Gold(I) metallocyclophosphazenes with antibacterial potency and antitumor efficacy. Synergistic antibacterial action of a heterometallic gold and silver-cyclophosphazene.

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One of the most important uses of phosphazenes today involves its biomedical applications. They can also be employed as scaffolds for the design and construction of a variety of ligands in order to coordinate them to metallic drugs. The coordination chemistry of the (amino)cyclotriphosphazene ligand, $[N_3P_3(NHCy)_6]$, towards gold(I) complexes has been studied. Neutral complexes, $[N_3P_3(NHCy)_6\{AuX\}_n]$ (X= Cl or C_6F_5; n= 1 or 2) (**1**-**4**), cationic complexes, $[N_3P_3(NHCy)_6\{Au(PR_3)\}_n](NO_3)_n$ (PR_3 = PPh_3, PPh_2Me, TPA; n=1, 2 or 3) (**6**-**12**) [TPA = 1,3,5-triaza-7-phosphaadamantane] and a heterometallic compound $[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**) have been obtained and characterized by various methods including single-crystal X-ray diffraction for **7**, which confirms the coordination of gold atoms to the nitrogens of the phosphazene ring. Compounds **1**, **4**, **6**-**13** were screened for *in vitro* cytotoxic activity against two tumor human cell lines, MCF7 (breast adenocarcinoma) and HepG2 (hepatocellular carcinoma), and for antimicrobial activity against five bacterial species including *Gram-positive*, *Gram-negative*, and Mycobacteria. Both the median inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC) values are among the lowest found for any gold or silver derivatives against the cell lines and particularly against the *Gram-positive* (*S. aureus*) strain and the mycobacteria used in this work. Structure-activity relationships are discussed in order to determine the influence of ancillary ligands and the number and type of metal atoms (silver or gold). Compounds **4** and **8** showed not only maximal potency on human cells but also some tumour selectivity. Remarkably, compound **13,** with both gold and silver atoms, showed outstanding activity against both *Gram-positive* and *Gram-negative* strains (nanomolar range), thus having a cooperative effect between gold and silver, with MIC values which are similar or lower than those of gentamicine, ciproflaxin and rifampicine. The broad spectrum antimicrobial efficacy of all these metallophosphazenes and particularly of heterometallic compound **13** could be very useful to obtain materials for surfaces with antimicrobial properties that are increasingly in demand.

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Electronic Supplementary Information (ESI) available: $^{31}P\{^1H\}$, ^1H, $^1H\{^{31}P\}$, $^{13}C\{^1H\}$ APT, HSQC (^{13}C-^1H) NMR spectra of compound **7** in $CDCl_3$ (Figures S1-S5). $^{31}P\{^1H\}$ and ^1H NMR spectra of compound **7** in DMSO over time until after 15 days (Figures S6-S9). $^{31}P\{^1H\}$, ^1H, $^{13}C\{^1H\}$ APT, HSQC (^{13}C-^1H) NMR spectra of compound **8** in $CDCl_3$ (Figures S10-S13). $^{31}P\{^1H\}$, ^1H, NMR spectra of compound **8** in DMSO (Figures S14 and S15). $^{31}P\{^1H\}$ NMR spectrum of compound **8** in DMSO after 7 days in solution (Figure S16). $^{31}P\{^1H\}$ NMR spectrum of starting product $[Au(ONO_2)PPh_3]$ in DMSO and that after 7 days in solution (Figure S17-S19). $^{31}P\{^1H\}$ and ^1H NMR spectra of compound **13** in $CDCl_3$ (Figures S20 and S21). $^{31}P\{^1H\}$ and ^1H NMR spectra of compound **13** measured in DMSO overtime (Figures S22-S25). Tables containing details of data collection and structure refinement (Table S1) and selected bond lengths and angles (Table S2) for compound **7**. Figures containing bactericidal activity of **4**, **11** and **13** at 2xMIC against *S. aureus, E. coli and P. aeruginosa* (Figure S26). Table containing the expected IC50 after 24 h for metallophosphazenes and their precursors exposure on MCF7, HepG2 and HDF cells under microscope analysis (Table S3). See DOI: 10.1039/x0xx00000x

Introduction

Since the discovery of cisplatin [1, 2] as one of the most powerful chemotherapeutic agents against ovarian and testicular cancer, a vast library of metal complexes has been synthesized and applied in the pharmacological field, mostly as anticancer agents, but also as anti-inflammatory, antimicrobial, antirheumatic, and antimalarial drugs. [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17] Among the non-platinum drugs, gold complexes have attracted major attention in inorganic medicinal chemistry and are gaining attention in all areas especially as anticancer agents. [18, 19, 20, 21, 22]

Ever since the work of R. Koch in the 19th century, who demonstrated that $K[Au(CN)_2]$ showed activity against *Mycobacterium tuberculosis*, a large number of gold complexes have been tested *in vitro* against a broad spectrum of bacteria, fungi and parasites. [20, 23, 24] Among them, gold(I) complexes have been the most studied, and those having phosphane and N-heterocyclic carbene ligands (NHCs) showed relevant antibacterial and antifungal activity. Over the last 10-15 years considerable research has focused on the potential anticancer properties of gold complexes leading to very promising

experimental drugs, not only in terms of their strong antiproliferation potency but also because of the variety of ligands that can be attached to the metal centres in various oxidation states, which in turn allows one to tune the cytotoxic effects with reduced side effects. [25] Auranofin, which was first approved in 1985 as an orally bioavailable antirheumatic drug, is currently one of the only three gold complexes approved for clinical use, which together with another Au(I) complex, myochrysine, are currently undergoing clinical trials as anticancer drugs. [26, 27] Several reviews on this topic have recently appeared. [25, 28, 29, 30, 31, 32] These surveys indicate that the stability of the ligands coordinated to the metal centre is a critical issue for the design of gold-based drugs. Thus, in the case of gold(I), ligands with relatively high bond dissociation energies, such as phosphanes, [20, 33] carbenes [19, 30] or alkynes, [18, 34, 35] are privileged moieties. Moreover, the ligand exchangeability of gold(I) species is thought to play a crucial role in the inhibition of biological molecules and in their antitumor activity. The efficacy of the complexes could be enhanced by choosing ligands that might have some anticancer activity themselves or might enhance the lipophility and thus the overall activity. It is now apparent that the rate of uptake of the gold complex into the cell, the extent to which thiol/selenol-containing proteins and enzymes can be inhibited by the gold species, as well as the solubility of these types of complexes, all influence the overall activity. [20] As for the antimicrobial activity of gold derivatives, most of the Au(I) complexes tested so far display antibacterial effects only on *Gram-positive* bacteria, with a few exceptions, [20, 36, 37, 38] and this selectivity has been explained by the different construction of the cell membrane. [39] Schultz and Wang recently showed that auranofin also has a potent bactericidal activity against *M. tuberculosis* and other clinically important *Gram-positive* bacteria and exerts its effects through a unique mechanism involving inhibition of bacterial thioredoxin reductase (TrxR) (not targeted by other antibiotics), which is an essential protein in many *Gram-positive* bacteria for maintaining the thiol-redox balance and protecting against reactive oxidative species. [40, 38] Similar potent inhibition of bacterial TrxR has been reported by Ott for Au(I) NHC complexes in combination with their high activity against several *Gram-positive* strains. [41, 42] Mead and co-workers have recently reported that auranofin and other gold(I) NHC compounds effectively and selectively inhibit the growth of the Gram-negative bacteria *Helicobacter pylori* (micromolar range) which, the same as many *Gram-positive* bacteria, lack the conventional redox couple GSH-GR normally present in most *Gram-negative* species. In addition, it was described that auranofin inhibited purified TrxR from *H. pylori*. [38] Regarding cancer, it is also generally accepted that the strong and selective inhibition of the thioredoxin reductase enzyme (TrxR) is of high relevance for the pharmacology of these metallodrugs, but much more research is still needed to fully elucidate the mechanism of the biological mode of action of gold-containing drugs against both microorganism and tumour cells. All attempts so far have been focused on the search for new metal complexes as promising clinical candidates, able to overcome the drawbacks of current clinical drugs including not only the limited spectrum of activity and high toxicity (producing significant side effects) but also resistance, poor water solubility, low bioavailability, and short circulating time. New complexes for better activity and lower toxicity need to be studied further, and systematic studies to establish clear structure-activity relationships are also needed.

Macromolecular pro-drugs are known to show excellent tumour targeting properties by the enhanced permeability and retention (EPR) effect, [43, 44, 45] and exhibit improved body distribution and prolonged blood circulation. [46] In this respect, drugs can be physically encapsulated by liposomes, nanocapsules or polymeric micelles or vesicles or, alternatively, conjugated to linear polymers or dendrimers by covalent bonding. Drug-polymer conjugates are an emerging area of drug delivery in order combat the hurdles related to the delivery of drugs, such as low solubility, protection against degradation, low bioavailability and high dose toxicity. Phosphazenes (PZs), $[NPR_2]$, offer a unique platform for developing advanced materials for biological applications, as they combine an intrinsic biodegradability with a versatile synthetic route, which allows for unprecedented structural diversity. [47, 48, 49, 50, 51, 52, 53] Synthetic "toolkit" methods for creating new structures via substitution of the oligomer or polymer precursor, $[NPCl_2]_n$, high skeletal stability (allowing reactions of organic side groups), tunable degradation, biocompatibility, flexibility of the backbone, high density of functional groups and established manufacturing processes can provide unconventional approaches in order to solve challenges in the drug delivery field. A variety of novel phosphazene drug carriers (both cyclic oligomers -CPZs- or polymers -PPZs-) have recently been developed in various forms, such as micelles, hydrogels, microspheres, and matrices, and these have proved to be useful for both formulation and conjugation with small molecular drugs to improve their therapeutic values, especially proteins and anticancer drugs. . [54, 55, 56, 57] Although there are still many problems to be overcome before the full therapeutic potential of amphiphilic PZs can be reached, there is great promise that amphiphilic PZ-based drug carriers will greatly benefit drug delivery applications. Specifically, cyclotriphosphazene (hexachlorocyclotriphosphazene, $N_3P_3Cl_6$), a six-membered ring system, represents an interesting biocompatible platform to develop six branches in 3D space to form, in turn, micelles or vesicles, dendrimers and networks such as hydrogels or microspheres. Over the last few years, cyclotriphosphazenes (CPZs) have been developed in several pharmacological domains [48, 58] and the use of dendrimers in general and dendrimers based on the cyclotriphosphazene core in particular has been said to represent a new strategy in nanomedicine. Besides, both PPZs and CPZs can be used as scaffolds for the design and construction of a variety of ligands, [59, 60, 61] to coordinate to metallic drugs. The facile substitution of P-Cl bonds in the cyclic trimer, $[N_3P_3Cl_6]$, allows the ready construction of CPZs carrying additional exocyclic donor functions, giving a library of multi-site coordination ligands. Furthermore, the ring nitrogen atoms have sufficient basicity to coordinate to metals depending on the electronic properties of the exocyclic substituents at phosphorus. [60] Indeed, we have

recently reported silver-metallophosphazenes in which the silver atoms are coordinated to ring nitrogen atoms using amino-substituents at phosphorus. [62, 63]

Despite the potential, there are only limited examples of the use of PZ derivatives in drug delivery and metallodrugs in particular. Some antibiotic, anti-viral, antitumoral or anti-malarial drugs have been conjugated to PPZ and CPZs. [47, 48, 49, 50] Several authors have prepared and studied the tumoral properties of cyclo- or polyphosphazene-platinum(II) conjugates. [64, 65, 66, 67, 68, 69, 70, 71] We have also contributed to this field by reporting the first silver phosphazenes with excellent antimicrobial and antitumoral properties. [62] Gold derivatives of PZs (cyclic or polymers) are also known. However, since the first gold-phosphazene complexes were reported in 1983 by Allcock et al., [72, 73] only a few exploratory examples have been described. [74, 75, 76, 77, 78] Methylphenylphosphazenes were used as a stabilizing medium in the solution-phase synthesis of gold nanoparticles by Wisian-Neilson et al. [79, 80] To the best of our knowledge, there are only two structurally characterized gold-phosphazene complexes, [76, 77] and there are no reports of gold-phosphazenes in which the gold atoms are coordinated to the nitrogens of the PZ ring. Nor are there reports of gold-phosphazenes with biological effects, such as antimicrobial or antitumoral properties. These results, together with our experience in both the chemistry of gold and silver, and phosphazenes, has prompted us to study: (1) the reactivity of the (amino)cyclotriphosphazene ligand, $[N_3P_3(NHCy)_6]$, in order to obtain gold(I) metallophosphazene complexes, (2) the antimicrobial or antitumoral properties of the gold-phosphazenes thus synthesized and (3) the comparison of the these properties with those of silver-aminophosphazenes, which we recently reported and whose only difference with those presented in this article is the metal atom, gold rather than silver. These studies constitute the basis for our main objective, which is the use of amphiphilic dendritic cyclophosphazenes as carriers of metallodrugs in order to combat the difficulties related to the delivery of drugs mentioned before. These studies are in progress in our laboratories.

