}Cl_3N_2O_3Pt$ (monoisotopic) $(M-O_2H_2+Cl)^-$: *m/z* (%) 527.0171, 528.0194, 529.0170, 530.0166, 531.0166. Observed: 527.0125 (50%) Δ(mDa) = 4.6; 528.0198 (55%) Δ(mDa) = 0.4; 529.0172 (100%) Δ(mDa) = 0.2; 530.0150 (40%) Δ(mDa) = 1.6; 531.0188 (43%) Δ(mDa) = 2.2. **Anal.** Calcd for $C_{11}H_{22}Cl_2N_2O_5Pt$: C, 25.01; H, 4.20; N, 5.30. Found: C, 24.99; H, 4.23; N, 5.28.

5.23. Synthesis of cis-{[(1S*, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]dec-8-yl)methylamine] dichloridodihydroxido}platinum(IV), **29**

A solution of **20** (45 mg, 0.20 mmol) in ethanol (1 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (89.8 mg, 0.20 mmol) in water (14 mL). The reaction mixture was stirred in the dark for 48 h, and then heated up to 80° for 1 h. The resulting precipitate was separated from the mother solution by filtration through a sintered-glass plate #4. The solid was washed with water and ethanol, and dried under vacuum to obtain product **29** (45.3 mg, yield = 43%). **IR** (ATR, neat): υ_{max} 3465 υ(O–H), 3191 υ(N–H), 2930, 2864 (υC–H), 1459 (δC–H), 1055 (υC–O–C), 1040 (δO–H), 566 (υPt–O and υPt–N), 341 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 1.27 (s, 3H; Me), 1.39 (s, 3H; Me), 1.50–1.65 (m, 2H; H1, H7), 1.80–2.20 (m, 4H; H8, H9 and H10), 3.10–3.55 (m, 6H; H2, H6, H1′ and H1″), 5.60–6.90 (m, 4H; NH). **MS** [Electrospray (−)]: *m/z* (%) 329 $(PtCl_3N_2)^-$, 336 $(PtCl_4)^-$, 527 $(M-O_2H_2+Cl)^-$. **HRMS** (Electrospray, 175 V). Calcd for $C_{12}H_{22}Cl_3N_2OPt^-$, (monoisotopic) $(M-O_2H_2+Cl)^-$: *m/z* (%) 525.0378, 526.0401, 527.0378, 528.0378, 529.0370. Observed: 525.0369 (50%) Δ(mDa) = 0.9; 526.0387 (59%) Δ(mDa) = 1.4; 527.0379 (100%) Δ(mDa) = 0.1; 528.0370 (62%) Δ(mDa) = 0.8; 529.0378 (66%) Δ(mDa) = 0.8. **Anal.** Calcd for $C_{12}H_{24}Cl_2N_2O_4Pt$: C, 27.38; H, 4.60; N, 5.32. Found: C, 27.36; H, 4.59; N, 5.35.

5.24. Synthesis of cis-{[(1S, 2S*, 6R*, 7R*, 8R*, 9R*)-4,4-dimethyl-9-aminomethyl-3,5-dioxatricyclo[5.2.2.0^{2,6}]undec-8-yl) methylamine]dichloridodihydroxido}platinum(IV),* ***30***

