Synthesis, structural characterisation and anti-proliferative activity of NHC gold amino acid and peptide conjugates†

Jessica Lemke, Antonio Pinto, Philip Niehoff, Vera Vasylyeva and Nils Metzler-Nolte*

Received 27th March 2009, Accepted 28th May 2009
First published as an Advance Article on the web 16th July 2009
DOI: 10.1039/b906140a

We report the synthesis of new NHC gold(I) and NHC gold(III) halide, amino acid and dipeptide complexes. Transmetallation of the *N*-phenylalanine-substituted NHC silver complex **3** with Me_2SAuCl yields the phenylalanine–NHC gold(I) conjugate **4a**. Halide exchange with LiBr and oxidation of **4a** with Br_2 in CH_2Cl_2 yields the phenylalanine–NHC Au(I) and Au(III) bromides **4b** and **4c**, respectively. Reaction of *N*-Boc protected cysteine methyl ester (Boc–Cys–OMe) or the dipeptide *N*-Boc–Leu–Cys–OMe with the NHC gold chloride **6a** yields the (NHC)Au–S complexed amino acid and dipeptide derivatives **8** and **9**. The NHC gold(III) complexes **4c** and **6c** were characterised by single crystal X-ray analysis. All of the tested gold carbene complexes showed significant anti-tumor activity on the HeLa, HepG2 and HT-29 cancer cell lines. The best compounds show activity comparable to the well-known anti-cancer drug cisplatin. There seems to be no clear cut structure–activity relationship in the compounds tested, nor did we observe a dependence on the metal oxidation state or the different halide substituents. Given the ease of preparation, stability and high activity of the compounds described herein, it may be possible to design tumor-specific anti-cancer agents based on NHC gold amino acid conjugates in the future.

Introduction

In the last decade, research on N-heterocyclic carbenes (NHCs) has developed strongly with applications mainly in organometallic catalysis, metathesis and lately, medicinal applications, as documented in this issue.[1–3] Strong interest exists in late transition metal NHC complexes, such as the coin metals silver and gold. Synthesis of NHC gold complexes allowed the testing of their application in fields ranging from luminescent devices[4] to potential drug candidates.[5] Furthermore, important general features regarding the nature of the NHC–metal bond were revealed.[6]

It is widely accepted that the replacement of phosphine ligands by the isolobal NHC ligands frequently improves the properties of the new compounds for practical applications, which are water and air-stable and easier to handle. Moreover, the imidazolium salts as the ligand precursor can be functionalised with almost any substituent, a rarely accessible feature in phosphines.

In medicinal applications, the focus is mostly concentrated on cationic NHC gold complexes, which are known to possess anti-tumor activity. Cationic gold carbenes are lipophilic compounds which are able to induce mitochondrial membrane permeabilisation of rat liver mitochondria, as shown by Berners-Price *et al*.[7–9] Raubenheimer *et al.* reported a promising ferrocenyl bis(carbene) gold(I) complex with an IC_{50} value in the range of cisplatin against Jurkat, CoLo320 DM and MCF 7 cells.[10] Also, a few simple neutral NHC gold halide complexes with anti-proliferative activity have been described.[11] However, none of these compounds were ever functionalised with biomolecules.

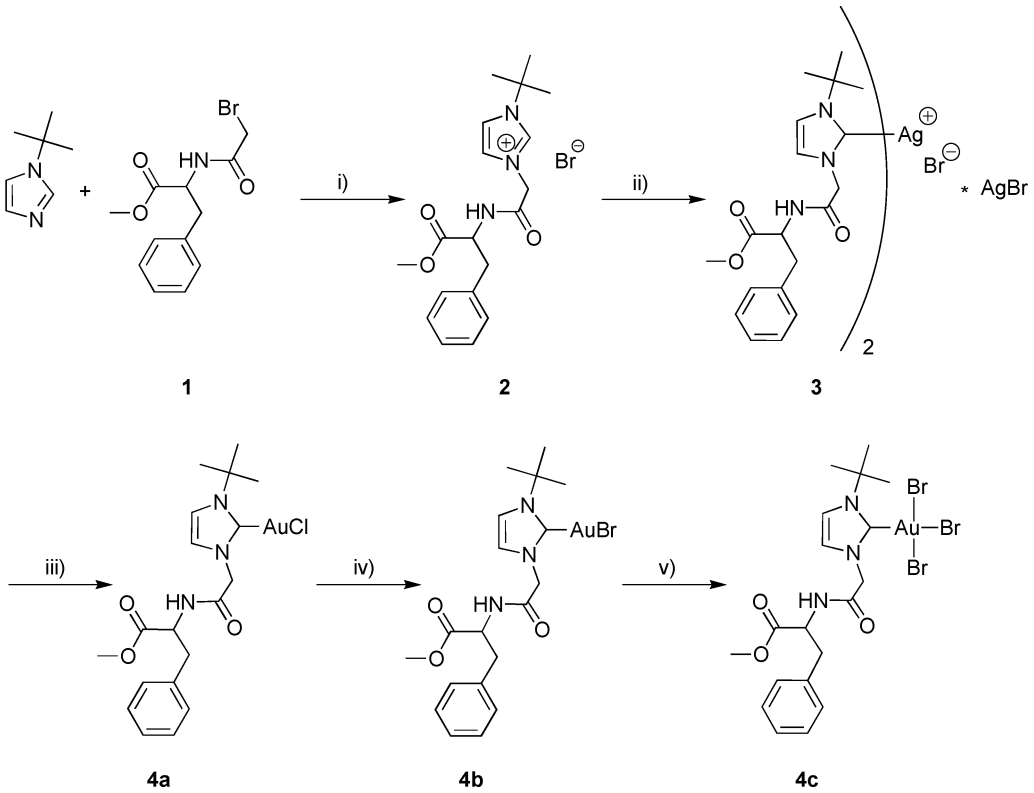
A small number of reports appeared in the literature, in which NHCs or NHC metal complexes were linked to biomolecules such as carbohydrates[12] and peptides.[13–15] In our group, we have synthesised a variety of organometal–peptide bioconjugates, mostly with metallocenes,[16–24] scorpionate[25] or cobalt–carbonyl complexes,[26] as well as with ruthenium NHC complexes.[27] The application of gold–phosphine peptide conjugates for the synthesis on solid phase has successfully been applied as well,[28] using the aurophilic behaviour of sulfur to couple a thiol-functionalised biomolecule to the gold centre.

Two examples of NHC gold conjugates with biomolecules were reported in the literature. The first is a carbene analogue of the anti-arthritic drug auranofin, which is a gold triethylphosphine complex coupled to 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolate as an ancillary ligand,[29] and the second a gold carbene complex of saccharin.[30] The ability of these compounds to act as potential chemotherapeutic agents due to their DNA-binding capacity is currently under study. To the best of our knowledge, no NHC gold conjugates with amino acids or peptides were reported to the present day.

Given the promise of NHC gold complexes for medicinal applications, we decided to extend our efforts in metal–bioconjugate systems to NHC gold complexes. In this paper, the synthesis of NHC gold(I) chloride and bromide, as well as the synthesis of NHC gold(III) bromide complexes are described, using symmetric or asymmetric NHC carbene precursors, where an amino acid is attached to a nitrogen atom of the imidazole moiety. Furthermore, NHC gold complexes, in which the metal atom is coordinated to the thiol group of cysteine are reported. Finally, a preliminary study of the cytotoxic activity of the NHC gold(I) and gold(III)

Lehrstuhl für Anorganische Chemie I - Bioanorganische Chemie, Fakultät für Chemie und Biochemie, Ruhr-Universität Bochum, Universitätsstrasse 150, D-44801 Bochum, Germany. E-mail: nils.metzler-nolte@rub.de; Fax: +49 (0)234 321 4378; Tel: +49 (0)234 322 8152

† CCDC reference numbers 725410 and 725411. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b906140a



Scheme 1 (i) THF, N_2, reflux, over night, (ii) CH_2Cl_2, Ag_2O, N_2, MS, over night, (iii) CH_2Cl_2, N_2, DMSAuCl, 6 h, (iv) acetone, LiBr 24 h, (v) Br_2, CH_2Cl_2, 3 h.

complexes, with halide ligands or thio-derivatives, has been undertaken and the results are reported.