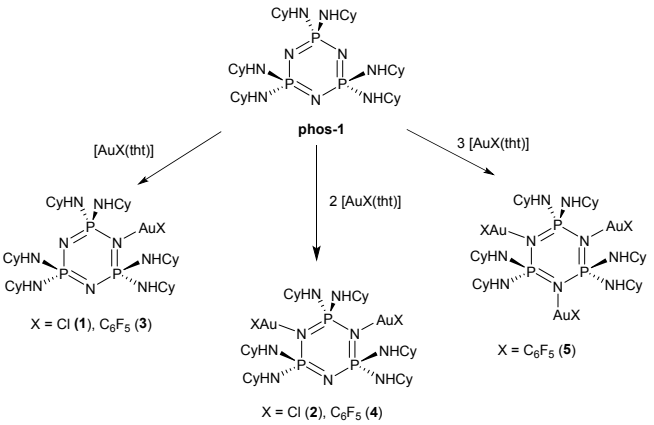
Following the success of auranofin, the search for novel gold drugs has afforded a large number of $[LAu(PR_3)]$ complexes that exhibit notable salutary effects. Complexes with $\{Au(PPh_3)\}^+$ moiety are specifically stable in biological media and readily exchange L with S- and Se-containing enzymes or proteins. Such an exchange leads to the rapid reduction of microbial loads or induction of apoptotic cell death at malignant sites. Mascharak *et al* have recently revised the advantages and medicinal applications of gold drugs with this moiety and have concluded that the use of the $\{Au(PPh_3)\}^+$ synthon in drug design could lead to novel chemotherapeutics for treatment of drug-resistant pathogens and cancers. [20]

Our aim was to study the coordination of {AuX} (X= Cl or C_6F_5) or $\{Au(PR_3)\}^+$ moieties to the phosphazene ring, to give neutral or cationic complexes respectively. Thus, herein, two series of gold metallophosphazenes will be described: neutral complexes, $[N_3P_3(NHCy)_6\{AuX\}_n]$ (X= Cl or C_6F_5; n= 1 or 2), and cationic complexes, $[N_3P_3(NHCy)_6\{Au(PR_3)\}_n](NO_3)_n$ (PR_3 = PPh_3, PPh_2Me, TPA; n=1, 2 or 3) [TPA = 1,3,5-triaza-7-phosphaadamantane]. A silver and gold heterometallic compound, $[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$, was also obtained. Studies of their cytotoxic activity towards the human breast adenocarcinoma (MCF7) and human hepatocellular carcinoma (HepG2) cell lines and against non-tumorigenic human fibroblasts were carried out. The antibacterial activity was also tested against the Gram-negative strains *Escherichia coli* and *Pseudomonas aeruginosa*, against the *Gram-positive Staphylococcus aureus* and against two *Mycobacterium tuberculosis complex* (MTBC) strains, *M. tuberculosis* H37Rv and *M. bovis* BCG Pasteur. Comparison with the corresponding antiproliferative and antimicrobial activity of cisplatin, auranofin, silver (I) nitrate and several well-known antibiotics was also performed.

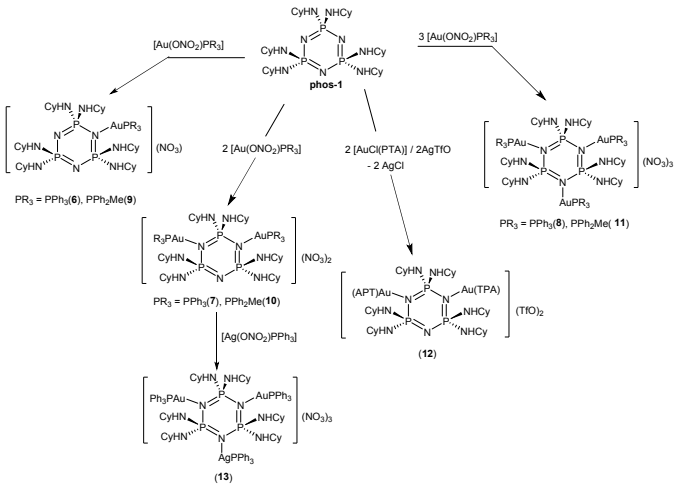
Results and Discussion

Synthesis and Characterisation of the Metallophosphazenes.

Synthetic methods are outlined in Schemes 1 and 2. Specifically, the reaction of hexakis(cyclohexylamino)cyclotriphosphazene, $[N_3P_3(NHCy)_6]$ (**phos-1**), with the gold(I) complexes [AuX(tht)] (X= Cl or C_6F_5) or $[Au(ONO_2)PR_3]$ (PR_3= PPh_3 or PPh_2Me), in dichloromethane and in different molar ratios of 1:1, 1:2 or 1:3 led to the new neutral or cationic metallophosphazenes **1**-**5** and **6**-**11** respectively. All compounds were obtained pure in high yield, except compound **5**. In the reaction of $[N_3P_3(NHCy)_6]$ with $[Au(C_6F_5)tht]$, in a molar ratio of 1:3 in order to obtain **5**, a mixture of **4** and **5** was always observed even if a higher excess of the starting gold complex was used (up to 1:4). In the reaction of $[N_3P_3(NHCy)_6]$ with [AuCl(tht)], in a molar ratio of 1:3, the metallophosphazene with three gold atoms was not observed, not even with a greater excess of starting gold complex.



Scheme 1. Synthetic methods to neutral metallophosphazenes



Scheme 2. Synthetic methods to cationic metallophosphazenes

All new metallophosphazenes **1-11** could be handled and stored under ambient conditions for long periods of time. They are soluble in dichloromethane, acetone, chloroform and dimethyl sulfoxide (DMSO) and only slightly soluble or insoluble in hexane or pentane, except **3** and **4**, which are soluble in hexane and diethyl ether. Cationic metallophosphazenes **6**-**11** are insoluble in diethyl ether, which permits the removal of the excess soluble gold starting complexes $[Au(ONO_2)PR_3]$ (PR_3= PPh_3 or PPh_2Me) if necessary. All of them are insoluble in water. Compound **12**, bearing the water-soluble phosphane ligand 1,3,5-triaza-7-phosphaadamantane (TPA), was prepared in an attempt to obtain a water-soluble metallophosphazene. However, **12** is practically insoluble in water. This compound was obtained by reaction of $[N_3P_3(NHCy)_6]$ with [AuCl(TPA)], in a molar ratio of 1:2, in the presence of AgTfO (TfO= SO_3CF_3). In this reaction, the metallophosphazene with three gold atoms $[N_3P_3(NHCy)_6\{Au(TPA)\}_3](TfO)_3$ was not observed, not even using a molar ratio 1:3:3 of the starting compounds, $[N_3P_3(NHCy)_6]$, [AuCl(TPA)] and AgTfO, respectively (see the Experimental Section). Heterometallic compound **13**, with gold and silver, was prepared from **7** by reaction with $[Ag(ONO_2)PPh_3]$ (see Scheme 2). The dichloromethane and chloroform solutions of compounds **4** and **6**-**13** were stable for more than a week at room temperature (RT), as was shown by $^{31}P\{^1H\}$ and ^1H NMR spectroscopy. However, those of **2** decompose to give **1** and metallic gold after approximately 12 h. The chloroform solutions of **1** and **3** were stable at least for 48 h at RT, but after three days some decomposition to metallic gold was observed. Heterometallic complex **13** had to be handled and stored protected from light.

All of the compounds **1-4** y **6**-**13** were characterized by elemental analysis, IR, ^1H, $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$ NMR spectroscopy, and mass spectrometry. The assignment of the signals in the ^1H and $^{13}C\{^1H\}$ NMR spectra was also inferred using ATP and 2D heteronuclear (HSQC ^1H-^{13}C) correlations. All these data are given in the Experimental Section and are consistent with the formulae and structures indicated, particularly with the coordination of metals to the ring nitrogen atoms. In addition, X-ray structural analysis of **7** confirmed the proposed structure. The $^{31}P\{^1H\}$ NMR spectra in solution for compounds **5**, **8** and **11**, which have three gold atoms, consisted of a singlet for the phosphorus atoms of the phosphazene ring. For all other compounds (**1**-**4, 6**, **7**, **9**, **10**, **12** and **13**), the spectra showed an AB_2 pattern in accordance with the nonequivalence of all three phosphorus atoms of the phosphazene ring. The signals were assigned in accordance with their multiplicity (see Table 1). In the compounds that have the same AuX or $AuPR_3$ unit but a different number of gold atoms, the signal corresponding to the same type of phosphorus was shifted upfield as the number of coordinated metal atoms increased. Moreover, in each compound, the phosphorus resonances were shifted downfield when the number of metals coordinated to adjacent nitrogen atoms increased, as was observed in other metallophosphazenes in which metals (such as silver or lithium) are coordinated to the backbone nitrogen atoms. [81, 82, 62] For **6**-**13**, these spectra also show a single signal from the phosphane ligands, which are all shifted from those in the starting complexes (See Table 1). [83,84,85,86] For **12**, the phosphane resonates at −55.42(s) ppm, which is consistent with a gold(I) compound containing TPA acting as a phosphorus-donor ligand. [84]

Table 1. $^{31}P\{^1H\}$ NMR Spectroscopic Data for complexes **1-13** and starting compounds **phos-1**, $[M(ONO_2)PR_3]$ (M=Au or Ag) for comparison. [a]

Compound	Spin system	δ[N-*P*-N]	δ[N-*P*-NAu] [^{2}J(P-P)] [b]	δ[AuN-*P*-NAu]	δ[*P*R$_3$]
$[N_3P_3(NHCy)_6]$ (**phos-1**)	A_3	14.44(s)	–	–	–
$[N_3P_3(NHCy)_6\{AuCl\}]$ (**1**)	AB_2	12.13("t")	14.39 ("d") [47.1]	–	–
$[N_3P_3(NHCy)_6\{AuCl\}_2]$ (**2**)	AB_2	–	11.82 ("d") [34.7]	17.92("t")	–
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}]$ (**3**)	AB_2	11.99("t")	14.78 ("d") [45.8]	–	–
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	AB_2	–	11.94 ("d") [36.1]	17.91("t")	–
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_3]$ (**5**)	A_3	–	–	14.80 (s)	–
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}](NO_3)$ (**6**)	AB_2	12.01("t")	14.48("d") [43.7]	–	32.40(s)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	AB_2	–	12.26 ("d") [34.3]	17.68("t")	32.20(s)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	A_3	–	–	13.10 (s)	31.77(s)
$[Au(ONO_2)PPh_3]$		–	–	–	27.48(s)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}](NO_3)$ (**9**)	AB_2	12.22("t")	14.79("d") [44.3]	–	17.06 (s)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	AB_2	–	12.56 ("d") [32.8]	17.51 ("t")	16.13 (s)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_3](NO_3)_3$ (**11**)	A_3	–	–	14.59 (s)	15.69(s)
$[Au(ONO_2)PPh_2Me]$		–	–	–	10.74(s)
$[N_3P_3(NHCy)_6\{AuTPA\}_2](TfO)_2$ (**12**)	AB_2	–	11.48("d") [32.7]	17.21("t")	−55.42(s)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	AB_2	–	14.88("d")[c] [33.1]	16.09("t")	31.71(s), 17.34(br)
$[Ag(ONO_2)PPh_3]$		–	–	–	18.35(dbr), $^1J_{Ag\text{-}P}$= 657.2[b]

[a] Data in $CDCl_3$. Values in ppm. [b] Values in Hz. [c] δ[AgN-P-NAu]

In the $^{19}F\{^1H\}$ NMR spectra of **3** and **4**, signals from the pentafluorophenyl units are also observed and are shifted from those in the starting complex, $[Au(C_6F_5)tht]$. [87]. The ^1H NMR spectrum for **12** shows a singlet resonance and two pseudo-doublets (AB spin system with ca. 13 Hz geminal H-H coupling) for the TPA protons, from the NCH_2P and NCH_2N methylene groups, respectively. This assignment was unambiguously confirmed by use of 2D heteronuclear HSQC ^1H-^{13}C correlations. The presence of a singlet for the NCH_2P methylene protons is unusual since two-bond P-H coupling would be expected, but a singlet has also been observed in other linear gold(I) complexes such as [Au(CCR)TPA], $[Au(C_6F_5)TPA]$ and [AuCl(TPA)] [88] The signals from the cyclohexylamino units in the ^1H NMR spectra for **1**-**4** and **6**-**13** are collected in Table 2. These signals, specifically those of N*H* and NH-C*H*, were also verified by two-dimensional heteronuclear HSQC ^1H-^{13}C correlations. In some cases, the N*H* protons resonate as a triplet because the coupling to the CH proton is similar to the coupling to the phosphorus atom, $^2J(H\text{-}P) = {}^3J(H\text{-}H)$, which was verified by recording the phosphorus-decoupled ^1H NMR spectrum. Most significantly, the ^1H NMR spectra of **8** and **11** show a single type of cyclohexylamino units as a result of the equivalence of all the amino side groups. For **1**-**4**, **6**, **7**, **9**, **10**, **12** and **13**, though, two types of NHCy units are observed (also seen in the $^{13}C\{^1H\}$ NMR spectra). Coordination at phosphazene in all complexes leads to a deshielding of the protons N*H*, as the number of metals coordinated to the adjacent nitrogen atoms rises, as observed in other metallophosphazenes with silver coordinated to N(ring) atoms. [62] In the Supporting Information, we have included the spectra for compound **7** and **8** as examples.