A solution of **21** (29.5 mg, 0.123 mmol) in ethanol (0.3 mL) was added to a solution of $K_2PtCl_4(OH)_2$ (55.2 mg, 0.123 mmol) in water (4 mL). The reaction mixture was stirred in the dark for 48 h, and then heated up to 80° for 1 h. Then the precipitate was separated from the mother solution by filtration through a sintered-glass plate. The product was washed with water and ethanol and dried under vacuum to obtain product **30** (20.2 mg, yield = 30%). **IR** (ATR, neat): 3436 υ(O–H), 3192 υ(N–H), 2910 (υC–H), 1455 (δC–H), 1045 (υC–O–C), 1038 (δO–H), 564 (υPt–O and υPt–N), 339 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 1.25 (s, 3H; Me), 1.36 (s, 3H; Me), 1.33–1.70 (m, 6H; H1, H7, H10 and H11), 1.84–1.90 (m, 2H; H8 and H9), 3.05–3.50 (m, 6H; H2, H6, H1′ and H1″), 5.45–6.25 (m, 4H; NH). **MS** [Electrospray (+)]: *m/z* (%) 524 $(M-OH+H)^+$, 563 $(M+Na)^+$. **Anal.** Calcd for $C_{13}H_{26}Cl_2N_2O_4Pt$: C, 28.90; H, 4.85; N, 5.18. Found: C, 28.88; H, 4.82; N, 5.21.

5.25. Synthesis of malonate silver salt, ***32***

A solution of malonic acid (23.7 mg, 0.228 mmol) and triethylamine (0.063 mL, 0.456 mmol) in ethanol (1 mL) was added dropwise to $AgNO_3$ (77 mg, 0.456 mmol) dissolved in the solvent mixture ethanol/acetonitrile 10/1 (3 mL). The solution was stirred for 2 h in the dark. The precipitate was separated by filtration and washed twice with ethanol and petroleum ether and dried under vacuum, in the dark, to obtain product **32** as a white solid (60.7 mg, yield = 84%). **IR** (ATR, neat): υ_{max} 1573 (υC–O), 1450 (δC–O), 1308 (υC–O). **Anal.** Calcd for $C_3H_2O_4Ag_2$: C, 11.34; H, 0.63. Found: C, 11.29; H, 0.61.

5.26. Synthesis of cis-{[(3-aminomethyl-7-oxabicyclo[2.2.1]hept-2-yl)methylamine]dihydroxidomalonato}platinum(IV), ***33***

Complex **25** (22.3 mg, 0.049 mmol) was suspended in milliQ water (3 mL) and silver malonate disalt (18.4 mg, 0.058 mmol) was added at once. The reaction mixture was stirred under argon atmosphere for 96 h at 45 °C, protected from light. The solution was filtrated and the mother liquor was concentrated to dryness to obtain the product **33** (15.7 mg, yield = 66%). **IR** (ATR, neat): υ_{max} 3400 υ(O–H), 3201 υ(N–H), 2915 (υC–H), 1717 (υCO), 1022 (υC–O), 1044 (δO–H), 927 (υC–O–C), 562 (υPt–O and υPt–N), 352 (υPt–Cl). **^{1}H NMR** (300 MHz, DMSO-d_6, 25 °C): δ 1.14–1.70 (m, 4H; H5, H6), 1.60–2.20 (m, 2H; H2, H3), 3.00–3.50 (m, 6H; H1′, H1″ and H2‴), 4.31–4.34 (m, 2H, H1, H4), 5.80–6.40 (m, 4H; NH). **MS** [Electrospray (−)]: *m/z* (%) 452 $(M-O_2H_3)^-$. **HRMS** (Electrospray, 175 V). Calcd for $C_{11}H_{17}N_2O_5Pt^-$, (monoisotopic) $(M-O_2H_3)^-$: *m/z* (%) 451.0770, 452.0792. Observed: 451.0767 (63%) Δ(mDa) = 0.3; 452.0783 (100%) Δ(mDa) = 0.9. **Anal.** Calcd for $C_{11}H_{20}N_2O_7Pt$: C, 27.11; H, 4.14; N, 5.75. Found: C, 27.13; H, 4.12; N, 5.76.