Results and discussion

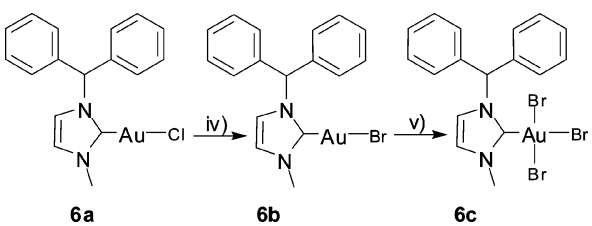
Syntheses

A set of neutral amino acid derived NHC gold complexes, namely 1-*tert*-butyl-3-(methylenecarbonyl-phenylalaninemethylester)-imidazol-2-ylidene gold halides, were designed to investigate their anti-tumor activity, and comparisons were made to their unfunctionalised neutral NHC gold analogues.

To exclude the formation of linear cationic bis(NHC) gold complexes, bulky *tert*-butyl substituents were placed on the nitrogen atoms of the imidazole ring. The imidazolium salt **2** was easily synthesised by reacting *tert*-butylimidazole with bromoacetyl-L-phenylalanine methylester **1**, which in turn was obtained using the mixed anhydride method where bromoacetic acid was first activated with isobutyl chloroformiate and *N*-methylmorpholine.[31] The imidazolium salt **2** was reacted with Ag_2O in CH_2Cl_2 to yield the Ag(I) bis(carbene) complex, which was then used as a transfer agent to synthesise the desired Au(I) complex in analogy to the reported procedure by Wang and Lin (Scheme 1).[32] The NHC silver complex **3** was obtained as a white, light-sensitive powder. Treatment of **3** with dimethylsulfide gold(I) chloride (Me_2SAuCl) in CH_2Cl_2 afforded the NHC gold(I) chloride **4a** as a white powder, along with a precipitate of AgBr, which was filtered off.

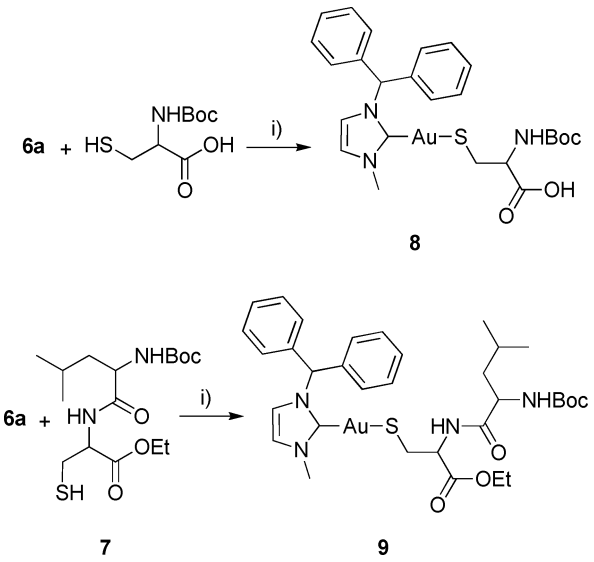
In order to achieve the halide exchange, the metathesis reaction described by Nolan was used without further modification,[33] yielding the desired NHC gold(I) bromide **4b**. The oxidation of this Au(I) complex to the desired Au(III) complex was performed with bromine in acetone at room temperature, resulting in the formation of **4c** as an orange powder.

For comparison, we also prepared symmetric NHC gold derivatives from the 1,3-di-*tert*-butyl imidazolium salt[34] ((^{t}Bu$_2$NHC)AuX, with X = Cl (**5a**), Br (**5b**), Br_3 (**5c**)),[29,30,35] as well as the chloro (1-methyl-3-diphenylmethane-imidazole-2-ylidene) gold(I) complex **6a**. Compound **6a** was synthesised with little variation of the literature procedure,[36] using Me_2SAuCl instead of tetrahydrothiophene gold(I) chloride (thtAuCl). The new NHC gold(I) bromide **6b** and NHC gold(III) bromide **6c** were obtained using the above described methods (Scheme 2). All prepared NHC gold complexes are air- and water-stable. Only **4a** shows a slight colour change when exposed to light, probably due to degradation.



Scheme 2 (iv) Acetone, LiBr 24 h, (v) Br_2, CH_2Cl_2, 3 h.

For peptide attachment to the NHC gold complexes *via* the gold atom, the dipeptide Boc–cysteine–leucine methylester **7** was prepared. The NHC gold(I) chloride **6a** was stirred with Boc–cysteine ethylester or **7** in CH_2Cl_2, containing NEt_3, to obtain the desired complexes **8** and **9** as white powders after filtration through Celite (Scheme 3).



Scheme 3 (i) NEt_3, CH_2Cl_2, N_2, over night.

NMR spectroscopy

The imidazolium salt **2** was fully characterised. As a typical spectroscopic feature, the acidic proton (NC*H*N) of **2** appears as a broad downfield shifted singlet at 9.2 ppm. The two vicinal protons of the imidazolium group show pseudo-triplets at 7.7 and 8.1 ppm (NC*H*=C*H*N), due to similar ^3J- and ^4J-coupling in deuterated DMSO, which is common for imidazolium salts.

The NHC silver(I) bromide **3** showed the absence of the acidic imidazole proton at 9.2 ppm due to complex formation. Further, the two remaining ring protons of the imidazole moiety were monitored as doublets at 7.1 and 7.3 ppm with a ^3J coupling constant of 2 Hz, in accordance with reported similar carbene complexes.[37] The ^{13}C NMR spectrum showed the expected silver-bound carbene signal at 180.1 ppm (N*C*(Ag)N).

The ^{13}C NMR of **4a** showed the gold–carbene (N*C*(Au)N) signal at 171.4 ppm. In contrast to **4a**, the carbene signal of **4b** appears 4.1 ppm downfield shifted at 175.1 ppm. This correlates with the lower Lewis acidity of the gold center, probably afforded by the lower electronegativity of bromine compared to chloride.[35] The ^{13}C NMR resonance of **4c** is shifted upfield by 39.3 ppm compared to the NHC gold(I) bromide **4b**. As expected, this shift arises from the increased acidity of Au(III) due to an increase in the oxidation state.[30]

Noteworthy for the complex series **6** is the downfield shift of the *C*(Au) resonance of the NHC gold(I) bromide **6b** from 172.9 ppm (NHC gold(I) chloride **6a**) to 176.3 ppm. In case of the NHC gold(III) bromide **6c**, a highfield shift of −35.0 ppm to 141.3 ppm could be observed. After functionalising **6a** with the cysteine-derivatives, the signal of the thiol proton in the ^{1}H NMR disappeared, proving the complexation. The ^{13}C NMR of **8** shows a further downfield shift of the *C*(Au) carbene carbon atom from 172.9 ppm to 185.4 ppm, and a shift to 185.1 ppm in the case of **9**.[35]

Single crystal X-ray structural studies†

Crystals of **4c** could be obtained by covering CH_2Cl_2 with a layer of pentane. The crystal structure of this amino acid derivative is shown in Fig. 1. Complex **4c** has a four-coordinate gold atom, in a square-planar environment, as expected for d^8 metals. The C(1)–Au(1)–Br(1) and Br(2)–Au(1)–Br(3) bonds are nearly linear, with angles of 176.1(4) and 177.84(7)°. The C(1)–Au(1) distance is 2.03(2) Å, which is in accordance with published NHC–Au(III) bromide complexes.[38] The Br–Au distances were found to be between 2.408(2) and 2.443(2) Å, with the Au–Br bond in *trans* being slightly longer than the other Au–Br bonds (Fig. 1).