Table 2. ^{1}H NMR Spectroscopic Data for **phos-1** and complexes **1**-**4** and **6**-**13.** [a]

Compound	δ[N*H*]	δ[NH-C*H*]	δ[NH(C_6H_{11})]
$[N_3P_3(NHCy)_6]$ (**phos-1**)	2.0 (br, 6H)	3.05 (m, 6H)	1.94, 1.65, 1.50,1.26, 1.10 (m, 60 H)
$[N_3P_3(NHCy)_6\{AuCl\}]$ (**1**)	2.38 (m, 4H), 2.09 (t, 2H)	3.13 (m, 4H) 3.03 (m, 2H)	2.04, 1.93, 1.71-1.64, 1.57-1.54, 1.33-1.07 (m, 60 H)
$[N_3P_3(NHCy)_6\{AuCl\}_2]$ (**2**)	2.76 (t, 2H), 2.53 (m, 4H)	3.34 (m, 2H) 3.14 (m, 4H)	2.16, 2.04-1.96, 1.73, 1.64-1.54, 1.34-1.07 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}]$ (**3**)	2.36 (m, 4H), 2.08 (m, 2H)	3.30 (m, 4H) 3.05 (m, 2H)	1.96, 1.70-1.50, 1.33-1.08 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	2.70 (t, 2H), 2.47 (m, 4H)	3.54 (m, 2H) 3.30 (m, 4H),	2.12, 2.09-1.99, 1.71, 1.58, 1.35-1.14 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}](NO_3)$ [b] (**6**)	3.56 (br, 4H), 2.17 (br, 2H)	3.06 (br, 6H)	1.92, 1.73-1.4, 1.25, 1.11 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ [b] (**7**)	5.8 (t, 2H), 3.77 (br, 4H)	3.09 (br, 6H)	1.98-1.88, 1.69-1.37,1.27, 1.17-1.01, 0.74 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ [b] (**8**)	6.46 (mbr, 6H)	2.96 (mbr, 6H)	1.93, 1.47, 1.37-1.28, 1.10-0.96 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}](NO_3)$ (**9**)	3.50 (br, 4H), 2.12 (br, 2H)	3.05 (br, 6H)	1.91, 1.73-1.4, 1.25-1.12 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	5.6 (br, 2H), 3.68 (br, 4H)	3.00 (br, 6H)	1.86, 1.58-1.43,1.31-0.87 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_2Me\}_3](NO_3)_3$ [b] (**11**)	6.12 (br, 6H)	2.97 (br, 6H)	1.93-1.84, 1.51-1.48, 1.40-1.29, 1.10-0.92 (m, 60H).
$[N_3P_3(NHCy)_6\{AuTPA\}_2](TfO)_2$ [b] (**12**)	4.50 (br, 2H), 3.23 (br, 4H)	3.01 (br, 6H)	1.91-1.79, 1.70, 1.58-1.55, 1.47-1.39, 1.26-1.10 (m, 60H)
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ [b] (**13**)	6.22 (tbr, 2H), 4.39 (br, 4H)	3.08 (br, 6H)	1.93, 1.54-1.26, 1.03 (m, 60H)

[a] Data in $CDCl_3$. Values in ppm. [b] The signals from phophane ligands are given in the Experimental Section.

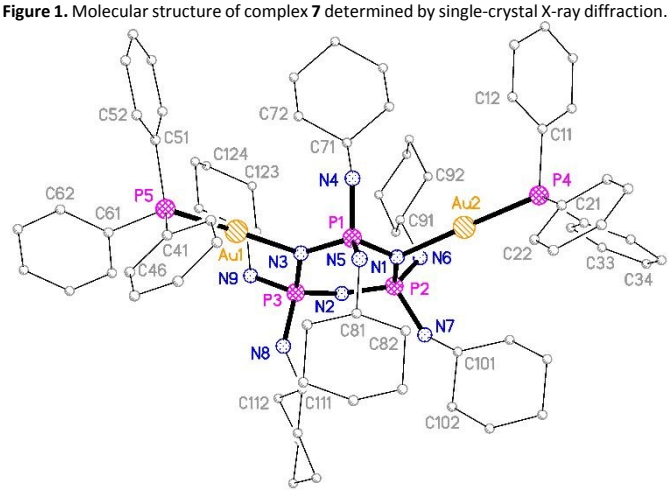
The IR spectra show absorptions attributable to the Au-Cl stretching band for **1** and **2,** to pentafluorophenyl groups for **3** and **4,** which are all slightly shifted from those in the starting complex [AuCl(tht)] or [Au(C_6F_5)tht], [87] and those from the nitrate or trifluoromethanesulfonate units for **6**-**11** and **13** or **12**, respectively. The part of the IR spectra of **6**-**11** and **13** corresponding to the stretching bands of nitrate is clearly different from that of the starting complexes, [Au(ONO_2)PPh_3], [Au(ONO_2)PPh_2Me] or [Ag(ONO_2)PPh_3], in which this group is bonded to gold or silver in a covalent manner. [83, 85, 86, 89] Complexes **6**-**11** and **13** have very similar bands in shape and position in the asymmetric N-O stretching region of nitrate (1450-1330 cm^{-1}) and are similar to those observed for **7**, for which the presence of ionic nitrate has been confirmed by single crystal X-ray structural analyses (as expected, considering the usual linear coordination to gold (I)). The triflate peaks in **12**, which could in principle also be used to distinguish covalent and ionic trifluoromethanesulfonate, are not very useful because an unambiguous assignment is hindered by the overlap of CF_3, SO_3 and phosphane vibrational modes. [90, 91] The bands of the characteristic phosphazene absorptions in the IR spectra, such as P=N and C-N (at 1186 and 1178 cm^{-1} for **phos-1**) change gradually after coordination of the metal, as previously observed in other metallophosphazenes with silver coordinated to the backbone nitrogen atoms. [82, 62] Besides, the bands in the 3000-3400 cm^{-1} region, which are assigned to the N-H stretching, are also different from those of the starting phosphazene, **phos-1**. Bearing in mind the usual linear coordination of gold(I) and the data discussed before, the shifts observed in our complexes are probably due to the disruption of part of the hydrogen bonding and not to the coordination of gold atom to nitrogen atoms of the N-H side group, as is verified by single crystal X-ray diffraction for **7**. It is worth noting that the molecular ions of almost all complexes were observed in the mass spectra.

Single crystals of **7**.$C_2H_4Cl_2$ were grown by slow diffusion of hexane into a solution of the complex in 1,2-dichloroethane. The X-ray analysis confirmed not only the presence of ionic trifluoromethanesulfonate, but also the coordination of gold to the ring nitrogen atoms (see Figure 1). Selected bond lengths and angles and details of the data collection and refinement are given in Tables in the Supporting Information. The gold(I) atoms show a nearly linear coordination with angles close to 180º [178.65(5) and 176.28(5)°]. The Au-N and Au-P bond lengths are typical. [77, 92, 93, 94] Metalation causes distortion of the cyclophosphazene ring skeleton. The P-N(ring) bonds associated with metal coordination are longer [av. 1.6408(19) Å] than the P-N(ring) bonds at the non-coordinating nitrogen centre [av. 1.5822(19) Å] and also the P-N(ring) bonds in the starting phosphazene $[N_3P_3(NHCy)_6]$ (**phos-1**) (1.598 Å). [95, 96] Such an N-P bond-length increase flanking the site of coordination (or protonation or alkylation) is known from

studies on cyclotriphosphazenes. [97, 98, 99, 100, 101, 102] This is consistent with the bonding model of Craig and Paddock. [103, 104] Accordingly, in such situations the lone pair on the ring nitrogen atom of the cyclophosphazenes is not available for π-bonding interactions within the ring, causing an increase in the affected bond distance. The exocyclic P-NHCy bond lengths [av. 1.627(2) Å] are longer than the P-N(ring) bond lengths but are shorter than the ideal P-N single-bond value of 1.77 Å. [105, 106] While the phosphazene rings of the free ligand, **phos-1**, are planar or close to planarity, [96] the coordination to gold causes the rings to pucker, which is obvious from the ring torsion angles [maximum absolute values of 33.55(18)º].

The NH groups of the ligand form classical hydrogen bonds to the nitrate anions: N4…O6 2.868(3) Å, N5…O4 2.984(3) Å, N6…O3 2.947(3) Å, N7…O2 3.138(3) Å, N8…O1 2.920(3) Å and N9…O2 3.017(3) Å.

Figure 1. Molecular structure of complex **7** determined by single-crystal X-ray diffraction.



Hydrogen atoms, anions and solvent molecules have been omitted for clarity. The radii are arbitrary.

Biological Evaluation: Antibacterial Potency and Antitumor Efficacy

All the complexes are insoluble in water but soluble in DMSO and in the DMSO/water mixtures used in the biological tests (cytotoxic and antibacterial ones). In the cytotoxicity assays, the solutions used contained a minimal amount of DMSO (≤ 0.1%). In the antibacterial tests, the solvent DMSO was also used as a control (in the same percentage used to dissolve the compounds) to confirm that it did not inhibit bacterial growth. While the tests were being performed, no precipitation of any compound at the concentration ranges assayed was observed. The DMSO solutions of all compounds remained colorless for much more than a week, with no observed decomposition to metallic gold, except that from **2**. The stability of compounds in DMSO was assessed by ^{31}P and ^{1}H NMR spectroscopy, compounds **1**, **4**, **6**, **7**, **9**, **10** and **12** being the more stable ones. The spectra of these compounds showed the expected signals, slightly displaced from those observed for the same complex in other solvents such as chloroform, which remain exactly the same after more than 7 days. In the Supporting Information, we have included these spectra for compound **7** as an example, measured over time until after 15 days, which is much longer than the time span of the biological assays. For **8** and **11**, though, their spectra in DMSO solution were different from those observed in chloroform, indicating a equimolar mixture of **8**, **7** and $[Au(ONO_2)PPh_3]$ (in case of **8**) and a mixture of **11**, **10** and $[Au(ONO_2)PPh_2Me]$ (in case of **11**). After 48 h, these spectra changed slightly because compounds $[Au(ONO_2)PR_3]$ (PR_3= PPh_3 or PPh_2Me) decompose by 5% to give $[Au(PR_3)_2]NO_3$ and metallic gold, as was proved using the starting complexes $[Au(ONO_2)PR_3]$. For **13**, their spectra in DMSO solution indicated a mixture of the starting complexes, **7** and $[Ag(ONO_2)PPh_3]$, which remained exactly the same for at least 7 days. In the Supporting Information, we have included these spectra for $[Au(ONO_2)PPh_3]$ and compounds **8** and **13**.

Among the new metallophosphazenes, we selected the most stable compounds (**1**, **4**, **6**-**13**) to carry out the biological studies and evaluate the structure-activity relationship. Under the same conditions, auranofin and cisplatin (two well-known chemotherapeutic drugs) and silver(I) nitrate, gentamicine, ciprofloxacin and rifampicine (several well-known antimicrobial drugs) were also tested and compared to all of the compounds studied. All the compounds analysed were dissolved in DMSO, not exceeding 0.1%, except cisplatin, which was dissolved in water.

Antibacterial Potency. The antibacterial activities of the new complexes **1**, **4**, **6**-**13** and their precursors (the phosphazene ligand, **phos-1**, and the stable silver and gold starting complexes) were tested against Gram-negative strains, *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 15442, *Gram-positive S. aureus* ATCC 11632, and, furthermore, **1**, **4**, **6**, **7**, **11**-**13** against two MTBC strains, M. tuberculosis H37Rv ATCC 27294 and M. bovis BCG Pasteur. The minimum inhibitory concentrations (MICs) obtained and those of $AgNO_3$, gentamicine, ciprofloxacin, rifampicine, silver sulfadiazine (AgSD) and auranofin are listed in Table 3.

Table 3. MICs (μM) Obtained for New Metallophosphazenes and Their Precursors against *Gram-Positive*, *Gram-Negative* and MTBC Strains.

COMPOUND	*S. aureus (Gram-Positive)*	*E. coli (Gram-Negative)*	*P. aeruginosa (Gram-Negative)*	*M. bovis BCG*	*M. tuberculosis H37Rv*
$[N_3P_3(NHCy)_6]$ (**phos-1**)	250±0	125±0	125±0	62.5±0	31.25±0
$[N_3P_3(NHCy)_6\{AuCl\}]$ (**1**)	0.49±0	7.8±0	62.5±0	0.97±0	0.49±0
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	≤0.12±0	3.9±0	31.25±0	1.95±0	≤0.12±0
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}](NO_3)$ (**6**)	0.24±0	3.9±0	62.5±0	0.49±0	0.24±0
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	≤0.12±0	3.9±0	31.25±0	0.49±0	0.24±0
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	≤0.12±0	3.9±0	31.25±0	–	–
$[Au(ONO_2)PPh_3]$ [a]	≤0.12±0	1.95±0	15.6±0	15.6±0	15.6±0
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}](NO_3)$ (**9**)	≤0.12±0	7.8±0	31.25±0		
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	≤0.12±0	3.9±0	15.6±0		
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_3](NO_3)_3$ (**11**)	≤0.12±0	0.97±0	15.6±0	≤0.12±0	≤0.12±0
$[N_3P_3(NHCy)_6\{AuTPA\}_2](TfO)_2$ (**12**)	0.49±0	1.95±0	31.25±0	≤0.12±0	0.49±0
[AuCl(TPA)]	3.9±0	15.6±0	62.5±0	31.25±0	31.25±0
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	≤0.12±0	≤0.12±0	≤0.12±0	3.9±0	1.95±0
$[N_3P_3(NHCy)_6\{Ag(PPh_3)\}_2](TfO)_2$ [b]	≤0.12	0.4	3.9	7.8	3.9
$[N_3P_3(NHCy)_6\{Ag(PPh_3)\}_3](TfO)_3$ [b]	0.24	0.97	15.6	≤0.12	0.97
$[Ag(OTf)PPh_3]$ [b]	0.97	3.9	15.6	15.6	31.25
$[Ag(ONO_2)PPh_3]$	3.9	15.6	15.6	–	–
$AgNO_3$ [b]	31.25	15.6	15.6	15.6	31.25
Gentamicine	1.95±0	3.9±0	3.9±0	–	–
Ciprofloxacin	0.24 ±0	≤0.12±0	0.24 ±0	–	–
Rifampicine	≤0.12±0	1.95±0	15.6±0	–	–
AgSD	44.8 [c]	22.4 [c]	13-90 [d]	nd [e]	nd [e]
Auranofin	0.18 [f]	46.1 [f]	368.5 [f]	nd [e]	0.74 [g]

[a] After 48 h in DMSO solution, $[Au(ONO_2)PPh_3]$ decompose in a 5% to give $[Au(PPh_3)_2]NO_3$ and metallic gold [b] Data taken from our reference [62] , measured under the same conditions as the other compounds and included for comparison [c] Data taken from reference [107] [d] Data taken from reference [108],where authors studied several strains of *P. aeruginosa*. MICs were transformed by us into μM. [e] Not determinated. [f] Data taken from reference [23]. MICs were transformed by into μM. [g] Data taken from reference [40]. MICs were transformed by us from mg/L into μM.