5.27. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.1]hept-2-yl) methylamine]dihydroxidomalonato}platinum(IV), ***34***

Complex **26** (8 mg, 0.0176 mmol) was suspended in milliQ water (2 mL) and silver malonate disalt (5.7 mg, 0.0179 mmol) was added at once. The reaction mixture was stirred under inert atmosphere for 52 h at 45 °C, protected from light. The solution was filtrated to remove AgCl and the mother liquid was concentrated to dryness to obtain the product **34** (7.1 mg, yield = 83%). **IR** (ATR, neat): υ_{max} 3350 (O–H), 3210 (υN–H), 2950, 2875 (υC–H), 1657 (υC=O), 1074 (υC–O), 1039 (δPt–O–H), 561 (υPt–O and υPt–N), 345 (υPt–Cl). **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C): δ 1.10–1.60 (m, 6H; H5, H6, H7), 1.70–2.10 (m, 4H; H1, H2, H3, H4), 2.60–3.80 (m, 6H; H1′, H1″ and H2‴), 5.70–6.00 (m, 4H; NH). **MS** [Electrospray (+)]: *m/z* (%) 474 $(M-O_2H_2+Na)^+$, 452 $(M-O_2H_2+H)^+$. **HRMS** (Electrospray, 175 V). Calcd for $C_{12}H_{21}N_2O_4Pt^+$, (monoisotopic) $(M-O_2H_2+H)^+$: *m/z* (%) 452.1143. Observed: 452.1137 (100%) Δ(mDa) = 0.6. **Anal.** Calcd for $C_{12}H_{22}N_2O_6Pt$: C, 29.69; H, 4.57; N, 5.77. Found: C, 29.71; H, 4.60; N, 5.73.

5.28. Synthesis of cis-{[(3-aminomethylbicyclo[2.2.2]oct-2-yl) methylamine]dihydroxidomalonato}platinum(IV), ***35***

Complex **27** (29.7 mg, 0.064 mmol) was suspended in milliQ water (2.5 mL) and silver malonate disalt (20.1 mg, 0.064 mmol) was added at once. The reaction mixture was stirred under argon atmosphere for 52 h at 45 °C, protected from light. The solution was filtrated and the mother liquor was concentrated to dryness to obtain the product **35** (17.5 mg, yield = 55%). **IR** (ATR, neat): υ_{max} 3430 υ(O–H), 3201, 3058 υ(N–H), 2930, 2864 (υC–H), 1700 (υC=O), 1080 (υC–O), 1040 (δPt–O–H), 563 (υPt–O and υPt–N), 347 (υPt–Cl). **^{1}H NMR** (300 MHz, DMSO-d_6, 25 °C): δ 1.20–1.90 (m, 12H; H1, H2, H4, H5, H6, H7 and H8), 2.61 (br s, 2H; H2‴), 3.10–3.40 (m, 4H; H1′, H1″), 5.55–5.80 (m, 4H; NH). **MS** [Electrospray (+)]: *m/z* (%) 466 $(M-O_2H_2+H)^+$. **HRMS** (Electrospray, 175 V). Calcd for $C_{13}H_{23}N_2O_4Pt^+$, (monoisotopic) $(M-O_2H_2+H)^+$: *m/z* (%) 466.1307. Observed: 466.1318 (100%) Δ(mDa) = 1.1. **Anal.** Calcd for $C_{13}H_{24}N_2O_6Pt$: C, 31.26; H, 4.84; N, 5.61. Found: C, 31.30; H, 4.88; N, 5.58.

6. Biological testing: materials and methods

6.1. Growth inhibitory activity

To determine the growth inhibitory activity of the tested compounds on human myeloid leukemia HL-60 cell line, 10^4 cells were plated into 96 well plates (Costar, Cambridge, UK) in 100 μL of complete RPMI medium and then treated with different concentrations of each drug. After 24 h or 72 h of incubation, the number of viable cells was determined using XTT assay. XTT was bioreduced by viable cells into formazan, and the amount of formazan present can be measured by reading the absorbance at 490 nm. The amount of formazan present was proportional to the number of living cells in culture. The absorbance of wells containing only the XTT reagent (the plate blank) was subtracted from all wells. The results were expressed as the percentage cell viability relative to vehicle-treated control cells (100%). Dose-response curves were plotted, and IC_{50} values (concentration of drug resulting in 50% reduction in cell viability) cells were obtained using Prism GraphPad4.