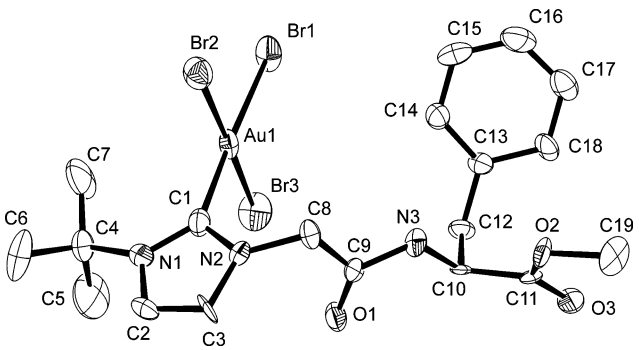


Fig. 1 ORTEP plot of **4c** with thermal ellipsoids drawn at 50% probability level. Hydrogen atoms were omitted for clarity. Selected bond lengths (Å) and angles (°): N1–C1 1.35(2), N2–C1 1.32(2), Au1–C1 2.02(2), Au1–Br3 2.408(2), Au1–Br2 2.418(2), Au1–Br1 2.443(2), C1–Au1–Br1 176.1(4), C1–Au1–Br2 88.0(5), C1–Au1–Br3 89.9(5), Br1–Au1–Br2 90.76(7), Br1–Au1–Br3 91.39(8), Br2–Au1–Br3 177.84(7).

An X-ray structure of **6c** could be obtained using the same method. The C(1)–Au(1) bond shows a distance of 2.016(8) Å and the Br–Au distances were as well found in the region of 2.4151(8) to 2.39(1) Å, with the longest Au–Br bond again being opposite to the carbene ligand. The angles between C(1)–Au(1)–Br(4) and Br(2)–Au(1)–Br(3) are also between 178.7(2)° and 176.25(4)° (Fig. 2).

Biological studies

In vitro cytotoxicity assays were performed to obtain an insight into the anti-tumor activity of the neutral gold carbenes. All NHC gold(I) chloride and NHC gold(III) bromide complexes, as well as complexes **8** and **9** were screened against the human cell lines HeLa (human cervix epitheloid carcinoma cell line), HT-29 (human caucasian colon adenocarcinoma grade II cell line) and HepG2 (human hepatocellular liver carcinoma cell line). Cell viability, which correlates with the metabolic activity of a cell, was determined by the resazurin assay.[39] Additionally, absolute cell numbers were determined by the crystal violet (CV) assay,[40] which can be applied after elution of resazurin. Both assays gave similar results (Table 1).

A known number of cells were exposed to increasing concentrations of the gold complexes on a 96-well tissue culture plate and incubated for a given period of time. Due to the poor solubility of the substances, DMSO stock solutions had to be used. Because of

Table 1 Cytotoxicity expressed as IC_{50} values (μM)

	HeLa		HepG2		HT-29	
	CV[a]	Resazurin	CV	Resazurin	CV	Resazurin
4a	45.5 ± 4.8	52.7 ± 5.0	61.4 ± 7.4	71.3 ± 5.9	63.8 ± 6.7	58.9 ± 4.3
4c	41.3 ± 10.4	36.2 ± 12.9	n.d.[b]	n.d.[b]	125.8 ± 49.7	282.5 ± 41.8
5a	10.0 ± 3.7	12.2 ± 5.1	119.0 ± 17.4	186.9 ± 34.8	37.8 ± 11.4	62.7 ±10.3
5c	4.5 ± 0.9	8.0 ± 1.3	37.9 ± 11.5	n.d.[b]	13.5 ± 5.8	48.5 ± 6.2
6a	3.3 ± 0.9	5.8 ± 1.9	4.7 ± 2.5	n.d.[b]	2.8 ± 1.7	5.2 ± 3.0
6c	27.3 ± 4.8	12.4 ± 3.0	28.5 ± 5.3	65.3 ± 6.2	12.7 ± 1.2	16.2 ± 1.6
8	3.4 ± 1.3	8.3 ± 1.4	15.2 ± 1.7	20.4 ± 0.9	10.5 ± 1.9	16.9 ± 1.7
9	17.3 ± 3	29.4 ± 1.8	28.1 ± 4.5	30.0 ± 2.6	26.8 ± 2.1	34.6 ± 1.8
Cisplatin	3.5 ± 2.5	7.3 ± 0.4	1.1 ± 0.1	0.9 ± 0.1	7.9 ± 1.2	12.3 ± 2.1

[a] CV = crystal violet. [b] n.d. = not determinable.

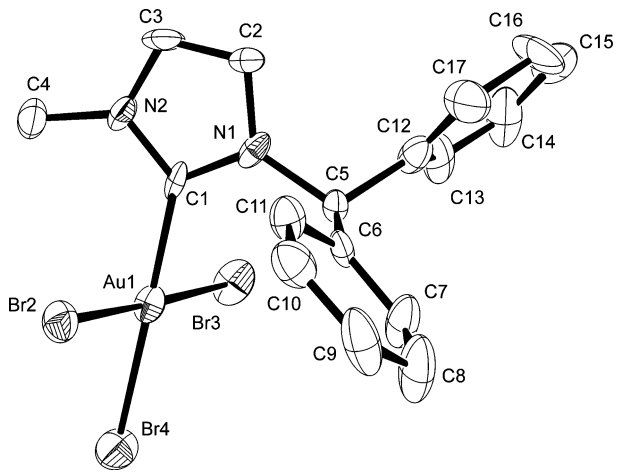


Fig. 2 ORTEP plot of **6c** with thermal ellipsoids drawn at 50% probability level. Hydrogen atoms were omitted for clarity. Selected bond lengths (Å) and angles (°): N1–C1 1.350(9), N2–C1 1.317(8), Au1–C1 2.016(8), Au1–Br2 2.4151(8), Au1–Br3 2.4220(9), Au1–Br4 2.4399(10), C1–Au1–Br4 178.71(19), C1–Au1–Br2 87.43(18), Br2–Au1–Br4 91.29(3), Br3–Au1–Br4 91.95(3), C1–Au1–Br3 89.34(18), Br2–Au1–Br3 176.25(4).

the cytotoxicity of DMSO in higher concentrations, final DMSO concentrations were limited to 0.5% in all samples. In Table 1, IC_{50} data are reported. Drug-free solvent controls were carried out, and data for cisplatin, a well-established anti-cancer drug, were included for comparison.[41]

Generally, all compounds show at least promising anti-proliferaitve activity, with IC_{50} values between *ca.* 100 to very low μM values. HeLa cells were the most sensitive, while the HT-29 cells were the least sensitive cells to the carbene complexes. Compounds **6a** and **8** showed the best activity, with consistently low μM values. The effect of the oxidation state of the gold atom could not be clarified. For the amino acid labelled gold complexes **4a** and **4c**, a decrease in activity could be detected for HepG2 and HT-29 cells. The opposite trend of anti-tumor activity was observed for the complexes **5a** and **5c**, where the gold(III) complex showed higher activity. In the cases of **6a** and **6c**, the anti-proliferative activity was higher for the gold(I) complex, which again opposes the results for **5a** and **5c**. As **6a** proved to be the most active gold complex, it was chosen for the syntheses of **8** and **9**.

For the amino acid and peptide conjugates **8** and **9**, an increase of activity compared to **6a** could not be detected. Compound **8**, with the additional amino acid Boc–cysteine, has IC_{50} values almost comparable to **6a**. Compound **9**, where the dipeptide is coupled to the gold complex, already exhibits a decrease of anti-tumor activity. Finally, of all compounds tested herein, compound **5a** exhibits the most pronounced cell line dependence, with excellent activity in HeLa cells, but more than 10-fold decreased activity in the HepG2 cell line.

Conclusions

Herein we have reported the synthesis of new NHC gold(I) and NHC gold(III) halide and amino acid complexes. Two of those gold(III) complexes with NHC ligands were characterised by single crystal X-ray analysis. All of the tested gold carbene complexes showed acceptable anti-tumor activity compared to the well-known anti-cancer drug cisplatin. Increased activity of the NHC gold(III) bromides **4c** and **5c** on some cell lines cannot be due to the presence of bromine, because the NHC gold(III) complex **6c** is 3–8 times less active than the chloride **6a** in all tested cell lines.