MIC values of tested metallophosphazenes indicated that all of them exhibit excellent antibacterial activity, particularly against *Gram-positive S. aureus* strain and MTBC strains. Their activity is much higher than that of $AgNO_3$ and silver sulfadiazine (AgSD) for the entire range of bacteria studied, except against *P. aeruginosa*. All of them have also an activity which is similar or better than auranofin, gentamicine, ciprofloxacin and rifampicine against *S. aureus* and much better than auranofin against *E. coli* and *P. aeruginosa.* The heterometallic (silver and gold) complex **13** is the only metallophosphazene that is more active than gentamicine, ciprofloxacin and rifampicine against *E. coli* and *P. aeruginosa*, for which **13** also exhibits an excellent activity, with a MIC value of ≤ 0.12 μM (≤ 0.26 mg/mL if the molar mass is taken into account). 0.12 μM was the minimum value of the concentration range tested. Leaving aside *P. aeruginosa*, the MICs of all metallophosphazenes range from ≤0.12 to 0.49 M against *S. aureus* (≤0.14 − 0.85 mg/mL) and from ≤0.12 to 7.8 μ M against *E. coli* (≤0.26 − 9.23 mg/mL), which are among the lowest values found for any gold or silver derivatives. [42, 62, 23, 24, 109, 110, 111] All of them exhibit much better activity than their phosphazene ligand, **phos-1**, and also than their metal precursors except **6**-**8**, which are as effective as their gold precursor, $[Au(ONO_2)PPh_3]$, against *S. aureus* and *E. coli*, but much better against the two MTBC strains. To the best of our knowledge, the biological properties of the gold precursors, $[Au(ONO_2)PR_3]$, have not been measured to date. All of the tested metallophosphazenes also exhibit outstanding activity against the two tested MTBC strains, with MICs lower than 0.97 μM, except that heterometallic complex **13** and **4**. Compound **13** has a MIC value of 3.9 and 1.95 μM against M. bovis BCG Pasteur and M. tuberculosis H37Rv, respectively, and **4** has a MIC value of 1.95μM against M. bovis BCG Pasteur, being only slightly higher than that of auranofin against M. tuberculosis. [40]

As regards the structure-activity relationships towards all bacterial strains in these metallophosphazenes, the following can be concluded: (1) Similar antibacterial activity is observed in triphenylphosphane derivatives to that in diphenylmethylphosphane or TPA derivatives (see Table 3; **7** is as potent as **10** and both of them only slightly more toxic than **12** against *S. aureus*, with **12** being slightly more potent than **7** and **10** against *E. coli*). The methyldiphenylphosphane derivative **11** is also more active than the analogous PPh_3 derivative **8** against *E. coli*. This last variation contrasts with the one observed in the above-mentioned silver metallophosphazenes reported by us (structurally similar but having silver instead of gold) against all bacterial strains used in this work, not only *E. coli*, in which the influence of the ligand in the sequence of efficacy was PPh_3 > PPh_2Me >TPA (reduction of activity with a reduction of lipophilicity). [62] (2) All the gold metallophosphazenes tested in this work have more antimicrobial activity against *Gram-positive* bacteria and MTBC

strains than *Gram-negative*. This property has previously been noted for auranofin [23, 40] and other gold(I) derivatives. [24, 42, 41] Indeed, most Au(I) complexes tested so far, with a few exceptions, [20, 36, 37, 38] display antibacterial effects only on *Gram-positive* bacteria, which has been attributed to the low permeability of the two-membrane cell wall of the *Gram-negative* bacteria. [39] Remarkably, all gold metallophosphazenes described here also show high potency against *E. coli*. P.K. Mascharak et al. have recently stated that the activity towards *Gram-negative* bacteria could be related to the presence of a [N-AuL] core, which could cause the N-donor ligand to exchange more effectively or rapidly in order to exert the drug action. [20] (3) In the activity against *Gram-negative* strains, there is an influence of the number of metal atoms linked to the phosphazene ring; the activity is enhanced by an increase in the number of metal atoms, as observed for the analogue silver metallophosphazenes mentioned before [62] (see Table 3; **11** is more active than **10** and **9**, and **8** and **7** slightly more than **6**). The MIC values for **7**-**11** and **13** against *S. aureus* were ≤0.12, which was the minimum measured value. (4) Regarding MTBC strains, there is no clear influence of the number of gold atoms linked to the phosphazene ring (**11** is more potent than **7** and **6**, but **6** and **7** have a similar activity). (5) The comparison with silver phosphazenes previously reported by us, whose only difference is the metal atom (MIC values are also shown in Table 3 for comparison), shows that they are more active against *Gram-negative* strains than gold phosphazenes, including *P. aeruginosa* ($[N_3P_3(NHCy)_6\{AgPPh_3\}_2](TfO)_2$ is more active than **7** and $[N_3P_3(NHCy)_6\{AgPPh_3\}_3](TfO)_3$ is more active than **8**). The opposite happens against MTBC strains [**7** is more active than the analogue of silver, $[N_3P_3(NHCy)_6\{AgPPh_3\}_2](TfO)_2$]. Remarkably, compound **13**, with both gold and silver, showed outstanding activity against both *Gram-positive* and *Gram-negative* strains, being much more active than gold complexes **6**-**12** and the analogous silver complexes ($[N_3P_3(NHCy)_6\{AgPPh_3\}_2](TfO)_2$ and $[N_3P_3(NHCy)_6\{AgPPh_3\}_3](TfO)_3$) against *Gram-negative* strains, including *P. aeruginosa*. **13** is also much more active than **7** and $[Ag(ONO_2)PPh_3]$ even though, as previously stated, **13** is a mixture of both compounds in DMSO solution. In this case it is clear that there is a synergistic or cooperative effect between the gold and silver metals, as observed in other heterometallic AuAg complexes. [92]

We also tested bactericidal activity of **4**, **11** and **13** by Kinetic killing assay against *S. aureus, E. coli* and *P. aeruginosa* strains (see Table S20 in the Supporting Information). The three compounds showed strong bactericidal activity reducing the cfus by several orders of magnitude. Particularly, for **11** and **13** we observed complete killing within 6 h in all cases.

The broad spectrum antimicrobial efficacy of all these metallophosphazenes may be explained by the lability of the ligands in these complexes. It was concluded that the antimicrobial properties of silver (I) or gold (I) complexes depend upon the fast rate of ligand exchange of the metal ion in the biological system (rather than solubility, charge or chirality), which correlates with the nature of the donor atoms coordinated to the metal. Weak M-N bonds play a key role in determining the wide-spectrum antibacterial activity of Ag(I) or Au(I) complexes because of the facile substitution of the corresponding labile ligands with S- or N-donor sites of amino acids or nucleotides in bacteria. [107, 20, 112, 113, 114] While the exact mechanism of action of gold species remains widely unknown, research suggests strongly that there are multiple mechanisms of action, which could benefit treatment of multi-drug resistance pathogens. Recent studies have shown that the inhibition of thioredoxin reductase (TrxR) (a protein that is essential in many *Gram-positive* bacteria for maintaining the thiol-redox balance and protecting against reactive oxidative species) plays a very important role for auranofin as a potent bacteria inhibitor. [40] *Gram-positive* bacteria have low glutathione levels (a thiol-containing tripeptide that also contributes to the regulation of the redox cellular milieu) and as a consequence, the functional Trx/TrxR system is critical for their growth and survival. Auranofin decreases the reducing capacity of target bacteria, thereby sensitizing them to oxidative stress, eventually leading to bacterial cell death. [40, 111] Similar potent inhibition of bacterial TrxR has been reported by Ott for Au(I) NHC complexes in combination with their high activity against several *Gram-positive* strains. [41, 42] Mead and co-workers have recently reported that auranofin and other gold(I) NHC compounds inhibit the growth of the *Gram-negative* bacteria helicobacter pylori in an effective and selective way. It was also described that auranofin inhibited purified TrxR from H. pylori. Interestingly, inhibition of bacterial TrxR has also been recently confirmed for an antimicrobial silver NHC complex. [115]

Antitumoral Efficacy. The cytotoxicity of the most stable complexes **1**, **4**, **6**-**13** and their precursors (the phosphazene ligand, **phos-1**, and the stable silver and gold starting complexes) has been evaluated in vitro against two tumour human cell lines, MCF7 and HepG2, by two different biomarkers, Alamar Blue (AB) and Neutral Red Uptake (NRU) assays after 48h exposure. At 24 h, the exposed cells were checked under an optical microscope, and the damage was observed at a similar range, which was calculated after 48 h (see the Supporting Information). By using both viability assays, median inhibitory concentration (IC50) values (Table 4a,b) were calculated from the dose-response curves by nonlinear regression analysis. IC50 values are the concentrations of a drug required to inhibit tumour cell proliferation by 50% compared to untreated cells.

Table 4. IC_{50} (μM) obtained by Alamar Blue and Neutral Red Uptake assays in (a) MCF7 and (b) HepG2 Cell Lines Exposed to the New Metallophosphazenes and Their precursors for 48 h. [i]

	Compound	Alamar Blue	Neutral Red Uptake
MCF7(a)	$N_3P_3(NHCy)_6$ **(phos-1)**	14.80±6.10	>25
	$[N_3P_3(NHCy)_6\{AuCl\}]$ (**1**)	4.83±0.3	4.88±0.65
	$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	2.75±0.07	1.32±0.07
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}](NO_3)$ (**6**)	3.87±0.47	2.71±0.19
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	2.97±0.55	1.86±0.58
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	1.36±0.1	1.31±0.07
	$[Au(ONO_2)PPh_3]$	3.81±0.82	5.30±0.21
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}](NO_3)$ (**9**)	5.59±0.1	5.47±0.6
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	2.59±0.37	3.12±0.1
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_3](NO_3)_3$ (**11**)	3.19±0.78	2.05±0.82
	$[N_3P_3(NHCy)_6\{AuTPA\}_2](TfO)_2$ (**12**)	7.77±0.17	5.98±0.51
	[AuCl(TPA)]	6.10±0.23	5.81±0.28
	$N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	4.04±0.18	3.20±0.59
	$[N_3P_3(NHCy)_6\{Ag(PPh_3)\}_2](TfO)_2$ [ii]	2.87±0.15	2.34±0.28
	$[N_3P_3(NHCy)_6\{Ag(PPh_3)\}_3](TfO)_3$ [ii]	1.60±0.05	2.14±0.65
	$[Ag(OTf)PPh_3]$ [b]	5.67±0.57	8.09±1.39
	cisplatin	56.82±4.23	23.71±1.24
	auranofin	2.58±0.15	2.19±0.19

	Compound	Alamar Blue	Neutral Red Uptake
HepG2 (b)	$N_3P_3(NHCy)_6$ **(phos-1)**	>25	>25
	$[N_3P_3(NHCy)_6\{AuCl\}]$ (**1**)	3.31±0.52	2.83±0.47
	$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	1.1±0.23	1.47±0.15
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}](NO_3)$ (**6**)	2.84±0.37	2.63±0.40
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	2.38 ±0.20	2.47±0.14
	$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	1.47±0.31	1.45±0.19
	$[Au(ONO_2)PPh_3]$	3.86±0.08	>8
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}](NO_3)$ (**9**)	4.58±0.30	4.37±1.60
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	3.07±0.14	3.55±0.60
	$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_3](NO_3)_3$ (**11**)	5.54±0.95	6.08±0.04
	$[N_3P_3(NHCy)_6\{AuTPA\}_2](TfO)_2$ (**12**)	5.09±0.37	6.51±0.51
	[AuCl(TPA)]	6.10±0.23	>8
	$N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	3.09±0.05	2.46±0.23
	$[N_3P_3(NHCy)_6\{AgPPh_3\}_2](TfO)_2$ [ii]	1.40±0.23	2.41±0.18
	$[N_3P_3(NHCy)_6\{AgPPh_3\}_3](TfO)_3$ [ii]	0.93±0.37	1.37±0.55
	$[Ag(OTf)PPh_3]$	4.45±0.37	7.69±1.96
	cisplatin	11.32±1.11	6.94±0.72
	auranofin	7.50±0.26	6.68±0.68

[i] All the compounds analysed were dissolved in DMSO, not exceeding 0.1%, except cisplatin, which was dissolved in water.
[ii] Data taken from our reference [62], measured under the same conditions as the other compounds and included for comparison.