6.2. Flow cytometry analysis of apoptosis

Apoptosis was evaluated by Annexin V–PI staining kit (Roche). $5{\cdot}10^5$ cells were treated with IC_{50} dose of cisplatin and evaluated complexes. After 24 h cells were washed and then re-suspended in binding buffer and stained with annexin-V–FITC antibody and propidium iodide (PI) for 10 min at room temperature. Bivariant analysis of FITC-fluorescence (FL-1) and PI-fluorescence (FL-2) gave different cell populations where FITC-negative and PI-negative were designated as viable cells; FITC-positive and PI-negative phenotype as apoptotic cells, and FITC-positive and PI-positive as late apoptotic or necrotic cells.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.042.

Abbreviations used

Magnitude/ Concept	Definition	Units
ATR	Attenuated total reflectance (IR system)	
cLogP	Logarithm of the calculated partition coefficient between *n*-octanol and water (for non-ionic species)	logarithmic units
cMR	Calculated molar refractivity	10^6 (m^3 mol^{-1})
IC_{50}	Inhibitory concentration 50	μM (micromolar)
Δ(mDa)	Observed *versus* calculated deviations in HRMS spectral lines	milliDalton
LogD	pH dependent partition coefficient (for ionic species at pH = 7.4)	logarithmic units
LogS	Water solubility logarithm	logarithmic units of molar concentration [M]
MW	Molecular weight	g mol^{-1}
μ	Dipolar moment	Debye
SASA	Solvent accessible surface area	$Å^2$
SES	Solvent excluded surface	$Å^2$
SEV	Solvent excluded volume	$Å^3$
tPSA	Topological polar surface area	$Å^2$
VdWSA	Van der Waals surface area	$Å^2$
VdWV	Van der Waals volume	$Å^3$