Nevertheless, introducing an amino acid to the imidazole moiety of the gold complexes leads to a decrease in biological activity. On the other hand, amino acid transporters are over-expressed on some tumor cell lines. By hijacking such amino acid transporters, these amino acid derivatives could be specific for tumor cells over normal, healthy cells.[42] In the case of the NHC gold complexes which were functionalised *via* a cysteine thiol group, a decrease of activity was observed for the dipeptide conjugate but not for the simple amino acid derivative. Comparing **6a**, **8** and **9**, the results show that the $[NHC–Au]^+$-fragment alone cannot be responsible for the cytotoxicity. Whether differences in cytotoxicity are due to differential uptake or different intracellular interactions requires further investigation. Clearly, the combination of very stable NHC metal complexes with biomolecule functionalisation holds great promise for further investigations.

Experimental section

General remarks

All reagents were purchased from commercial sources and used as received. L-Amino acids were purchased from IRIS Biotech or Novabiochem. Antibiotics, colour dyes and cell culture media

were purchased from PAA, Riedel-de-Haën and Sigma-Aldrich. Solvents were distilled over $CaCl_2$ (CH_2Cl_2) or taken from the solvent purification system MBraun SPS (THF). Glassware was used oven dry. NMR spectra were recorded on a Bruker DPX 250 (^{1}H at 250 MHz) spectrometer. The NMR chemical shifts (δ) are reported in ppm relative to the residual proton chemical shifts of the deuterated solvent set relative to external TMS. Microanalysis was performed on a Analytik Jena Multi EA 3100. Electrospray ionisation mass spectra (ESI) were recorded with a Bruker Esquire 6000 instrument. FAB mass spectra were recorded on a Finnigan VG Autospec (glycerol or 3-nitrobenzylalcohol (NBA) as matrix). Di-*tert*-buylimidazolium chloride,[34] *tert*-butylimidazol,[43] chloro-(1,3,-di-*tert*-butylimidazol-2-ylidene) gold(I) **5a**,[29] bromo-(1,3,-di-*tert*-butylimidazol-2-ylidene) gold(III) **5b**,[35] trisbromo-(1,3,-di-*tert*-butylimidazol-2-ylidene) gold(III) **5c**,[33] 1-methyl-3-diphenylmethyl-imidazolium chloride[36] and chloro-(1-methyl-3-diphenylmethyl-imidazol-2-ylidene) gold(I)[36] **6a** and (Me_2S)AuCl,[44] were prepared according to the literature. X-Ray data were collected using a Bruker-axs SMART 1000 CCD diffractometer. Structure solution was performed with direct methods (SHELXS-97),[45] and refined against F^2 with all measured reflections (SHELXL-97,[45] Platon-Squeeze).[46]

Methyl 2-(2-bromoacetamido)-3-phenyl-methylpropanoate (1)

To a stirred solution of bromoacetic acid (1.4 g, 10 mmol) in THF was added *N*-methylmorpholine (1.12 mL, 10 mmol), followed by the addition of isobutyl chloroformiate (1.32 mL, 10 mmol), resulting in a precipitation of a white solid. In a second flask L-phenylalanine-methylester hydrochloride (2.16 g, 10 mmol) was suspended in THF (50 mL) containing triethylamine (1.38 mL, 10 mmol). Both suspensions were mixed and stirred for 1 h at room temperature. After removal of the white precipitates by filtration, the solvent was removed under reduced pressure and the residual oil dissolved in $CHCl_3$ (100 mL). The solution was washed with H_2O (50 mL) and the aqueous solution was back-extracted with $CHCl_3$ (3 × 50 mL). The combined $CHCl_3$ solutions were dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the product as a white solid (2.51 g, 84%). ^{1}H NMR (DMSO-d_6, 200 MHz, 25 °C): δ 7.34–7.16 (3H, m, $H_{Ar,Phe}$), 7.09–7.05 (2H, m, $H_{Ar,Phe}$), 6.83 (1H, d, $^3J_{H,H}$ = 7.1 Hz, N*H*), 4.87–4.72 (1H, m, $C_{\alpha,Phe}H$), 3.79 (1H, d, $^2J_{H,H}$ = 1.4 Hz, Br–CH_2–CO), 3.68 (3H, s, CO_2CH_3), 3.10 (2H, dq, $^2J_{H,H}$ = 13.75 Hz, $^3J_{H,H}$ = 7.25 Hz, $C_{\beta,Phe}H$). ^{13}C NMR (DMSO-d_6, 50 MHz, 25 °C): δ 171.3 (CO_2CH_3), 165.1 (*C*O), 135.3 ($C_{q,Phe}$), 129.2 ($C_{Ar,Phe}$), 128.6 ($C_{Ar,Phe}$), 127.3 ($C_{Ar,Phe}$), 53.7 ($C_{\alpha,Phe}$), 52.5 (CO_2CH_3), 37.6 ($C_{\beta,Phe}$), 28.6 (Br–*C*H_2–CO). MS (FAB$^+$): m/z = 300.0, 302.0 [M$^+$]. Anal. calc. for $C_{12}H_{14}BrNO_3$ (m = 300.15): C, 48.02; H, 4.70; N, 4.67. Found: C, 50.44; H, 5.12; N, 4.74.

(1-*tert*-Butyl-3-(2-(2-acetamido)-3-phenylalanine methylester) imidazolium bromide (2)

To a solution of *tert*-butylimidazol (372 mg, 3 mmol) in dry THF (10 mL) was added dropwise a solution of **1** (897 mg, 3 mmol) in THF (10 mL). After complete addition, the mixture was refluxed for 24 h, during which the solution turned pale orange. The solvent was removed under reduced pressure and the residue was washed with pentane and dried *in vacuo* to give a white-orange hygroscopic powder (580 mg, 46%). ^{1}H NMR (DMSO-d_6, 250 MHz, 25 °C): δ 9.37 (1H, br. s, N–C*H*=N), 9.18 (1H, d, $^3J_{H,H}$ = 7.41 Hz, NH_{Phe}), 8.05 (1H, t, $^3J_{H,H}$ = 1.77 Hz, N–C*H*=CH–N), 7.69 (1H, t, $^3J_{H,H}$ = 1.77 Hz, N–CH=C*H*–N), 7.37–7.14 (5H, m, $C_{Ar,Phe}H$), 5.1 (2H, s, N–CH_2–CO), 4.60–4.48 (1H, m, $C_{\alpha,Phe}H$), 3.62 (3H, s, CO_2CH_3), 3.02 (2H, dq, $^2J_{H,H}$ = 13.75 Hz, $^3J_{H,H}$ = 7.41 Hz, $C_{\beta,Phe}H$), 1.60 (9H, s, N–C(CH_3)$_3$). ^{13}C NMR (DMSO-d_6, 63 MHz, 25 °C): δ 171.4 (CO_2CH_3), 165.2 (*C*O), 136.7 (N–*C*H=N), 135.3 ($C_{q,Phe}$), 129.2 ($C_{Ar,Phe}$), 128.6 ($C_{Ar,Phe}$), 127.3 ($C_{Ar,Phe}$), 123.9 (N–*C*H=CH–N), 119.6 (N–CH=*C*H–N), 59.6 (N–*C*(CH_3)$_3$), 54.2 (N–*C*H_2–CO), 51.8 ($C_{\alpha,Phe}$), 50.2 (CO_2CH_3), 36.8 ($C_{\beta,Phe}$), 29.0 (N–C(*C*H_3)$_3$). MS (ESI$^+$): m/z = 344.16 [M – Br]$^+$. Anal. calc. for $C_{19}H_{26}BrN_3O_3$ (m = 424.34): C, 53.78; H, 6.18; N, 9.90. Found: C, 54.39; H, 5.85; N, 9.56.