All the tested metallophosphazenes showed good antitumor activities towards MCF7 cell line with IC_{50} values lower than 5.98 μM (see Table 4a) using the biomarker NRU, which proved to be a more sensitive assay than AB. The best of these were complexes **4**, **8** and **11**, with IC_{50} values lower than 2.05 μM. All of them are more cytotoxic than their phosphazene ligand (**phos-1**) and their metal precursors, except **12**, which is less toxic than its precursor [AuCl(TPA)]. Besides, all of them, including precursors, are much more cytotoxic than cisplatin. Remarkably, **4**, **8** and **11** are just as cytotoxic as or slightly more so than auranofin.

Regarding the cytotoxicity observed in HepG2 cells, the biomarker AB was somewhat more sensitive in comparison to NRU. In the AB assay, except for **9**, **11** and **12**, the obtained IC_{50} values were lower than 3.32 μM, **4** and **8** being the most cytotoxic compounds, with values of 1.10, and 1.47 μM, respectively. Once more, all metallophosphazenes showed lower IC_{50} values than their precursors and cisplatin. Besides, all metallophosphazenes are more cytotoxic than auranofin, the new gold phosphazenes being highly effective as antitumor agents *in vitro*.

Regarding the structure-activity relationships towards both cell lines, not outstanding differences in activity between these gold phosphazenes could be noticed. However, the following can be concluded: (1) the metal atom exerts cytotoxic activity and there also seems to be a small influence of the number of metal atoms linked to the phosphazene ring (see Table 4a,b; **8** is more potent than **7** and **6**, and **10** more than **9** in both biomarkers and cell lines, although **11** is no more toxic than **10** and **9**, especially in HepG2 cells); (2) Unlike the antibacterial activity, there is no synergistic or cooperative effect between the gold and silver metals for the cytotoxic properties. Heterometallic complex **13** is less active than both gold and silver analogues having the same phosphane ligand (gold complexes **7** and **8**, and silver complexes $[N_3P_3(NHCy)_6\{AgPPh_3\}_2](TfO)_2$ and $[N_3P_3(NHCy)_6\{AgPPh_3\}_3](TfO)_3$, see Table 4a,b). (3) The ligands coordinated to gold also have a small influence on the cytotoxicity, which is higher in triphenylphosphane and pentafluorophenyl derivatives than in those with diphenylmethylphosphane or TPA (**8** is more potent than **11, 4** and **7** are more active than **10** and **12**, and **6** is more than **9**).
The anticancer activity of gold(I) complexes has been summarized in recent reviews [25, 28, 20] and the IC_{50} values obtained for some of the tested gold-phosphazenes are among the lowest found for any gold derivatives against MCF7 and HepG2 cell lines, taking into account the experimental conditions (measured at 48 h). [34, 35] Phosphazenes **4** and **8** proved to be just as or more potent than the analogue silver metallophosphazenes mentioned before, whose IC_{50} values are also shown in Table 4a, b for comparison.

Non-tumorigenic human dermal fibroblasts (HDF) were also incubated with the compounds **4**, **7**, **8**, **10**, **13** and auranofin under similar conditions for 48 h for comparative purposes. The results are summarized in Tables 5 and 6. Compounds **4** and **8** show some tumour selectivity for the used cells, which could be higher for other tumoral cells.

No clear influence of the ancillary phosphane ligand is observed in the selectivity and heterometallic compound **13** is even more cytotoxic against non-tumorigenic cells.

Table 5. IC_{50} (μM) Obtained by Alamar Blue (AB) assays in HDF Cell Lines Exposed to **4**, **7**, **8**, **10** and **13** for 48 h and Tumour Selectivity Index.

Compound	HDF	IC_{50}(HDF)/ IC_{50}(MCF7)	IC_{50}(HDF)/ IC_{50}(HepG2)
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	3.95 ± 0.34	1.4	3.6
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	3.96 ± 0.41	1.3	1.7
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	2.86 ± 0.05	2.1	1.95
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	3.02 ± 0.12	1.2	0.98
$N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	2.23 ± 0.21	0.6	0.7
auranofin	8.91±1.01	3.5	1.2

Table 6. IC_{50} (μM) Obtained by Neutral Red Uptake (NRU) assays in HDF Cell Lines Exposed to **4**, **7**, **8**, **10** and **13** for 48 h and Tumour Selectivity Index.

Compound	HDF	IC_{50}(HDF)/ IC_{50}(MCF7)	IC_{50}(HDF)/ IC_{50}(HepG2)
$[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_2]$ (**4**)	> 8	> 6.1	> 5.4
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2](NO_3)_2$ (**7**)	4.1 ± 0.54	2.2	1.7
$[N_3P_3(NHCy)_6\{Au(PPh_3)\}_3](NO_3)_3$ (**8**)	3.62 ± 0.28	2.8	2.5
$[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_2](NO_3)_2$ (**10**)	5.5 ± 0.15	1.8	1.6
$N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**)	2.4 ± 0.05	0.75	0.97
auranofin	5.77±0.83	2.6	0.86

Experimental Section

General Data.

Instrumentation and general experimental techniques (elemental analysis, IR and mass spectroscopy) were as described earlier. [77, 62] NMR spectra were recorded on a Brucker AV 400 spectrometer. Chemical shifts are quoted relative to $SiMe_4$ (TMS, ^1H and ^{13}C, external) and H_3PO_4 (85%) (^{31}P, external). HSQC ^1H-^{13}C correlation spectra were obtained using standard procedures. FAB and MALDI-TOFF mass spectra were recorded using a Micromass Autospec spectrometer with m-nitrobenzyl alcohol (NBA) as matrix (for FAB), and ditranol or DCTB (trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) as matrix (for MALDI). Starting cyclotriphosphazene, $[N_3P_3(NHCy)_6]$ [62] and metal complexes [AuCl(tht)], [87] $[Au(C_6F_5)tht]$, [87] $[Au(ONO_2)PR_3]$ (PR_3= PPh_3, PPh_2Me) [83], [AuCl(TPA)] [84] and $[Ag(ONO_2)PPh_3]$ [85] were prepared according to published procedures. Culture medium, fetal bovine serum and cell culture reagents were obtained from Gibco and Corning (Spain). Chemicals for the different assays were provided by VWR International Eurolab and Merck. Plastic material for the cytotoxicity assays were supplied by Fisher Scientific.

Synthesis and Spectroscopic Characterization Data.

Synthesis of $[N_3P_3(NHCy)_6\{AuCl\}_n]$ [n=1 **(1)**, 2 **(2)**]. [chloride(2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ*N*)gold(I) (**1**), dichloride(μ-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^2N^1,N^3)digold(I) (**2**)]. To a solution of $[N_3P_3(NHCy)_6]$ (72.4 mg, 0.1 mmol) in dichloromethane (15 mL) was added [AuCl(tht)] (0.1 mmol, 32.1 mg, for **1** or 0.2 mmol, 64.1 mg, for **2**) and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to *ca.* 1 mL and the addition of pentane (10 mL) led to the precipitation of **1** or **2** as white solids.

1: Yield: 43 mg, 45%. Anal. Calcd (%) for $C_{36}H_{72}AuClN_9P_3$ (956.36): C, 45.21; H, 7.59; N, 13.18; Found: C, 45.50; H, 7.72; N, 13.31. IR (ATR): 3400(w, sh), 3316 (w, br) cm^{-1} (N-H); 1223 (vs), 1186 (s) cm^{-1} (P=N and C-N); 1096(vs, br) cm^1 (P-NHR), 342 (s) cm^{-1} (Au-Cl). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 14.39 ("d", 2P), 12.13 ("t",1P) (AB_2 system, $^2J_{AB}$= 47.1 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 3.13 (m, 4H; NH-C*H*), 3.03 (m, 2H; NH-C*H*), 2.38 (m, 4H; N*H*), 2.09 (t, $^3J_{H-H}$= $^2J_{H-P}$= 9.2 Hz, 2H; N*H*), 2.04 (m, 4H; NH(C_6H_{11}), 1.93 (m, 8H; NH(C_6H_{11})), 1.71-1.64 (m, 12H; NH(C_6H_{11})), 1.57-1.54 (m, 6H; NH(C_6H_{11})), 1.33-1.07 (m, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 50.43 (s, 4C; NH-*C*H), 49.90 (s, 2C; NH-*C*H), 36.45, 36.30, 25.64, 25.53, 25.38, 25.31, 25.17 (s, 30C; *C*H_2). MALDI-TOF (dithranol): m/z (%) = 1644.1 (55) $[(phos\text{-}1)_2Au]^+$, 724.5 (100) $[phos\text{-}1]^+$.

2: Yield: 71 mg, 60%. Anal.Calcd (%) for $C_{36}H_{72}Au_2Cl_2N_9P_3$ (1188.78): C, 36.37; H, 6.10; N, 10.60; Found: C, 36.1; H, 6.35; N, 10.45. IR (ATR): 3382 (vw), 3281 (m), 3262 (m) cm^{-1} (N-H); 1251 (vs), 1228 (m) cm^{-1} (P=N and C-N); 1073 (vs), 1052 (vs) cm^{-1} (P-NHR), 343 (s) cm^{-1} (Au-Cl). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 17.92 ("t", 1P), 11.82 ("d",2P) (AB_2 system, $^2J_{AB}$= 34.7 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 3.34 (m, 2H; NH-C*H*), 3.14 (m, 4H; NH-C*H*), 2.76 (t, $^3J_{H-H}$= $^2J_{H-P}$= 10.4 Hz, 2H; N*H*), 2.53 (m, 4H; N*H*), 2.16 (m, 4H; NH(C_6H_{11}), 2.04-1.96 (m, 8H; NH(C_6H_{11})), 1.73 (m, 12H; NH(C_6H_{11})), 1.64-1.54 (m, 6H; NH(C_6H_{11})), 1.34-1.07 (m, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 51.31 (s, 2C; NH-*C*H), 50.96 (s, 4C; NH-*C*H), 36.36, 36.23, 25.41, 25.34, 25.32, 25.24 (s, 30C; *C*H_2). MALDI-TOF (dithranol): m/z (%) = 1188.6 (1) $[M]^+$, 2110.6 (1) $[(phos\text{-}1)_2Au_3Cl_2]^+$, 1876.7 (3) $[(phos\text{-}1)_2Au_2Cl]^+$, 1644.6 (15) $[(phos\text{-}1)_2Au]^+$, 956.7 (3) $[(phos\text{-}1)AuCl+H]^+$, 724.5 (100) $[phos\text{-}1]^+$.

Synthesis of $[N_3P_3(NHCy)_6\{Au(C_6F_5)\}_n]$ [n=1**(3)**, 2**(4)**, 3**(5)**]. [(2,2,4,4,6,6-hexakis-(cyclohexylamino)cyclotriphosphazene-κ*N*)pentafluorophenylgold(I) (**3**), (μ-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^2N^1,N^3)bis(pentafluorophenyl)digold(I) (**4**) and (μ_3-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^3N^1,N^3,N^5)-tris(pentafluorophenyl)trigold(I) (**5**)]. To a solution of $[N_3P_3(NHCy)_6]$ (72.4 mg, 0.1 mmol) in dichloromethane (15 mL) was added $[Au(C_6F_5)tht]$ (0.1 mmol, 45.2 mg, for 3; 0.2 mmol, 90.4 mg, for **4** or 0.3 mmol, 135.7 mg, for **5**) and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to *ca.* 0.5 mL. Addition of pentane (10 mL) led to the precipitation of **3** or **4** as white solids. For compound **5**, evaporation of the solvent gave a white solid, which proved to be a mixture of **4**, **5** and $[Au(C_6F_5)tht]$. Compound **5** was not obtained pure even using an excess of

30% of the starting gold complex. In the reaction mixture, **4** was always observed.

3: Yield: 44 mg, 40%. Anal.Calcd (%) for $C_{42}H_{72}AuF_5N_9P_3$ (1087.96): C, 46.37; H, 6.67; N, 11.59; Found: C, 46.85; H, 7.01; N, 11.35. IR (ATR): 3394(w, sh), 3353 (w, br), 3255 (w, br) cm^{-1} (N-H); 1224 (vs), 1183 (s) cm^{-1} (P=N and C-N); 1086 (vs), 1058 (s) cm^{-1} (P-NHR); 1042 (s), 953 (vs), 803 (s) cm^{-1} (C_6F_5). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 14.78 ("d", 2P), 11.99 ("t", 1P) (AB_2 system, $^2J_{AB}$= 45.8 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 15.24 ("d", 2P), 12.67 ("t", 1P) (AB_2 system, $^2J_{AB}$= 44.9 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 3.30 (m, 4H; NH-C*H*), 3.05 (m, 2H; NH-C*H*), 2.36 (m, 4H; N*H*), 2.08 (m, 2H; N*H*), 1.96 (m, 12H; NH(C_6H_{11})), 1.70-1.50 (m, 18H; NH(C_6H_{11})), 1.33-1.08 (m, 30H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 3.23 (m, 6H; NH-C*H*), 3.08 (t, $^3J_{H-H}$= $^2J_{H-P}$= 9.0 Hz, 2H; N*H*), 2.86 (m, 4H; N*H*), 2.17 (m, 6H; NH(C_6H_{11})), 1.97 (m, 2H; NH(C_6H_{11})), 1.85 (m, 4H; NH(C_6H_{11})), 1.62-1.48 (m, 18H; NH(C_6H_{11})), 1.22-1.06 (m, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = C_6F_5 not well detected, 50.35 (s, 4C; NH-*C*H), 50.00 (s, 4C; NH-*C*H), 36.52, 36.44, 36.39, 25.72, 25.64, 25.34, 25.27 (s, 30C; CH_2). ^{19}F NMR ($CDCl_3$): δ = −115.92 (m, 2F; *o*-F), −161.35 (t, $^3J(F_p\text{-}F_m)$= 20.1 Hz, 1F; *p*-F), −163.59 (m, 2F; *m*-F). MS (FAB^+): m/z (%) = 1088 (4) $[M]^+$, 724.5 (100) $[M-AuC_6F_5]^+$, 1284 (1) $[M+Au]^+$, 1450 (1) $[M+AuC_6F_5]^+$, 1644.1 (5) $[(phos\text{-}1)_2Au]^+$, 2008 (3) $[(phos\text{-}1)_2Au_2C_6F_5]^+$.