References

[1] M. Peyrone, Ueber die Einwirkung des Ammoniaks auf Platinchlorür, Ann. Chem. Pharm. 51 (1844) 1–29.
[2] B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode, Nature 205 (1965) 698–699.
[3] R.B. Weiss, M.C. Chistian, New cisplatin analogues in development. A review, Drugs 43 (1993) 360–377.
[4] M.A. Fuertes, C. Alonso, J.M. Pérez, Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance, Chem. Rev. 103 (2003) 645–662.
[5] (a) A.M. Montaña, C. Batalla, The rational design of anticancer platinum complexes: the importance of the structure–activity relationship, Curr. Med. Chem. 16 (2009) 2235–2260;
(b) J. De Mier-Vinué, A.M. Montaña, V. Moreno, Platinum complexes with cytotoxic activity: models for structure-activity relationship studies, in: J.M. Pérez, M.A. Fuertes, C. Alonso (Eds.), Metal Compounds in Cancer Chemotherapy, Research Signpost, Kerala, India, 2005, pp. 47–154.
[6] E.R. Jamieson, J.J. Lippard, Structure, recognition and processing of cisplatin-DNA adducts, Chem. Rev. 99 (1999) 2467–2498.
[7] J. Reedijk, A.M.J. Fichtinger-Shepman, A.T. Van Oosterom, P. Van de Putte, Platinum amine coordination compounds as anti-tumor drugs. Molecular aspects of the mechanism of action, Struct. Bond. 67 (1987) 53–89.
[8] P.M. Takahara, C.A. Frederick, S.J. Lippard, Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin, Nature 377 (1995) 649–652.
[9] A. Gelasco, J.J. Lippard, NMR solution structure of a DNA dodecamer duplex containing a *cis*-diammineplatinum(II) d(GpG) intrastrand crosslink, the major adduct of the anticancer drug cisplatin, Biochemistry 37 (1998) 9230–9239.
[10] D. Yang, S.S.G.E. Van Boom, J. Redjick, J.H. Van Boom, A.H.J. Wang, Structure and isomerization of an intrastrand cisplatin-cross-linked octamer DNA duplex by NMR analysis, Biochemistry 34 (1995) 12912–12929.
[11] D.T. Witiak, D.P. Rotella, J.A. Filippi, J. Gallucci, Stereocontrolled syntheses for the six diastereomeric 1,2-dihydroxy-4,5-diaminocyclohexanes: Pt(II) complexes and P-388 antitumor properties, J. Med. Chem. 30 (1987) 1327–1336.
[12] M. Noji, K. Okamoto, Y. Kidani, T. Tashiro, Relation of conformation to antitumor activity of Pt (II) complexes of 1,2-cyclohexanediamine and 2-(aminomethyl)cyclohexylamine isomers against leukemia P388, J. Med. Chem. 24 (1981) 508–515.
[13] J. De Mier-Vinué, M. Gay, A.M. Montaña, R.I. Sáez, V. Moreno, J. Kasparkova, O. Vrana, P. Heringova, V. Brabec, A. Boccarelli, M. Coluccia, G. Natile, Synthesis, biophysical studies, and antiproliferative activity of platinum(II) complexes having 1,2-bis(aminomethyl)carbobicyclic ligands, J. Med. Chem. 51 (2008) 424–431.
[14] A.M. Montaña, R.I. Sáez, M. Gay, J. De Mier, Bicyclic Cis-platinum (II) Complexes Having Anticancer Activity, WO-2009/080853-A1.
[15] (a) N. Havis, D.R. Walters, F.M. Cook, D.J. Robins, Synthesis, fungicidal activity and effects on polyamine metabolism of novel cyclic diamines, J. Agric. Food. Chem. 45 (1997) 2341–2344;
(b) D. Varech, J. Jacques, Relative size of some substituents determined from the equilibrium between *cis*- and *trans*-2,3-disubstituted bicyclo[2.2.2]octanes, Bull. Soc. Chim. Fr. 1 (1973) 224–231;
(c) O. De Lucchi, N. Piccolrovazzi, G. Licini, G. Modena, G. Valle, Synthesis of anti-sesquinorbornene and related polycyclic alkenes via reductive elimination of vicinal dicyano derivatives, Gazz. Chim. Ital. 117 (1987) 401–408.
[16] M. Schröder, Osmium tetraoxide *cis* hydroxylation of unsaturated substrates, Chem. Rev. 80 (1980) 187–213.