{[1-*tert*-Butyl-3-(2-(2-acetamido)-3-phenylalanine methylester) imidazol-2-ylidene)] AgBr}$_2$ (3)

To a dried Schlenk tube charged with molecular sieves (4 Å, 50 mg) was added **2** (258 mg, 0.61 mmol) and Ag_2O (74 mg, 0.32 mmol). The mixture was backflashed three times with N_2 and then dry CH_2Cl_2 was added (30 mL). The flask was closed and shaken over night in the dark. The solution was filtered through Celite and the solvent was removed under reduced pressure. The residue was recrystallised from THF–hexane (10 mL : 30 mL) at 4 °C to yield a white powder (173 mg, 54%). ^{1}H NMR (CD_3CN, 250 MHz, 25 °C): δ = 7.32 (1H, d, $^3J_{H,H}$ = 1.7 Hz, N–C*H*=CH–N), 7.27–7.19 (6H, m, $C_{Ar,Phe}H$, NH_{Phe}), 7.14 (1H, d, $^3J_{H,H}$ = 1.7 Hz, N–CH=C*H*–N), 4.87 (2H, s, N–CH_2–CO), 4.71–4.58 (1H, m, $C_{\alpha,Phe}H$), 3.62 (3H, s, CO_2CH_3), 3.05 (2H, dq, $^2J_{H,H}$ = 13.80 Hz, $^3J_{H,H}$ = 6.87 Hz, $C_{\beta,Phe}H$), 1.68 (9H, s, N–C(CH_3)$_3$). ^{13}C NMR (CD_3CN, 63 MHz, 25 °C): δ 180.1 (N*C*N), 167.8 (CO_2CH_3), 156.4 (*C*ONH), 138.1 ($C_{q,Phe}$), 129.9 ($C_{Ar,Phe}$), 129.5 ($C_{Ar,Phe}$), 127.8 ($C_{Ar,Phe}$), 122.5 (N–*C*H=CH–N), 120.3 (N–CH=*C*H–N), 58.8 (N–*C*(CH_3)$_3$), 55.3 (N–*C*H_2–CO), 54.4 ($C_{\alpha,Phe}$), 52.9 (CO_2CH_3), 38.2 ($C_{\beta,Phe}$), 29.8 (N–C(*C*H_3)$_3$). MS (Fab$^+$): 794.4 [M – $AgBr_2$]$^+$ (100%). Anal. calc. for $C_{38}H_{50}Ag_2Br_2N_6O_6$ (m = 1062.39): C, 42.96; H 4.74; N, 7.91. Found: C, 44.45; H, 5.19; N, 8.40.

(1-*tert*-Butyl-3-(2-(2-acetamido)-3-phenylalanine methylester) imidazol-2-ylidene) gold(I) chloride (4a)

To a dry Schlenk tube was added **3** (258 mg, 0.09 mmol) and (Me_2S)AuCl (54 mg, 0.18 mmol). The mixture was backflashed three times with N_2 and then dry CH_2Cl_2 was added (20 mL). The suspension was stirred over night in the dark, during which the colour changed to grey-violet. The mixture was filtered through Celite and the solvent was removed under reduced pressure. The residue was recrystallised from CH_2Cl_2–pentane (1 : 5) at –20 °C to yield **4a** as a white powder (215 mg, 74%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.36–7.08 (6H, m, N–C*H*=CH–N, $C_{Ar,Phe}H$), 7.01 (1H, d, $^3J_{H,H}$ = 2.0 Hz, N–CH=C*H*–N), 6.44 (1H, d, $^3J_{H,H}$ = 7.0 Hz, NH_{Phe}), 4.95 (1H, d, $^3J_{H,H}$ = 6.1 Hz, N–CH_2–CO), 4.87–4.79 (1H, m, $C_{\alpha,Phe}H$), 3.72 (3H, s, CO_2CH_3), 3.12 (2H, dq, $^2J_{H,H}$ = 13.80 Hz, $^3J_{H,H}$ = 5.8 Hz, $C_{\beta,Phe}H$), 1.82 (9H, s, N–C(CH_3)$_3$). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 172.0 (CO_2CH_3), 171.4 (N*C*N), 165.8 (*C*ONH), 136.3 ($C_{q,Phe}$), 129.9 ($C_{Ar,Phe}$), 129.2 ($C_{Ar,Phe}$), 127.6 ($C_{Ar,Phe}$), 122.7 (N–*C*H=CH–N), 119.5 (N–CH=*C*H–N), 59.7 (N–*C*(CH_3)$_3$, 55.4 (N–*C*H_2–CO), 54.1 ($C_{\alpha,Phe}$), 53.0 (CO_2CH_3), 38.2

($C_{\beta,Phe}$), 31.8 (N–C(CH_3)$_3$). MS (FAB$^+$): 540.1 [M – Cl]$^+$ (95%), 484.1 [M – Cl-*tert*-butyl + H]$^+$ (20), 344.2 [M – AuCl]$^+$ (100). Anal. calc. for $C_{19}H_{25}AuClN_3O_3$ (m = 575.85): C, 39.63; H, 4.38; N 7.30. Found: C, 39.9; H, 4.21; N, 7.34.

(1-*tert*-Butyl-3-(2-(2-acetamido)-3-phenylalanine methylester) imidazol-2-ylidene) gold(I) bromide (4b)

Compound **4a** (215 mg, 0.37 mmol) was dissolved in acetone (1 mL) and LiBr (273 mg, 3.18 mmol) was added. The reaction was stirred for 24 h in the dark. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (2 mL) and $MgSO_4$ was added. The salts were filtered off using a short plug of silica and the solvent was removed under reduced pressure. The residue was recrystallised from CH_2Cl_2–pentane at –20 °C to yield **4b** as a white powder (70 mg, 30%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.36–7.12 (6H, m, N–CH=CH–N, $C_{Ar,Phe}H$), 7.05 (1H, d, $^3J_{H,H}$ = 2.1 Hz, N–CH=CH–N), 6.55 (1H, d, $^3J_{H,H}$ = 7.0 Hz, NH_{Phe}), 4.99 (1H, d, $^3J_{H,H}$ = 1.9 Hz, N–CH_2–CO), 4.88–4.81 (1H, m, $C_{\alpha,Phe}H$), 3.72 (3H, s, CO_2CH_3), 3.21–3.01 (2H, m, $C_{\beta,Phe}H$), 1.81 (9H, s, N–C(CH_3)$_3$). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 175.1 (NCN), 172.0 (CO_2CH_3), 166.2 (CONH), 136.2 ($C_{q,Phe}$), 129.9 ($C_{Ar,Phe}$), 129.2 ($C_{Ar,Phe}$), 127.6 ($C_{Ar,Phe}$), 120.7 (N–CH=CH–N), 119.5 (N–CH=CH–N), 59.8 (N–C(CH$_3$)$_3$), 55.3 (N–CH$_2$–CO), 54.1 ($C_{\alpha,Phe}$), 53.1 (CO_2CH_3), 38.1 ($C_{\beta,Phe}$), 31.9 (N–C(CH_3)$_3$). MS (FAB$^+$): 540.0 [M – Br]$^+$ (100%), 344.2 [M – AuBr]$^+$ (30). Anal. calc. for $C_{19}H_{25}AuBrN_3O_3$ (m = 620.30): C, 36.79; H, 4.06; N 6.77. Found: C, 36.11; H, 4.24; N 6.98.

(1-*tert*-Butyl-3-(2-(2-acetamido)-3-phenylalanine methylester) imidazol-2-ylidene) gold(III) bromide (4c)

To a solution of **4b** (36 mg, 58 μmol) in CH_2Cl_2 (2 mL) was added bromine (11 mg, 70 μmol). The reaction was stirred for 3 h in the dark and the solvent was removed under reduced pressure, as well as the excess of bromine. The residue was recrystallised from CH_2Cl_2–pentane (1 : 5) at –20 °C to yield **4c** as an orange powder (41 mg, 90%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.40–7.14 (7H, m, N–CH=CH–N, N–CH=CH–N, $C_{Ar,Phe}H$), 6.62 (1H, d, $^3J_{H,H}$ = 7.0 Hz, NH_{Phe}), 5.09–4.87 (3H, q + m, N–CH_2–CO, $C_{\alpha,Phe}H$), 3.74 (3H, s, CO_2CH_3), 3.22–3.01 (2H, m, $C_{\beta,Phe}H$), 1.85 (9H, s, N–C(CH_3)$_3$). ^{13}C NMR (CD_3CN, 63 MHz, 25 °C): δ 172.1 (CO_2CH_3), 165.1 (CONH), 137.4 ($C_{q,Phe}$), 135.8 (NCN), 130.6 ($C_{Ar,Phe}$), 129.6 ($C_{Ar,Phe}$), 128.0 ($C_{Ar,Phe}$), 127.1 (N–CH=CH–N), 124.0 (N–CH=CH–N), 62.7 (N–C(CH$_3$)$_3$), 55.0 (N–CH$_2$–CO), 53.7 ($C_{\alpha,Phe}$), 52.9 (CO_2CH_3), 38.3 ($C_{\beta,Phe}$), 31.7 (N–C(CH_3)$_3$). MS (FAB$^+$): 540.0 [M – 3Br]$^+$ (100%), 344.1 [M – AuBr]$^+$ (20). Anal. calc. for $C_{19}H_{25}AuBr_3N_3O_3$ (m = 780.10): C, 29.25; H, 3.23; N, 5.39. Found: C, 28.72, H, 3.45; N, 6.00.