4: Yield: 87 mg, 87%. Anal. Calcd (%) for $C_{48}H_{72}Au_2F_{10}N_9P_3$ (1451.98): C, 39.71; H, 5.00; N, 8.68; Found: C, 39.25; H, 5.10; N, 8.40. IR (ATR): 3409 (w), 3395 (w) cm^{-1} (N-H); 1243 (s), 1233 (s) cm^{-1} (P=N and C-N); 1078 (vs), 1059 (s) cm^{-1} (P-NHR); 1051 (s), 954 (s), 805 (s) cm^{-1} (C_6F_5).$^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 17.91 ("t", 1P), 11.94 ("d", 2P) (AB_2 system, $^2J_{AB}$= 36.1 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 19.59 ("t", 1P), 11.87 ("d", 2P) (AB_2 system, $^2J_{AB}$= 39.5 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 3.54 (m, 2H; NH-C*H*), 3.30 (m, 4H; NH-C*H*), 2.70 (t, $^3J_{H-H}$= $^2J_{H-P}$= 10.4 Hz, 2H; N*H*), 2.47 (m, 4H; N*H*), 2.12 (m, 4H; NH(C_6H_{11})), 2.09-1.99 (m, 8H; NH(C_6H_{11})), 1.71 (m, 12H; NH(C_6H_{11})), 1.58 (m, 6H; NH(C_6H_{11})), 1.35-1.14 (m, 30H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 4.23 (t, $^3J_{H-H}$= $^2J_{H-P}$= 11.4 Hz, 2H; N*H*), 3.92 (m, 4H; N*H*), 3.25 (m, 2H; NH-C*H*), 3.21 (m, 4H; NH-C*H*), 2.22 (m, 4H; NH(C_6H_{11})), 2.09-2.01 (m, 8H; NH(C_6H_{11})), 1.83-1.50 (m, 18H; NH(C_6H_{11})), 1.28-1.03 (m, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = C_6F_5 not well detected, 51.13 (s, 2C; NH-*C*H), 50.77 (s, 4C; NH-*C*H), 36.37, 25.48, 25.42, 25.25, 25.16, 25.10 (s, 30C; CH_2). ^{19}F NMR ($CDCl_3$): δ = −116.13 (m, 2F; *o*-F), −160.45(t, $^3J(F_p\text{-}F_m)$= 20.1 Hz, 1F; *p*-F), −163.19 (m, 2F; *m*-F). MS (FAB^+): m/z (%) = 1452 (20) $[M]^+$, 724.5 (100) $[M-Au_2(C_6F_5)_2]^+$, 1088 (25) $[M-AuC_6F_5]^+$, 1284 (10) $[M-C_6F_5]^+$, 1644.1 (1) $[(phos\text{-}1)_2Au]^+$, 2008 (3) $[(phos\text{-}1)_2Au_2C_6F_5]^+$.

5: $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 14.80 (s, 3P; N_3P_3 ring). ^{19}F NMR ($CDCl_3$): δ = −116.33 (m, 2F; *o*-F), −159.47 (t, $^3J(F_p\text{-}F_m)$= 20.1 Hz, 1F; *p*-F), −162.72 (m, 2F; *m*-F).

Synthesis of $[N_3P_3(NHCy)_6\{Au(PPh_3)\}_n](NO_3)_n$ [n=1**(6)**, 2**(7)** , 3**(8)**]. [(2,2,4,4,6,6-hexakis-(cyclohexylamino)cyclotriphosphazene-κ*N*)(triphenylphosphane)gold(I) nitrate (**6**), (μ-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^2N^1,N^3)-bis(triphenylphosphane)digold(I) bis(nitrate) (**7**) and (μ_3-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^3N^1,N^3,N^5)-tris(triphenylphosphane)-trigold(I) tris(nitrate) (**8**)]. To a solution of $[N_3P_3(NHCy)_6]$ (72.4 mg, 0.1 mmol) in dichloromethane (10 mL) was added $[Au(ONO_2)PPh_3]$ (0.1 mmol, 52.1 mg, for **6**; 0.2 mmol, 104.2 mg, for **7** or 0.33 mmol, 171.9 mg, for **8**) and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to *ca.* 2 mL. Addition of hexane (15 mL) led to the precipitation of **6**, **7** or **8** as white solids, which were washed with diethyl ether (3 x 2 mL) to remove the excess of $[Au(ONO_2)PPh_3]$ in case of **8**.

6: Yield: 110 mg, 88.6%. Anal.Calcd (%) for $C_{54}H_{87}AuN_{10}O_3P_4$ (1245.20): C, 52.09; H, 7.04; N, 11.25; Found: C, 52.60; H, 7.95; N, 11.20. IR (ATR): 3409(vw), 3320(w, sh) 3258 (w, br) cm^{-1} (N-H); 1221 (vs), 1188 (s) cm^{-1} (P=N and C-N); 1099 (vs), 1059 (m) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 32.40 (s, 1P; PPh_3); 14.48 ("d", 2P), 12.01 ("t", 1P) (AB_2 system, $^2J_{AB}$= 43.7 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 32.27 (s, 1P; PPh_3); 14.27 ("d", 2P), 12.38 ("t", 1P) (AB_2 system, $^2J_{AB}$= 44.7 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.62-7.46 (m, 15H, C_6H_5); 3.56 (br, 4H; N*H*), 3.06 (br, 6H; NH-C*H*), 2.17 (br, 2H; N*H*), 1.92 (m, 12H; NH(C_6H_{11})), 1.73-1.4 (m, 18H; NH(C_6H_{11})), 1.25 (m, 12H; NH(C_6H_{11})), 1.11 (m, 18H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 7.68-7.57 (m, 15H, C_6H_5); 3.95 (br, 4H; N*H*), 3.21 (br, 2H; N*H*), 3.02 (br, 4H; NH-C*H*), 2.88 (br, 2H; NH-C*H*), 1.91 (br, 12H; NH(C_6H_{11})), 1.58-1.44 (mbr, 18H; NH(C_6H_{11})), 1.14 (br, 18H; NH(C_6H_{11})), 1.02 (br, 12H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR ($CDCl_3$, APT): δ = 134.30 (d, *J(P-C)*= 13.2 Hz, 6C; CAr), 132.09 (s, 3C; CAr), 129.44 (d, *J(P-C)*= 11.8 Hz, 6C; CAr), C*ipso* not detected, 50.50 (s, 4C; NH-*C*H), 50.06 (s, 2C; NH-*C*H), 36.32, 25.73, 25.44 (s, 30C; CH_2). $^{13}C\{^1H\}$ NMR (DMSO, APT): δ = 133.85 (d, *J(P-C)*= 13.8 Hz, 6C; CAr), 132.27 (s, 3C; CAr), 129.41 (d, *J(P-C)*= 11.6 Hz, 6C; CAr), 128.61 (d, *J(P-C)*= 61.4 Hz, 3C; C*ipso*), 49.68 (s, 4C; NH-*C*H), 49.43 (s, 2C; NH-*C*H), 35.69, 35.50, 25.15, 25.01 (s, 30C; CH_2). MS (FAB^+): m/z (%) = 1183 (100) $[M]^+$, 724.5 (80) $[M-AuPPh_3]^+$, 459 (42) $[AuPPh_3]^+$.

7: Yield: 141 mg, 79.8%. Anal.Calcd (%) for $C_{72}H_{102}Au_2N_{11}O_6P_5$ (1766.45): C, 48.96; H, 5.82; N, 8.72; Found: C, 49.12; H, 5.66; N, 8.65. IR (ATR): 3209 (w, br), 3051 (vw) cm^{-1} (N-H); 1251(m), 1230 (s, br) cm^{-1} (P=N and C-N); 1102 (s), 1081 (vs), 1051 (m) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 32.20 (s, 2P; PPh_3); 17.68 ("t", 1P), 12.26 ("d", 2P) (AB_2 system, $^2J_{AB}$= 34.3 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 32.12 (s, 2P; PPh_3); 18.11 ("t", 1P), 10.83 ("d", 2P) (AB_2 system, $^2J_{AB}$= 33.5 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.62-7.48 (m, 30H, C_6H_5); 5.8 (t, $^3J_{H-H}$= $^2J_{H-P}$= 11,4 Hz, 2H; N*H*), 3.77 (br, 4H; N*H*), 3.09 (br, 6H; NH-C*H*), 1.98-1.88 (m, 12H; NH(C_6H_{11})), 1.69-1.37 (m, 18H; NH(C_6H_{11})), 1.27 (m, 12H; NH(C_6H_{11})), 1.17-1.01 (m, 14H; NH(C_6H_{11})), 0.74 (m, 4H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 7.71-7.57 (m, 30H, C_6H_5); 5.15 (t, $^3J_{H-H}$= $^2J_{H-P}$= 11,1 Hz, 2H; N*H*), 4.57 (mbr, 4H; N*H*), 3.03 (br, 6H; NH-C*H*), 2.06-1.93 (m, 12H; NH(C_6H_{11})), 1.63-1.31 (m, 18H; NH(C_6H_{11})), 1.19 (m, 12H; NH(C_6H_{11})), 1.06 (m, 14H; NH(C_6H_{11})), 0.73 (m, 4H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 134.32 (d, *J(P-C)*= 13.7 Hz, 12C; CAr), 132.17 (s, 6C; CAr), 129.48 (d, *J(P-C)*= 11.9 Hz, 12C; CAr), 128.63 (d, *J(P-C)*= 63.0 Hz, 6C; C*ipso*), 51.06 (s, 2C; NH-*C*H), 50.99 (s, 4C; NH-*C*H), 36.32, 36.18, 25.58, 25.43, 25.36, 25.02 (s, 30C; CH_2). $^{13}C\{^1H\}$ NMR (DMSO, APT): δ = 133.87 (d, *J(P-C)*= 13.8 Hz, 12C; CAr), 132.61 (s, 6C; CAr), 129.52

(d, *J(P-C)*= 11.8 Hz, 12C; CAr), 127.88 (d, *J(P-C)*= 63.1 Hz, 6C; C*ipso*), 50.58 (s, 2C; NH-*C*H), 50.18 (s, 4C; NH-*C*H), 36.10, 35.62, 25.00, 24.91, 24.68 (s, 30C; *C*H_2). MS (FAB^+): m/z (%) = 1641 (8) $[M]^+$, 1183 (100) $[M-AuPPh_3]^+$, 986 (8) $[M-Au_2PPh_3]^+$, 724.5 (18) $[M-Au_2(PPh_3)_2]^+$, 459 (93) $[AuPPh_3]^+$.

8: Yield: 215 mg, 94%. Anal.Calcd (%) for $C_{90}H_{117}Au_3N_{12}O_9P_6$ (2287.71): C, 47.25; H, 5.15; N, 7.35; Found: C, 47.75; H, 5.30; N, 7.42. IR (ATR): 3148 (w, br), 3055 (vw) cm^{-1} (N-H); 1320 (m, sh), 1310 (s), 1300 (s, sh) cm^{-1} (P=N and C-N); 1119 (m, sh), 1099 (vs), 1090 (s, sh) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 31.77 (s, 3P; PPh_3); 13.10 (s, 3P; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.59-7.54 (m, 45H, C_6H_5); 6.46 (mbr, 6H; N*H*), 2.96 (mbr, 6H; NH-C*H*), 1.93 (m, 12H; NH(C_6H_{11})), 1.47 (mbr, 12H; NH(C_6H_{11})), 1.37-1.28 (m, 18H; NH(C_6H_{11})), 1.10-0.96 (m, 18H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 134.27 (d, *J(P-C)*= 13.5 Hz, 18C; CAr), 132.41 (d, *J(P-C)*= 2.3 Hz, 9C; CAr), 129.68 (d, *J(P-C)*= 12.1 Hz, 18C; CAr), 127.86 (d, *J(P-C)*= 64.3 Hz, 9C; C*ipso*), 50.07 (s, 6C; NH-*C*H), 36.39, 25.34, 25.05 (s, 30C; *C*H_2). MALDI-TOF (DCTB): m/z (%) = 2100.5 (9) $[M]^+$, 1641.9 (59) $[M-AuPPh_3]^+$, 1182.86 (100) $[M]^+$.