[17] N. Katagiri, M. Tomura, T. Haneda, C. Kaneko, Stereospecific synthesis of carbocyclic analog of oxazinomycin by retrograde aldol carbon-carbon bond fission under reductive conditions, J. Chem. Soc. Chem. Commun. 19 (1987) 1422–1423.
[18] (a) M. Karplus, Vicinal proton coupling in nuclear magnetic resonance, J. Am. Chem. Soc. 85 (1963) 2870–2871;
(b) L.M. Jackman, S. Sternhell, Application of NMR Spectroscopy in Organic Chemistry, second ed., Pergamon Press, New York, 1969, pp. 281–283;
(c) A.M. Montaña, K.M. Nicholas, ^1H and ^{13}C NMR determination of the relative stereochemistry of the diastereoisomers resulting from the reaction of silyl enol ethers with 1-ethoxy-1-propargylium dicobalt hexacarbonyl cation, Magn. Reson. Chem. 28 (1990) 486–495;
(d) A.M. Montaña, S. Ribes, P.M. Grima, F. García, General method of assignment of relative stereochemistry in C-1 substituted 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one by ^1H and ^{13}C NMR correlations, Magn. Reson. Chem. 36 (3) (1998) 174–180;
(e) A.M. Montaña, F. García, P.M. Grima, Complete assignment of ^1H and ^{13}C NMR data and establishment of the relative stereochemistry of C1-functionalized 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives, Magn. Reson. Chem. 37 (7) (1999) 507–511;
(f) A.M. Montaña, M. Cano, General model of assignment of the relative stereochemistry in the Nicholas reaction products, resulting from chiral dicobalthexacarbonyl-1-alkoxy-propargylium cations, Magn. Reson. Chem. 40 (4) (2002) 261–272.
[19] S. Ren, L. Cai, B. Segal, Synthesis and characterization of monodeoxynucleotide tethered platinum-(II) and -(IV) complexes, J. Chem. Soc. Dalt. Trans. 9 (1999) 1413–1422.
[20] L.E. Cox, D.G. Peters, Electronic and vibrational spectra for *trans*-dihydroxotetrachloroplatinate (IV), Inorg. Chem. 9 (8) (1970) 1927–1930.
[21] B. Shelimov, J.F. Lambert, M. Che, B. Didillan, Application of NMR to interfacial coordination chemistry: a ^{195}Pt NMR study of the interaction of hexachloroplatinic acid aqueous solutions with alumina, J. Am. Chem. Soc. 121 (1999) 545–556.
[22] (a) C.M. Giandomenico, M.J. Abrams, B.A. Murrer, J.F. Vollano, M.I. Rheinheimer, S.B. Wyer, G.E. Bossard, J.D. Higgins III, Carboxylation of kinetically inert platinum(IV) hydroxy complexes. An entree into orally active platinum(IV) antitumor agents, Inorg. Chem. 34 (1995) 1015–1021;
(b) S.O. Dunham, R.D. Larsen, E.H. Abbott, Nuclear magnetic resonance investigation of the hydrogen peroxide oxidation of platinum(II) complexes. Crystal and molecular structures of sodium *trans*-dihydroxobis(malonato) platinate(IV) hexahydrate and sodium *trans*-dihydroxobis(oxalato)platinate(IV) hexahydrate, Inorg. Chem. 32 (1993) 2049–2055;
(c) K. Nakamoto, Infrared and Raman Spectra of Inorganic Coordination Compounds, fourth ed., John Wiley, New York, 1986, p. 383.
[23] (a) A.D. Allen, T. Theophanides, Platinum(II) complexes: infrared spectra in the 300-800-cm^{-1} region, Can. J. Chem. 42 (7) (1964) 1551–1554;
(b) J.R. Ferraro, Low-Frequency Vibrations of Inorganic and Coordination Compounds, Plenum Press, New York, 1971, p. 46.
[24] J.D. Hoeschele, H.D.H. Showalter, A. Kraker, W. Elliot, B. Roberts, J. Kampft, Synthesis, structural characterization, and antitumor properties of a novel class of large-ring platinum(II) chelate complexes incorporating the cis-1,4-diaminocyclohexane ligand in a unique locked boat conformation, J. Med. Chem. 37 (1994) 2630–2636.
[25] A. Jakob, H. Schmidt, B. Walfort, G. Rheinwald, S. Frühauf, S. Schulz, T. Gessen, H.Z. Long, Tri-*n*-butylphosphine silver(I) complexes with carboxylate-,