(1-Diphenylmethyl-3-(methyl imdazole-2-ylidene) gold(I) bromide (6b)

Compound **6a** (35 mg, 73 μmol) was dissolved in acetone (1 mL) and LiBr (53 mg, 620 μmol) was added. The reaction was stirred for 24 h in the dark. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (2 mL) and $MgSO_4$ was added. The salts were filtered off through a short plug of silica and the solvent was removed under reduced pressure. The residue was recrystallised from CH_2Cl_2–pentane at –20 °C to yield **6b** as a white powder (25 mg, 65%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.40–7.35 (6H, m, $C_{Ar}H$), 7.30 (1H, s, NCH(Ph)$_2$), 7.19–7.13 (4H, m, $C_{Ar}H$), 6.97 (1H, d, $^3J_{H,H}$ = 1.8 Hz, N–CH=CH–N), 6.83 (1H, d, $^3J_{H,H}$ = 1.8 Hz, N–CH=CH–N), 3.85 (3H, s, N–CH_3). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 176.3 (NCN), 138.8 ($C_{q,Phe}$), 129.4 ($C_{Ar,Phe}$), 129.1 ($C_{Ar,Phe}$), 128.9 ($C_{Ar,Phe}$), 122.5 (N–CH=CH–N), 120.2 (N–CH=CH–N), 68.5 (N–CH(Ph)$_2$), 49.0 (N–CH$_3$). MS (Fab$^+$): 693.1 [M + carbene]$^+$ (60%), 445.0 [M – Br]$^+$ (25). Anal. calc. for $C_{17}H_{16}AuBrN_2$ (m = 525.20): C, 38.88; H, 3.07; N, 5.33. Found: C, 38.10; H, 3.24; N, 4.99.

(1-Diphenylmethyl-3-(methyl imdazol-2-ylidene) gold(III) bromide (6c)

To a solution of **6b** (15 mg, 28.6 μmol) in CH_2Cl_2 (2 mL) was added bromine (4 mg, 34.4 μmol). The reaction was stirred for 3 h in the dark and the solvent was removed under reduced pressure, as well as the excess of bromine. The residue was recrystallised from CH_2Cl_2–pentane (1 : 5) at –20 °C to yield **6c** as an orange powder (10 mg, 51%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.44–7.39 (6H, m, $C_{Ar}H$), 7.28–7.18 (5H, m, NCH(Ph)$_2$, $C_{Ar}H$), 7.16 (1H, d, $^3J_{H,H}$ = 2.0 Hz, N–CH=CH–N), 6.95 (1H, d, $^3J_{H,H}$ = 2.0 Hz, N–CH=CH–N), 3.92 (3H, s, N–CH_3). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 141.3 (NCN), 137.0 ($C_{q,Phe}$), 129.6 ($C_{Ar,Phe}$), 129.6 ($C_{Ar,Phe}$), 129.3 ($C_{Ar,Phe}$), 125.1 (N–CH=CH–N), 123.2 (N–CH=CH–N), 68.5 (N–CH(Ph)$_2$), 38.9 (N–CH$_3$). MS (Fab$^+$): 445.0 [M – 3Br]$^+$ (100%). Anal. calc. for $C_{17}H_{16}AuBr_3N_2$ (m = 685.01): C, 29.81; H, 2.35; N, 4.09. Found: C, 27.93; H, 2.37; N, 3.50.

Boc–leucine–cysteine–OEt (7)

To a stirred solution of Boc–leucine (0.72 g, 2 mmol) in THF (20 mL) was added *N*-methylmorpholine (0.22 mL, 2 mmol) followed by the addition of isobutyl chloroformiate (0.26 mL, 2 mmol), resulting in a precipitation of a white solid. In a second flask, cysteine ethylester hydrochloride (0.37 g, 2 mmol) was suspended in THF (20 mL) containing triethylamine (0.28 mL, 2 mmol). Both suspensions were mixed and stirred for 1 h at room temperature. After removal of the white precipitates by filtration, the solvent was removed under reduced pressure and the residual oil dissolved in $CHCl_3$ (50 mL). The solution was washed with water (30 mL) and the aqueous solution was back-extracted with $CHCl_3$ (3 × 30 mL). The combined $CHCl_3$ solutions were dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the product as a white solid (0.58 g, 80%). ^{1}H NMR (DMSO-d_6, 250 MHz, 25 °C): δ 8.17 (1H, d, $^3J_{H,H}$ = 7.8 Hz, NH_{Leu}), 6.93 (1H, d, $^3J_{H,H}$ = 8.2 Hz, NHBoc), 4.55–4.39 (1H, m, $C_{\alpha,Cys}H$), 4.14–4.09 (3H, m, CO_2–CH_2–CH_3, $C_{\alpha,Leu}H$), 2.91–2.65 (2H, m, $C_{\beta,Cys}H$), 1.68–1.52 (3H, m, $C_{\beta,Leu}H$, $C_{\gamma,Leu}H$), 1.43 (10H, s, Boc, $S_{Cys}H$), 1.30 (3H, t, $^3J_{H,H}$ = 6.8 Hz, CO_2–CH_2–CH_3), 0.86 (6H, dd, $^3J_{H,H}$ = 6.8 Hz, $^2J_{H,H}$ = 4.1 Hz, $C_{\delta,Leu}H$). ^{13}C NMR (DMSO-d_6, 63 MHz, 25 °C): δ 172.9 (CO_2Et), 170.1 (CO_{Leu}), 155.4 (CO_{Boc}), 78.1 (C(CH$_3$)$_3$), 60.9 (CO_2–CH_2–CH$_3$), 54.0 ($C_{\alpha,Cys}$), 52.4 ($C_{\alpha,Leu}$), 40.6 ($C_{\beta,Leu}$), 27.8 (C(CH_3)$_3$), 25.1 ($C_{\beta,Cys}$), 24.0, 22.6 ($C_{\delta,Leu}$), 21.3 ($C_{\gamma,Leu}$), 14.0 (CO_2–CH_2–CH_3). MS (ESI$^+$): 385.13 [M + Na]$^+$, 334.14 [M – C_2H_5]$^+$, 307.10 [M – *tert*-butyl]$^+$. Anal. calc. for $C_{16}H_{30}N_2O_5S$ (m = 362.49): C, 53.01; H, 8.34; N, 7.73; S, 8.85. Found: C, 53.19; H, 8.36; N, 7.67; S, 8.88.

(1-Diphenylmethyl-3-(methyl imdazol-2-ylidene) gold(I) Boc–cysteine (8)

To a solution of **6a** (40 mg, 83 μmol) and Boc–cysteine (18.4 mg, 3 μmol) in dry CH_2Cl_2 (5 mL) was added triethylamine (13 μL, 91.7 μmol) and stirred over night. The mixture was filtered through Celite and recrystallised from CH_2Cl_2–pentane (1 : 5) at –20 °C to yield **8** as a white powder (33 mg, 60%). ^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.41–6.76 (15H, m, NCH(*Ph*)$_2$, N*H*Boc, NH_{Cys}, N–C*H*(PH)$_2$, N–CH=C*H*–N, N–C*H*=CH–N), 4.40–4.26 (1H, m, C$_{\alpha,Cys}H$), 3.86 (2H, m, C$_{\beta,Cys}H$), 3.04 (3H, s, N–CH_3), 1.37 (9H, s, C(CH_3)$_3$). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 185.4 (N*C*N), 174.1 (*C*O$_2$H), 155.8 (*C*O$_{Boc}$), 139.1 (*C*$_{q,Ar}$), 129.5 (*C*$_{Ar}$), 129.3 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 122.6 (N–*C*H=CH–N), 120.0 (N–CH=*C*H–N), 79.3 (*C*(CH$_3$)$_3$), 68.36 (*C*H(Phe)$_2$), 57.6 (*C*$_{\beta,Cys}$), 46.2 (*C*$_{\alpha,Cys}$), 38.7 (N–*C*H$_3$), 28.6 (C(*C*H$_3$)$_3$). MS (ESI$^-$, acetonitrile): 664.03 [M – H]$^-$. Anal. calc. for $C_{25}H_{30}AuN_3O_4S$ (m = 665.56): C, 45.12; H, 4.54; N, 6.31; S, 4.82. Found: C, 46.02; H, 5.89; N, 6.62; S, 3.84.