Synthesis of $[N_3P_3(NHCy)_6\{Au(PPh_2Me)\}_n](NO_3)_n$ [n=1**(9)**, 2**(10)**, 3**(11)**]. [(2,2,4,4,6,6-hexakis-(cyclohexylamino)cyclotriphosphazene-κ*N*)(methyldiphenylphosphane)gold(I) nitrate (**9**), (μ-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^2N^1,N^3)bis(methyldiphenylphosphane)digold(I) bis(nitrate) (**10**) and (μ_3-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-κ^3N^1,N^3,N^5)-tris(methyldiphenylphosphane)trigold(I) tris(nitrate) (**11**)]. To a solution of $[N_3P_3(NHCy)_6]$ (72.4 mg, 0.1 mmol) in dichloromethane (10 mL) was added $[Au(ONO_2)PPh_2Me]$ (0.1 mmol, 45.9 mg, for **9**; 0.2 mmol, 91.8 mg, for **10** or 0.33 mmol, 151.5 mg, for **11**) and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to *ca.* 1 mL. Addition of hexane (15 mL) led to the precipitation of **9**, **10** or **11** as white solids, which were washed with diethyl ether (3 x 2 mL) to remove the excess of $[Au(ONO_2)PPh_2Me]$ in case of **11**.

9: Yield: 98 mg, 82.8%. Anal.Calcd (%) for $C_{49}H_{85}AuN_{10}O_3P_4$ (1183.13): C, 49.74; H, 7.24; N, 11.84. Found: C, 49.30; H, 7.31; N, 11.60. IR (ATR): 3407 (vw), 3238 (w, br) cm^{-1} (N-H); 1216 (vs), 1190 (vs) cm^{-1} (P=N and C-N); 1108 (s, sh), 1092 (vs) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 17.06 (s, 1P; PPh_2Me); 14.79 ("d", 2P), 12.22 ("t", 1P) (AB_2 system, $^2J_{AB}$= 44.3 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 17.03 (s, 1P; PPh_2Me); 14.49 ("d", 2P), 12.56 ("t", 1P) (AB_2 system, $^2J_{AB}$= 43.2 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.67-7.62, 7.48-7.47 (m, 10H, C_6H_5); 3.50 (br, 4H; N*H*), 3.05 (br, 6H; NH-C*H*), 2.29 (d, $^2J_{P-H}$= 10.4 Hz, 3H; PPh_2Me), 2.12 (br, 2H; N*H*), 1.91 (m, 12H; NH(C_6H_{11})), 1.73-1.4 (m, 18H; NH(C_6H_{11})), 1.25-1.12 (m, 30H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 7.82-7.77, 7.60-7.55 (m, 10H, C_6H_5); 3.89 (br, 4H; N*H*), 3.23 (br, 2H; N*H*), 3.03 (br, 4H; NH-C*H*), 2.89 (br, 2H; NH-C*H*), 2.27 (d, $^2J_{P-H}$= 8.8 Hz, 3H; PPh_2Me), 1.93-1.87 (br, 12H; NH(C_6H_{11})), 1.61-1.46 (br, 18H; NH(C_6H_{11})), 1.14-1.04 (br, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR ($CDCl_3$, APT): δ = 132.82 (d, *J(P-C)*= 13.2 Hz, 4C; CAr), 131.77 (sbr, 2C; CAr), 129.32 (d, *J(P-C)*= 11.2 Hz, 4C; CAr), C*ipso* not detected, 50.43 (s, 4C; NH-*C*H), 50.03 (s, 2C; NH-*C*H), 36.29, 25.44 (s, 30C; *C*H_2), 14.21 (d, $^1J(P-C)$= 39.2 Hz, 1C; *C*H_3). $^{13}C\{^1H\}$ NMR (DMSO, APT): δ = 132.67 (d, *J(P-C)*= 13.5 Hz, 4C; CAr), 132.02 (s, 2C; CAr), 130.80 (d, *J(P-C)*= 60.3 Hz, 2C; C*ipso*), 129.18 (d, *J(P-C)*= 11.6 Hz, 4C; CAr), 49.81 (s, 4C; NH-*C*H), 49.44 (s, 2C; NH-*C*H), 35.81, 35.68, 35.49, 25.07 (s, 30C; *C*H_2), 13.22 (d, $^1J(P-C)$= 39.2 Hz, 1C; *C*H_3). MALDI-TOF (DCTB): m/z (%) = 1120.82 (100) $[M]^+$.

10: Yield: 150 mg, 91.5%. Anal.Calcd (%) for $C_{62}H_{98}Au_2N_{11}O_6P_5$ (1642.31): C, 45.34; H, 6.01; N, 9.38; Found: C, 44.96; H, 6.37; N, 9.10. IR (ATR): 3213 (w, br), 3055 (vw) cm^{-1} (N-H); 1264 (m, sh), 1233 (vs, br) cm^{-1} (P=N and C-N); 1100 (sh,s), 1073 (vs), 1045 (s, sh) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 16.13 (s, 2P; PPh_2Me); 17.51 ("t", 1P), 12.56 ("d", 2P) (AB_2 system, $^2J_{AB}$= 32.8 Hz; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 17.41 (s, 2P; PPh_2Me); 18.23 ("t", 1P), 11.17 ("d", 2P) (AB_2 system, $^2J_{AB}$= 34.1 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.62-7.57, 7.46 (m, 20H, C_6H_5); 5.6 (br, 2H; N*H*), 3.68 (br, 4H; N*H*), 3.00 (br, 6H; NH-C*H*), 2.24 (d, $^2J_{P-H}$= 11.0 Hz, 6H; PPh_2Me), 1.86 (m, 12H; NH(C_6H_{11})), 1.58-1.43 (m, 18H; NH(C_6H_{11})), 1.31-0.87 (m, 30H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 7.84-7.79, 7.65-7.56 (m, 20H, C_6H_5); 5.07 (t, $^3J_{H-H}$= $^2J_{H-P}$= 11.2 Hz, 2H; N*H*), 4.47 (mbr, 4H; N*H*), 3.02 (br, 6H; NH-C*H*), 2.32 (d, $^2J_{P-H}$= 10.7 Hz, 6H; PPh_2Me), 2.07-1.92 (m, 12H; NH(C_6H_{11})), 1.61-1.43 (m, 18H; NH(C_6H_{11})), 1.27-0.93 (m, 30H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR ($CDCl_3$, APT): δ = 132.68 (d, *J(P-C)*= 13.2 Hz, 8C; CAr), 131.95 (s, 4C; CAr), 130.12 (d, *J(P-C)*= 61.0 Hz, 4C; C*ipso*), 129.38 (d, *J(P-C)*= 11.8 Hz, 8C; CAr), 50.87 (s, 4C; NH-*C*H), 50.36 (s, 2C; NH-*C*H), 36.12, 25.00 (s, 30C; *C*H_2), 14.08 (d, $^1J(P-C)$= 40.1 Hz, 2C; *C*H_3). $^{13}C\{^1H\}$ NMR (DMSO, APT): δ = 132.73 (d, *J(P-C)*= 13.5 Hz, 8C; CAr), 132.28 (s, 4C; CAr), 130.16 (d, *J(P-C)*= 62.6 Hz, 4C; C*ipso*), 129.27 (d, *J(P-C)*= 11.8 Hz, 8C; CAr), 50.41 (s, 4C; NH-*C*H), 50.37 (s, 2C; NH-*C*H), 35.97, 35.63, 25.08, 25.02 (s, 30C; *C*H_2), 13.06 (d, $^1J(P-C)$= 40.6 Hz, 2C; *C*H_3). MALDI-TOF (DCTB): m/z (%) = 1518.73 (26) $[M]^+$, 1121.47 (100) $[M]^+$.

11: Yield: 198 mg, 94%. Anal.Calcd (%) for $C_{75}H_{111}Au_3N_{12}O_9P_6$ (2101.50):C, 42.86; H, 5.32; N, 8.00; Found: C, 42.50; H, 5.8; N, 7.72. IR (ATR): 3173 (w, br), 3055 (vw) cm^{-1} (N-H); 1323 (s, br), 1295 (m, sh) cm^{-1} (P=N and C-N); 1094 (vs, br) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 15.69 (s, 3P; PPh_2Me); 14.59 (s, 3P; N_3P_3 ring). $^{31}P\{^1H\}$ NMR (DMSO): δ = 17.50 (s, 3P; PPh_2Me); 13.76 (s, 3P; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.65-7.51 (m, 30H, C_6H_5); 6.12 (br, 6H; N*H*), 2.97 (br, 6H; NH-C*H*), 2.31 (d, $^2J_{P-H}$= 11.2 Hz, 9H; PPh_2Me) 1.93-1.84 (m, 12H; NH(C_6H_{11})), 1.51-1.48 (m, 12H; NH(C_6H_{11})), 1.40-1.29 (m, 18H; NH(C_6H_{11})), 1.1-0.92 (m, 18H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 7.86-7.57 (m, 30H, C_6H_5); 5.59 (br, 6H; N*H*), 3.06 (br, 6H; NH-C*H*), 2.38 (d, $^2J_{P-H}$= 11.2 Hz, 9H; PPh_2Me) 2.01 (br, 12H; NH(C_6H_{11})), 1.61-1.59 (m, 12H; NH(C_6H_{11})), 1.44 (m, 6H; NH(C_6H_{11})), 1.27 (m, 12H; NH(C_6H_{11})), 1.1-0.92 (m, 18H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 132.74 (d, *J(P-C)*= 13.1 Hz, 12C; CAr), 132.20 (d, *J(P-C)*= 2.5 Hz, 6C; CAr), 129.65 (d, *J(P-C)*= 12.0 Hz, 12C; CAr), 129.58 (d, *J(P-C)*= 64.3 Hz, 6C; C*ipso*), 50.31 (s, 6C; NH-*C*H), 36.21, 25.26, 24.98 (s, 30C; *C*H_2), 13.94 (d, $^1J(P-C)$= 40.9 Hz, 3C; *C*H_3). MS (FAB^+): m/z (%) = 1916 (4) $[M]^+$, 1518.5 (12) $[M-AuPPh_2Me]^+$, 1121.7 (100) $[M-Au_2(PPh_2Me)_2]^+$, 397 (20) $[AuPPh_2Me]^+$.

Synthesis of $[N_3P_3(NHCy)_6\{Au(TPA)\}_2](TfO)_2$ (12). [(μ-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-

κ²*N*¹,*N*³)bis(1,3,5-triaza-7-phosphaadamantane-κ*P*)digold(I) bis(trifluoromethanesulfonate) (**12**)]. To a mixture of $[N_3P_3(NHCy)_6]$ (72.4 mg, 0.1 mmol) and AuCl(TPA) (85,7 mg, o.22 mmol) in methanol (15 mL) was added AgTfO (56.5 mg, 0.22 mmol) and the mixture was stirred at room temperature for 1.5 h protected from the light. AgCl was filtered off and the solvent was removed under reduced pressure to *ca.* 1 mL. Addition of hexane (15 mL) led to the precipitation of **12** as a white solid.

Yield: 121.1 mg, 70 %. Anal.Calcd (%) for $C_{50}H_{96}Au_2F_6N_{15}O_6P_5S_2$ (1730.32): C, 34.71; H, 5.59; N, 12.14; S, 3.71; Found: C, 35.21; H, 5.87; N, 12.01; S, 3.34. IR (ATR): 3286 (w, br) cm^{-1} (N-H); 1223 (vs, br) cm^{-1} (P=N and C-N); 1071 (s) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 17.21 ("t", 1P), 11.48 ("d", 2P) (AB_2 system, $^2J_{AB}$= 32.7 Hz; N_3P_3 ring); −55.42 (s, 2P; TPA). $^{31}P\{^1H\}$ NMR (DMSO): δ = 18.39 ("t", 1P), 11.44 ("d", 2P) (AB_2 system, $^2J_{AB}$= 31.6 Hz; N_3P_3 ring); −55.98 (s, 2P; TPA). ^1H NMR ($CDCl_3$): δ = 4.61 ("d", $^2J_{H\text{-}H}$= 13.2 Hz, 6H; NC*H*$_2$N), 4.5 (br, 2H; N*H*), 4.48 ("d", $^2J_{H\text{-}H}$= 13.2 Hz, 6H; NC*H*$_2$N), 4.39 (s, 12H; NC*H*$_2$P), 3.23 (br, 4H; N*H*), 3.01 (br, 6H; NH-C*H*), 1.91-1.79 (m, 12H; NH(C_6H_{11})), 1.70 (br, 12H; NH(C_6H_{11})), 1.58-1.55 (m, 6H; NH(C_6H_{11})), 1.47-1.39 (m, 4H; NH(C_6H_{11})), 1.26-1.10 (m, 26H; NH(C_6H_{11})). ^1H NMR (DMSO): δ = 4.84 (br, 2H; N*H*), 4.66 ("d", $^2J_{H\text{-}H}$= 12.8 Hz, 6H; NC*H*$_2$N), 4.41 ("d", $^2J_{H\text{-}H}$= 12.8 Hz, 6H; NC*H*$_2$N), 4.29 (s, 12H; NC*H*$_2$P), 3.30 (br, 4H; N*H*), 2.91 (br, 6H; NH-C*H*), 1.98-1.87 (m, 12H; NH(C_6H_{11})), 1.77-1.71 (m, 12H; NH(C_6H_{11})), 1.58-1.56 (m, 6H; NH(C_6H_{11})), 1.19-1.10 (m, 30H; NH(C_6H_{11})). $^{19}F\{^1H\}$ NMR (DMSO): δ = −77.72 (s). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 73.42 (d, $^3J(P\text{-}C)$= 8.4 Hz, 6C; NCH$_2$N), 52.47 (d, $^1J(P\text{-}C)$= 24.1 Hz, 6C; NCH$_2$P), 51.96 (s, 2C; NH-*C*H), 51.04 (s, 4C; NH-*C*H), 36.26, 36.14, 26.18, 25.54, 25.44, 25.19 (s, 30C; *C*H$_2$). MALDI-TOF (dithranol): m/z (%) = 1432.2 (10) $[M]^+$, 1078 (100) $[M-AuTPA]^+$, 882 (20) $[M-Au_2TPA]^+$, 724.2 (25) $[M-Au_2(TPA)_2]^+$, 354 (90) $[AuTPA]^+$.