tropolonate- or *N*-hydroxyphthalimide building blocks. Synthesis and their use as spin-on precursors, Z. Anorg. Allg. Chem. 631 (2005) 1079–1986.

[26] (a) G.B. Deacon, R.J. Phillips, Relationships between the carbon-oxygen stretching frequencies of carboxylato complexes and the type of carboxylate coordination, Coord. Chem. Rev. 33 (1980) 227–250;
(b) A.R. Khokhar, S. Al-Baker, Z.H. Siddik, Synthesis and antitumor activity of 1,2-diaminocyclohexane platinum(IV) complexes, J. Inorg. Biochem. 54 (1994) 39–47.

[27] (a) B.D. Markovic, V.D. Dobricic, S.M. Vladimirov, O.A. Cudina, V.M. Savic, K.D. Karljikovic-Rajic, Investigation of solvolysis kinetics of new synthesized fluocinolone acetonide C-21 esters. An *in vitro* model for prodrug activation, Molecules 16 (2011) 2658–2671;
(b) C.H. Choi, Y.J. Cha, C.S. An, K.J. Kim, K.C. Kim, S.P. Moon, Z.H. Lee, Y.D. Min, Molecular mechanisms of heptaplatin effective against cisplatin-resistant cancer cell lines: less involvement of metallothionein, Cancer Cell Int. 4 (6) (2004) 1–12.

[28] (a) A.M. Montaña, Química Orgánica Estructural, in: Estereoquímica y Propiedades Moleculares, second ed., vol. II, Pearson Prentice-Hall, Madrid, 2012, pp. 1319–1381;
(b) I.F. Tannock, D. Rotin, Acid pH in tumors and its potential for therapeutic exploitation, Cancer Res. 49 (1989) 4373–4384;
(c) M. Stubbs, P.M. McSheehy, J.R. Griffiths, C.L. Bashford, Causes and consequences of tumor acidity and implications for treatment, Mol. Med. Today 6 (2000) 15–19;
(d) L.E. Gerweck, K. Seetharaman, Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of Cancer, Cancer Res. 56 (1996) 1194–1198.

[29] N. Graf, S.J. Lippard, Advanced redox activation of metal-based prodrugs as a strategy for drug delivery, Drug Deliv. Rev. 64 (11) (2012) 993–1004.

[30] (a) S. Kerpel-Fronius, Cisplatin and its analogues for cancer chemotherapy, in: J. Fischer, C.R. Ganellin (Eds.), Analogue-based Drug Discovery, Wiley-VCH Verlag, Weinheim, Germany, 2006, pp. 385–394;
(b) F. Caponigro, M. Basile, V. de Rosa, N. Normanno, New drugs in cancer therapy, Anticancer Drugs 16 (2005) 211–221;
(c) M. Galanski, B.K. Keppler, Searching for the magic bullet: anticancer platinum drugs which can be accumulated or activated in the tumor tissue, Anti-Cancer Agents Med. Chem. 7 (2007) 55–73.

[31] G. Caron, G. Ermondi, M.B. Gariboldi, E. Monti, E. Gabano, M. Ravera, D. Osella, The relevance of polar surface area (PSA) in rationalizing biological properties of several *cis*-diamminemalonatoplatinum(II) derivatives, Chem. Med. Chem. 4 (2009) 1677–1685.