(1-Diphenylmethyl-3-(methyl imdazol-2-ylidene) gold(I) cysteine-leucine ethylester (9)

To a solution of **6a** (40 mg, 83 μmol) and **7** (31.5 mg, 87 μmol) in dry CH_2Cl_2 (5 mL) was added triethylamine (13 μL, 91.7 μmol) and stirred over night. The mixture was filtered through Celite and recrystallised from CH_2Cl_2–pentane (1 : 5) at –20 °C to yield **9** as a white powder (17 mg, 25%).^{1}H NMR (CD_2Cl_2, 250 MHz, 25 °C): δ 7.47–7.04 (13H, m, NCH(*Ph*)$_2$, N*H*Boc, NH_{Cys}, N–C*H*(PH)$_2$), 6.97 (1H, d, $^3J_{H,H}$ = 1.9 Hz, N–C*H*=CH–N), 6.81 (1H, d, $^3J_{H,H}$ = 1.9 Hz, N–CH=C*H*–N), 4.61–4.50 (1H, m, C$_{\alpha,Cys}H$), 4.22–3.97 (3H, m, C$_{\alpha,Leu}H$, CO$_2$–CH_2–CH$_3$), 3.84 (3H, s, N–CH_3), 3.35–3.12 (2H, m, C$_{\beta,Cys}H$), 1.75–1.56 (3H, m, C$_{\gamma,Leu}H$, C$_{\beta,Leu}H$), 1.40 (9H, s, C(CH_3)$_3$), 1.36 (3H, t, $^3J_{H,H}$ = 7.3 Hz, CO$_2$–CH$_2$–CH_3), 0.88 (6H, dd, $^3J_{H,H}$ = 6.1 Hz, $^2J_{H,H}$ = 10.5 Hz, C$_{\delta,Leu}H$). ^{13}C NMR (CD_2Cl_2, 63 MHz, 25 °C): δ 185.1 (N*C*N), 172.1 (*C*O$_2$Et), 171.0 (*C*O$_{Leu}$), 155.3 (*C*O$_{Boc}$), 138.8 (*C*$_{q,Ar}$), 129.9 (*C*$_{Ar}$), 128.9 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 122.2 (N–*C*H=CH–N), 119.6 (N–CH=*C*H–N), 79.4 (*C*(CH$_3$)$_3$), 68.3 (*C*H(Phe)$_2$), 68.0 (CO$_2$–*C*H$_2$–CH$_3$), 55.0 (*C*$_{\beta,Cys}$), 45.8 (*C*$_{\alpha,Cys}$), 42.3 (*C*$_{\alpha,Leu}$), 38.2 (N–*C*H$_3$), 28.1 (C(*C*H$_3$)$_3$), 24.7 (*C*$_{\beta,Leu}$), 22.9 (*C*$_{\delta,Leu}$), 21.8 (*C*$_{\gamma,Leu}$), 14.1 (CO$_2$–CH$_2$–*C*H$_3$). MS (Fab$^+$): 693.2 [NHC$_2$ – Au]$^+$ (85%), 830.3 [M + Na]$^+$, 1251.2 [M + NHC–Au]$^+$. Anal. calc. for $C_{33}H_{45}AuN_4O_5S$ (m = 806.78): C, 49.13; H, 5.62; N, 6.94; S, 3.97. Found: C, 45.7; H, 6.52; N, 7.32; S 3.06.

Crystal structure determination†

Crystals of **4c** and **6c** were mounted on a glass capillary in perfluorinated oil and measured in a cold gas stream. The intensities were measured with a Bruker-axs-SMART diffractometer (Mo Kα radiation, λ = 0.7170 Å, ω scan). The structure was solved by direct methods (SHELXS-97), and refined against F^2 with all measured reflections (SHELXL-97). All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were included in calculated positions.

Crystal data of 4c. $C_{19}H_{25}AuBr_3N_3O_3$, M = 780.12, monoclinic, a = 8.452(4), b = 9.212(4), c = 15.756(7) Å, β = 92.894(9)°, V = 1225.2(9) Å^3, T = –60 °C, space group $P2_1$, Z = 2, $2\theta_{max}$ = 50°, Flack-parameter = 0.00(2), ρ(calc) = 2.115 mg m^{-3}, 262 parameters, μ = 10.919 mm^{-1}, 6721 measured reflections, 3852 unique (R_{int} = 0.0497) which were used in all calculations. The final R_1 = 0.0594 for 3540 observed reflections ($I > 2\sigma(I)$) and wR_2 = 0.1619 for all data.

Crystal data of 6c. $C_{17}H_{16}AuBr_3N_2$, M = 685.01, monoclinic, a = 9.6826(5), b = 12.2416(4), c = 20.3950(8) Å, β = 91.488(4)°, V = 2416.6(2) Å^3, T = –80 °C, space group $P2_1/n$, Z = 4, $2\theta_{max}$ = 50.2°, ρ(calc) = 1.883 mg m^{-3}, 208 parameters, μ = 11.048 mm^{-1}, 14 411 measured reflections, 4284 unique (R_{int} = 0.062) which were used in all calculations. The final R_1 = 0.0307 for 2497 observed reflections ($I > 2\sigma(I)$) and wR_2 = 0.0472 for all data.

Cell culture and IC_{50} values

HeLa, HepG2 and HT-29 cells were kept in culture with RPMI 1640 medium supplemented with 10% FCS (fetal calf serum), 2 mM L-glutamin and the antibiotic penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) in 5% CO_2-atmosphere. *In vitro* cytotoxicity of gold compounds was studied on HeLa, HT-29 and HepG2 cells. Cell viability, which correlates with the metabolic activity of a cell, was determined by the resazurin assay.[39] In addition to the cell viability, absolute cell numbers were determined by the crystal violet assay,[40] which can be applied after elution of resazurin. The cells were seeded in 96-well microtiterplates (MTP) coated with 0.2% of gelatine. After seeding, the cells were grown for 24 h under standard conditions. The compounds were dissolved in culture medium with 0.5% DMSO and applied to the cells in various concentrations for 48 h. Every concentration was tested six-fold. Before resazurin was added to the cells they were washed three times with phenol red-free RPMI-1640 medium. Then, phenol red-free RPMI-medium with 10% resazurin was added. Absorbance at 600 nm was directly measured with a Tecan Sapphire² microplate reader (Tecan, Germany) at 37 °C. After 2 h of incubation at 37 °C and 5% CO_2, the measurement was repeated. The decrease in absorbance gave the viability. Resazurin was removed and the cells were fixated with 4% *para*formaldehyde (PFA) in PBS (phosphate buffered saline) for 15 min at room temperature. PFA was eluted twice with PBS and membranes were permeabilised by 0.1% Triton-X100 in PBS for 10 min. Afterwards, an aqueous 0.04% crystal violet solution was added to the cells and the MTP was mechanically shaken for 1 h. The cells were washed seven times with H_2O, and crystal violet was eluted with 96% ethanol for 4 h. The absorbance was determined at 570 nm, after subtraction of 24 h pre-substance incubation absorbance values, cell biomass could be calculated. In case the compound showed a promising cytotoxic activity, the inhibitory concentration at 50% growth (IC_{50}) for both assays was calculated. Therefore, a series of concentration suitable for the estimated IC_{50} was applied to the cells according to the procedure described above. The obtained viability or cytotoxicity data were plotted against the concentration in half-logarithmical scale and a sigmoidal function fit was performed with Origin 7 (Originlab, Northampton, USA) until the fit converged. IC_{50}-values were directly calculated from the fit function.