Synthesis of $[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (13)

[(μ$_3$-2,2,4,4,6,6-hexakis(cyclohexylamino)cyclotriphosphazene-1κ*N*¹,1κ*N*³,2κ*N*⁵)-tris(triphenylphosphane)digold(I)silver(I) tris(nitrate) (**13**)]. To a solution of $[N_3P_3(NHCy)_6]\{Au(PPh_3)\}_2](NO_3)_2$ (**7**) (88.3 mg, 0.05 mmol) in dichloromethane (10 mL) was added $[Ag(ONO_2)PPh_3]$ (0.05 mmol, 21.6 mg) and the mixture was stirred at room temperature for 15 min protected from the light. The solvent was removed under reduced pressure to *ca.* 1 mL. Addition of hexane (15 mL) led to the precipitation of **13** as a white solid. Yield: 85.7 mg, 78 %. Anal.Calcd (%) for $C_{90}H_{117}AgAu_2N_{12}O_9P_6$ (2198,61):C, 49.17; H, 5.36; N, 7.64; Found: C, 49.01; H, 5.68; N, 7.30. IR (ATR): 3221 (w, br), 3056 (vw) cm^{-1} (N-H); 1320 (m, sh), 1308 (s, sh), 1295 (s, br) cm^{-1} (P=N and C-N); 1096 (vs), 1082 (vs, sh) cm^{-1} (P-NHR). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 31.71 (s, 2P; Au *P*Ph$_3$); 17.34 (br, 1P; Ag*P*Ph$_3$); 16.09 ("t", 1P), 14.88 ("d", 2P) (AB_2 system, $^2J_{AB}$= 33.1 Hz; N_3P_3 ring). ^1H NMR ($CDCl_3$): δ = 7.61-7.45 (m, 45H, C_6H_5); 6.22 (tbr, $^3J_{H\text{-}H}$= $^2J_{H\text{-}P}$= 12.4 Hz,2H; N*H*), 4.39 (br, 4H; N*H*), 3.08 (br, 6H; NH-C*H*), 1.93 (m, 12H; NH(C_6H_{11})), 1.54-1.26 (m, 30H; NH(C_6H_{11})), 1.03 (m, 18H; NH(C_6H_{11})). $^{13}C\{^1H\}$ NMR (($CDCl_3$, APT): δ = 134.09 (d, *J(P-C)*= 13.5 Hz, 12C; CAr, AuP*Ph*$_3$), 133.96 (d, *J(P-C)*= 16.0 Hz, 6C; CAr, AgP*Ph*$_3$), 132.71 (s, 3C; CAr, AgP*Ph*$_3$), 132.38 (d, *J(P-C)*= 2.5 Hz, 6C; CAr, AuP*Ph*$_3$), 129.62 (d, *J(P-C)*= 12 Hz, 12C; CAr, AuP*Ph*$_3$), 129.43 (d, *J(P-C)*= 10.8 Hz, 6C; CAr, AgP*Ph*$_3$), 128.16 (d, *J(P-C)*= 63.3 Hz, 6C; C*ipso*, AuP*Ph*$_3$), C*ipso* from AgP*Ph*$_3$ not detected, 51.46 (br, 4C; NH-*C*H), 49.59 (br, 2C; NH-*C*H), 36.45, 36.28, 36.01, 25.48, 25.18, 25.05 (30C; *C*H$_2$). MALDI-TOF (DCTB): m/z (%) = 1642.41 (6) $[M-AgPPh_3]^+$, 1183.92 (100) $[M-AgAu(PPh_3)_2]^+$, 721.39 (71) $[M-AgAu_2(PPh_3)_3-2H]^+$.

X-Ray Structure Determination of compound 7

The crystals of **7** was mounted on a glass fibre and transferred to the cold gas stream of a Bruker SMART 1000 CCD diffractometer. Measurements were made at -130°C. Absorption corrections were based on multi-scans. The structures were refined anisotropically using the program SHELXL-2017. [116] Hydrogen atoms of the NH groups were refined freely but with N–H distance restraints ("SADI"). Other hydrogens were included using a riding model starting from calculated positions. The solvent molecule is disordered. Despite the use of appropriate restraints, the dimensions and the U values of this molecule are unsatisfactory and should be interpreted with caution.

Deposition Number CSD-2145600 contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

Cell culture protocols.

To assess the cellular cytotoxicity and the antitumoral potential, human breast adenocarcinoma (MCF7) and human hepatocellular carcinoma (HepG2) epitelial cell lines were used. Both of them are commonly used in toxicological and in tumoral studies. Both were recognized as good experimental models, the first one, MCF7 cell line, due to its exquisite hormone sensitivity through expression of oestrogen receptor, making it an ideal model to study hormone response and a great breast cancer representative. [117] The second one, HepG2 cells, retained inducibility and activities of several phase I and phase II xenobiotic metabolising enzymes. [118] Also, the HDF cell line was used as non-tumoral cell line to evaluate the selectivity of the compounds in comparison to tumoral cell lines. It is a well stablished non-tumoral model being used in biomedical assays. HDF is considered an immortalized non-tumoral cell line, which can be used as a cellular model relatively easily, with an uncomplicated maintenance and use, and without genetic engineering [119] Cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MCF7 cells were cultured in monolayer in Dulbecco´s Minimum Essential Medium (DMEN) supplemented with 10% of fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin (1%) and 2 mM L-glutamine (1%). The passages used for MCF7 are between 7-12. For HepG2 cells, Minimum Essential Medium (MEM) was used as main culture medium and similar nutrients and proportions above mentioned for MCF7. The passages we used were from 9-14. For HDF cells, passages from 3-7 were used and

the media and nutrients selected were similar to MCF7, but FBS were added at 20%. Cells were routinely grown at 37ºC and 5% CO_2 in a humidified atmosphere.

Cytotoxicity assays.

For cytotoxicity assays, the compounds were dissolved in DMSO. DMSO concentration did not exceed 0.1% and appropriate controls of solvent were tested in all the experiments. Under the same conditions, auranofin and cisplatin have also been tested, except that cisplatin was dissolved in water.

The exposure concentrations for the metallophosphazenes were 0-8 μM, after a wide range assay (0-200 μM) to determine the specific spectrum and the possible precipitation of compounds (data not shown). **Phos-1** was tested from 0 to 25 μM, and metal precursors were assayed from 0 to 80 μM. MCF7 and HepG2 cells were seeded at a density of $8x10^4$ and $1x10^5$ cells/mL in 100 μL 96 wells microplates and allowed to attach for 24 h prior to the addition of the compounds under study. In each assay, all the compounds were tested in sixtuplicate with three independent experiments being conducted. The period of exposure of the metal complexes to the cell lines selected was 48h at 37ºC and 5% CO_2.

The cell viability was evaluated as described earlier. [62]

Antimicrobial activity assays

Determination of MIC for Gram positive and Gram negative strains. The antibacterial activities of all compounds were tested against the *Gram-negative* strains Escherichia coli ATCC 10536, Pseudomonas aeruginosa ATCC 15442 and the *Gram-positive* Staphylococcus aureus ATCC 11632. Bacteria were stored as glycerol stocks at -80ºC and streaked onto Luria – Bertani plates prior to each experiment. Colonies from these newly prepared plates were inoculated into 5 mL LB media and the tubes were incubated at 37ºC. The overnight cultures were diluted to obtain a final concentration in the experiment of approximately $5x10^5$ cfus/mL. Stock solutions of each compound in DMSO were prepared at a concentration of 1 mM. Minimum inhibitory concentrations (MIC) were calculated as described earlier. [62] The range of the final concentrations tested spanned from 0.12 to 250 μM. Each experiment was performed twice.

Determination of MIC for Mycobacterium tuberculosis complex (MTBC) strains. The compounds were assayed against two MTBC strains, M. tuberculosis strain H37Rv ATCC 27294 and M. bovis BCG Pasteur. In this work, we also utilized M. bovis BCG, which is commonly used as a model organism for the study of M. tuberculosis because it is not virulent vaccine strain and the BCG genome shares a very high degree of similarity with that of M. tuberculosis. The anti-MTBC activity of all compounds was determined as described earlier. [62]

Kinetic killing assay. For kinetic killing assays, exponentially growing bacterial cultures were diluted in fresh media to obtain 106 cfu/mL. The compounds were added to the culture at 2 x MIC concentrations. The number of cfus at the start of the experiment was estimated by plating appropiate dilutions of the bacterial cultures onto LB agar plates. The effect of each compound was determined by plating for cfus at the indicated time points and counting the resulting number of colonies.

Conclusions

The nitrogen atoms of the cyclotriphosphazene ring in $[N_3P_3(NHCy)_6]$ have been shown to be basic enough to coordinate one, two or three gold groups "AuL" depending on the molar ratio used. Thus, the first gold-phosphazenes in which the gold atoms are coordinated to the nitrogens of PZ skeleton have been obtained. Specifically, neutral complexes, $[N_3P_3(NHCy)_6\{AuX\}_n]$ (X= Cl or C_6F_5; n= 1 or 2) (**1**-**4**), cationic ones, $[N_3P_3(NHCy)_6\{Au(PR_3)\}_n](NO_3)_n$ (PR_3 = PPh_3, PPh_2Me, TPA; n=1, 2 or 3) (**6**-**12**) [TPA = 1,3,5-triaza-7-phosphaadamantane] and a heterometallic compound $[N_3P_3(NHCy)_6\{Au(PPh_3)\}_2\{Ag(PPh_3)\}](NO_3)_3$ (**13**) have been synthesized and characterized. The most stable metallophosphazenes **1**, **4**, **6**-**13** exhibited an excellent broad spectrum antibacterial activity, being particularly active against *Gram-Positive S. aureus* bacteria and against the MTBC strains used in this work. The antibacterial activity of all complexes is much higher than that of $AgNO_3$ and AgSD for the entire range of bacteria studied, except against *P. aeruginosa.* The MIC values are among the lowest values found for any gold or silver derivatives. The heterometallic (silver and gold) complex **13** is the only metallophosphazene that is more active than $AgNO_3$ and AgSD against *P. aeruginosa*, for which **13** also exhibits an excellent activity, with a MIC value of ≤ 0.12 μM (≤ 0.26 μg/mL). The broad-spectrum antimicrobial efficacy of all these metallophosphazenes may be explained by the lability of the ligands in these complexes. As for the structure-activity relationships, the following can be concluded: (1) There is no a clear influence of the ancillary ligand (PPh_3, PPh_2Me or TPA) on the antibacterial activity; (2) The activity against *Gram-negative* strains is enhanced when increasing the number of gold atoms; (3) As for the comparison with silver phosphazenes previously reported by us, which only differ in the metal atom, they are more active against *Gram-negative* strains than gold phosphazenes, including *P. aeruginosa.* The opposite happens against MTBC strains. Remarkably, compound **13,** with both gold and silver, showed outstanding activity against both *Gram-positive* and *Gram-negative* strains, which makes it clear that there is a synergistic or cooperative effect between the gold and silver metals, as observed in other heterometallic AuAg complexes.

All tested metallophosphazenes have a good antitumoral activity toward MCF7 and HepG2 cell lines. All of them are more effective than auranofin towards HepG2 cell line. **4**, **8** and **11** are just as cytotoxic as or slightly more so than auranofin towards MCF7 cell line, with IC_{50} values lower than 2.05 μM. Unlike with the antibacterial activity, there was no cooperative cytotoxicity between gold and silver and the ancillary ligands coordinated to gold also have an small influence on the cytotoxicity, which is

higher when lipophilicity increases. The cytotoxicity is produced at the same range of inhibition of the growth of *E. coli* and it seems to increase with the number of gold atoms.

Notably, the antibacterial effects against *Gram-positive* strains were stronger than the growth-inhibiting effects against human cell lines. For **13**, the antibacterial effects were also stronger, not only against *S. aureus* but also against all the bacteria used in this work. Comparing the IC_{50} values of the tested metallophosphazenes against HDF Cell line with the MICs values of the same complexes against *Staphylococcus aureus*, it is observed that there is a very notable difference between them, being the IC_{50}/MIC ratio between 20 and 66 depending on the compound. For **13**, this ratio is > 18 against all bacteria used. For auranofin, this ratio against *S. aureus* and *M. tuberculosis H37Rv* is 32 and aprox. 8, respectively. Despite its apparently low *in vitro* therapeutic index, auranofin has been suggested as a candidate for drug repurposing in antibacterial therapy. [40] Thus, the IC_{50}/MIC ratio for our metallophosphazenes indicate that it could be possible to use them in the appropriate concentration as antibacterial drugs with non-cytotoxic effects.

On the other hand, the broad spectrum antimicrobial efficacy of all these metallophosphazenes and particularly of heterometallic compound **13** could be very useful to obtain materials for surfaces with antimicrobial properties that are increasingly in demand.

The outstanding biological activities of these complexes are worth studying further to determine action mechanisms and to elucidate the possibility of new biological targets.

Author contributions

Conceptualization and visualization: J.J.; formal analysis, investigation, and methodology: E.G., I.O., S.M., M.L., E.V., G.R., P.J., and J.J.; project administration, and supervision: J.J.; funding acquisition, and resources: G.R. and L.O.; writing-original draft: J.J.; validation, writing-review and editing: I.O., S.M., M.L., E.V., G.R., P.J., L.O. and J.J.

Conflicts of interest

There are no conflicts to declare

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