[32] (a) M. Galanski, M.A. Jakupec, B.K. Keppler, Update of the preclinical situation of anticancer platinum complexes: novel design strategies and innovative analytical approaches, Curr. Med. Chem. 12 (2005) 2075–2094;
(b) M.D. Hall, S. Amjadi, M. Zhang, P.J. Beale, T.W. Hambley, The mechanism of action of platinum(IV) complexes in ovarian cancer cell lines, J. Inorg. Biochem. 98 (2004) 1614–1624;
(c) L.R. Kelland, B.G. Murrer, G. Abel, C.M. Giandomenico, P. Mistry, K.R. Harrap, Ammine/amine platinum(IV) dicarboxylates: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines, Cancer Res. 52 (1992) 822–828;
(d) K. Morikawa, M. Honda, K. Endoh, T. Matsumoto, K. Akamatsu, H. Mitsui, M. Koizimi, Synthesis of platinum complexes of 2-aminomethylpyrrolidine derivatives for use as carrier ligands and their antitumor activities, Chem. Pharm. Bull. 38 (1990) 930–935;
(e) S. Shamsuddin, I. Takahashi, Z.H. Siddik, A.R. Khokhar, Synthesis, characterization, and antitumor activity of a series of novel cisplatin analogs with *cis*-1,4-diaminocyclohexane as nonleaving amine group, J. Inorg. Biochem. 61 (1996) 291–301;
(f) Y.E. Kwon, K.J. Whang, Y.J. Park, K.H. Kim, Synthesis, characterization and antitumor activity of novel octahedral Pt(IV) complexes, Bioorg. Med. Chem. 11 (2003) 1669–1676;
(g) R. Kizu, T. Nkanishi, S. Yakamoto, K. Hayakawa, A. Matsuzawa, M. Eriguchi, Y. Takeda, N. Akiyama, Y. Kidani, Significance of water solubility in the gastrointestinal absorption of *trans*-bis(*n*-valerato)(1*R*,2*R*-cyclohexanediamine)(oxalato)platinum(IV), an orally active antitumor platinum complex, and its analogues, Anticancer Drugs 9 (1998) 167–174;
(h) T.W. Hambley, A.R. Battle, G.B. Deacon, E.T. Lawrenz, G.D. Fallon, B.M. Gatehouse, L.K. Webster, S. Rainone, Modifying the properties of platinum(IV) complexes in order to increase biological effectiveness, J. Inorg. Biochem. 77 (1999) 3–12;
(i) S.R. Ali Khan, S. Huang, S. Shamsuddin, S. Inutsuka, K.H. Whitmire, Z.H. Siddik, A.R. Khokhar, Synthesis, characterization and cytotoxicity of new platinum(IV) axial carboxylate complexes: crystal structure of potential antitumor agent [Pt^{IV}(*trans*-1*R*,2*R*-diaminocyclohexane)*trans*(acetate)$_2$$Cl_2$], Bioorg. Med. Chem. 8 (2000) 515–521;
(j) S. Shamsuddin, C.C. Santillan, J.L. Stark, K.H. Whitmire, Z.H. Siddik, A.R. Khokhar, Synthesis, characterization, and antitumor activity of new platinum(IV) *trans*-carboxylate complexes: crystal structure of [Pt(*cis*-1,4-DACH)*trans*-(acetate)$_2$$Cl_2$], J. Inorg. Biochem 71 (1998) 29–35.

[33] Calculated by the ChemAxon's Marvin Sketch 6.1.6 software, http://www.chemaxon.com.

[34] I.V. Tetko, I. Jaroszewicz, J.A. Platts, J. Kuduk-jarowska, Calculation of lipophilicity for Pt(II) complexes: experimental comparison of several methods, J. Inorg. Biochem. 102 (2008) 1424–1437.

[35] (a) P. Dobes, J. Rezac, J. Fanfrlik, M. Otyepka, P. Hobza, Semiempirical quantum mechanical method PM6-DH2X describes the geometry and energetics of CK2-inhibitor complexes involving halogen bonds well, while the empirical potential fails, J. Phys. Chem. B 115 (26) (2011) 8581–8589;
(b) J.J.P. Stewart, MOPAC: a semiempirical molecular orbital program, J. Comput. Aided Mol. Des. 4 (1990) 1–105;
(c) J.J.P. Stewart, Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements, J. Mol. Mod. 13 (2007) 1173–1213.

[36] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B. 37 (1988) 785–789.

[37] A.D. Becke, Density-functional thermochemistry III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652.

[38] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, A.D. Malick, K.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, A.G. Baboul, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andrés, C. González, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian 03, Revision E.01-SMP, Gaussian Inc, Wallingford, CT, 2004.

[39] W.J. Hehre, R. Ditchfield, J.A. Pople, Self-consistent molecular orbital methods XII. Further extensions of Gaussian-type basis sets for use in molecular orbital studies of organic molecules, J. Chem. Phys. 56 (1972) 2257–2261.

[40] P.J. Hay, W.R. Wadt, Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals, J. Chem. Phys. 82 (1985) 299–310.

[41] J. Tomasi, B. Mennucci, R. Cammi, Quantum mechanical continuum solvation models, Chem. Rev. 105 (2005) 2999–3093.