Acknowledgements

Financial support from the Ruhr-University Research School to J. L. is gratefully acknowledged. This work is supported by

the Research Units "Biological Function of Organometallic Compounds" (DFG FOR 630, www.rub.de/for630) and "Molecular Aggregation" (DFG FOR 618).

References

1 D. Enders, O. Niemeier and A. Henseler, *Chem. Rev.*, 2007, **107**, 5606–5655.
2 A. J. Arduengo, in *Comprehensive Organic Functional Group Transformations II*, ed. A. R. Katritzky and R. J. K. Taylor, Elsevier Pergamon, New York, 2005, pp. 1103–1125.
3 H. G. Raubenheimer and S. Cronje, *Chem. Soc. Rev.*, 2008, **37**, 1998–2011.
4 M. V. Baker, P. J. Barnard, S. J. Berners-Price, S. K. Brayshaw, J. L. Hickey, B. W. Skelton and A. H. White, *Dalton Trans.*, 2006, 3708–3715.
5 P. J. Barnard, L. E. Wedlock, M. V. Baker, S. J. Berners-Price, D. A. Joyce, B. W. Skelton and J. H. Steer, *Angew. Chem., Int. Ed.*, 2006, **45**, 5966–5970.
6 X. Hu, I. Castro-Rodriguez, K. Olsen and K. Meyer, *Organometallics*, 2004, **23**, 755–764.
7 P. J. Barnard, M. V. Baker, S. J. Berners-Price and D. A. Day, *J. Inorg. Biochem.*, 2004, **98**, 1642–1647.
8 J. L. Hickey, R. A. Ruhayel, P. J. Barnard, M. V. Baker, S. J. Berners-Price and A. Filipovska, *J. Am. Chem. Soc.*, 2008, **130**, 12570–12571.
9 M. M. Jellicoe, S. J. Nichols, B. A. Callus, M. V. Baker, P. J. Barnard, S. J. Berners-Price, J. Whelan, G. C. Yeoh and A. Filipovska, *Carcinogenesis*, 2008, **29**, 1124–1133.
10 U. E. I. Horvath, G. Bentivoglio, M. Hummel, H. Schottenberger, K. Wurst, M. J. Nell, C. E. J. van Rensburg, S. Cronje and H. G. Raubenheimer, *New J. Chem.*, 2008, **32**, 533–539.
11 S. Ray, R. Mohan, J. K. Singh, M. K. Samantaray, M. M. Shaikh, D. Panda and P. Ghosh, *J. Am. Chem. Soc.*, 2007, **129**, 15042–15053.
12 F. Tewes, A. Schlecker, K. Harms and F. Glorius, *J. Organomet. Chem.*, 2007, **692**, 4593–4602.
13 G. Xu and S. R. Gilbertson, *Org. Lett.*, 2005, **7**, 4605–4608.
14 J. F. Jensen, K. Worm-Leonhard and M. Meldal, *Eur. J. Org. Chem.*, 2008, 3785–3797.
15 K. Worm-Leonhard and M. Meldal, *Eur. J. Org. Chem.*, 2008, 5244–5253.
16 L. Barisic, M. Dropucic, V. Rapic, H. Pritzkow, S. I. Kirin and N. Metzler-Nolte, *Chem. Commun.*, 2004, 2004–2005.
17 J. T. Chantson, M. V. V. Falzacappa, S. Crovella and N. Metzler-Nolte, *ChemMedChem*, 2006, **1**, 1268–1274.
18 U. Hoffmanns and N. Metzler-Nolte, *Bioconjugate Chem.*, 2006, **17**, 204–213.
19 J. L. Jios, S. I. Kirin, N. N. Buceta, T. Weyhermueller, C. O. Della Vedova and N. Metzler-Nolte, *J. Organomet. Chem.*, 2007, **692**, 4209–4214.
20 S. I. Kirin, H.-B. Kraatz and N. Metzler-Nolte, *Chem. Soc. Rev.*, 2006, **35**, 348–354.
21 S. D. Koester, J. Dittrich, G. Gasser, N. Huesken, I. C. Henao Castaneda, J. L. Jios, C. O. Della Vedova and N. Metzler-Nolte, *Organometallics*, 2008, **27**, 6326–6332.
22 N. Metzler-Nolte, *Chimia*, 2007, **61**, 736–741.
23 F. Noor, R. Kinscherf, G. A. Bonaterra, S. Walczak, S. Woelfl and N. Metzler-Nolte, *ChemBioChem*, 2009, **10**, 493–502.
24 A. Gross and N. Metzler-Nolte, *J. Organomet. Chem.*, 2008.
25 M. C. Kuchta, A. Gross, A. Pinto and N. Metzler-Nolte, *Inorg. Chem.*, 2007, **46**, 9400–9404.
26 A. Neukamm Merja, A. Pinto and N. Metzler-Nolte, *Chem. Commun.*, 2008, 232–234.
27 J. Lemke and N. Metzler-Nolte, *Eur. J. Inorg. Chem.*, 2008, 3359–3366.
28 J. Caddy, U. Hoffmanns and N. Metzler-Nolte, *Z. Naturforsch., B*, 2007, **62**, 460–466.
29 M. V. Baker, P. J. Barnard, S. J. Berners-Price, S. K. Brayshaw, J. L. Hickey, B. W. Skelton and A. H. White, *J. Organomet. Chem.*, 2005, **690**, 5625–5635.
30 P. de Fremont, E. D. Stevens, M. D. Eelman, D. E. Fogg and S. P. Nolan, *Organometallics*, 2006, **25**, 5824–5828.
31 O. Brosch, PhD thesis, *Ruhr-University Bochum*, 1999.
32 H. M. J. Wang and I. J. B. Lin, *Organometallics*, 1998, **17**, 972–975.
33 P. De Fremont, R. Singh, E. D. Stevens, J. L. Petersen and S. P. Nolan, *Organometallics*, 2007, **26**, 1376–1385.
34 Y. Chu, H. Deng and J.-P. Cheng, *J. Org. Chem.*, 2007, **72**, 7790–7793.
35 M. V. Baker, P. J. Barnard, S. K. Brayshaw, J. L. Hickey, B. W. Skelton and A. H. White, *Dalton Trans.*, 2005, 37–43.
36 S. K. Schneider, W. A. Herrmann and E. Herdtweck, *Z. Anorg. Allg. Chem.*, 2003, **629**, 2363–2370.
37 A. Neveling, G. R. Julius, S. Cronje, C. Esterhuysen and H. G. Raubenheimer, *Dalton Trans.*, 2005, 181–192.
38 M. K. Samantaray, K. Pang, M. M. Shaikh and P. Ghosh, *Dalton Trans.*, 2008, 4893–4902.
39 J. O'Brien, I. Wilson, T. Orton and F. Pognan, *Eur. J. Biochem.*, 2000, **267**, 5421–5426.
40 G. Bernhardt, H. Reile, H. Birnboeck, T. Spruss and H. Schoenenberger, *J. Cancer Res. Clin. Oncol.*, 1992, **118**, 35–43.
41 B. Lippert, *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, Verlag Helvetica Chimica Acta, Zürich, 1999.
42 Y. Liu, J. K. Pak, P. Schmutz, M. Bauwens, J. Mertens, H. Knight and R. Alberto, *J. Am. Chem. Soc.*, 2006, **128**, 15996–15997.
43 R. E. Cowley, R. P. Bontchev, E. N. Duesler and J. M. Smith, *Inorg. Chem.*, 2006, **45**, 9771–9779.
44 M.-C. Brandys, M. C. Jennings and R. J. Puddephatt, *J. Chem. Soc., Dalton Trans.*, 2000, 4601–4606.
45 G. M. Sheldrick, *SHELXS-97, Program for solution of crystal structures*, University of Göttingen, Germany, 1997.
46 A. L. Spek, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 1990, **46**, C34.

