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FULL PAPER

Reversible covalent and supramolecular functionalization of water-soluble Au(I)-complexes

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Abstract: The ligation of Au(I)-metalloamphiphiles with biomolecules is reported, using water-soluble Au(I)-*N*-alkynyl substituted maleimide complexes. For this purpose, two different polar ligands were applied: (1) a neutral, dendritic tetraethylene glycol functionalized phosphane and (2) a charged, sulfonated *N*-heterocyclic carbene (NHC). The retro Diels-Alder reaction of a furan protected maleimide-Au(I)-complex, followed by cycloaddition with a diene functionalized biotin under mild conditions leads to a novel Au(I)-metalloamphiphile. The strong streptavidin-biotin binding affinity in buffered aqueous solution of the resulting biotin Au(I)-alkynyl-phosphane conjugate remains intact. The cytotoxicity of the biotinylated Au(I)-complex against a T47D human breast cancer cell line is higher than for cisplatin.

Introduction

The chemoselective modification of biological macromolecules is a steadily expanding field in life sciences with regard to a wide-range of applications in imaging, diagnostics, therapy and the elucidation of biological function.[1] In recent years, chemists have developed numerous strategies for the conjunction with drugs, contrast agents, fluorescent labels or affinity tags using bioconjugation reactions such as Cu^{I}-catalyzed[2] or strain-promoted[3] alkyne-azide cycloaddition (CuAAC or SPAAC, respectively), native chemical[4] and Staudinger ligations,[5] metal-catalyzed olefin cross metathesis[6] and cross-coupling reactions,[7] direct cysteine arylation using organometallic palladium reagents,[8] as well as Diels-Alder[9] and the inverse electron demand Diels-Alder cycloaddition.[10] Maleimides are commonly employed as dienophiles in normal-electron-demand Diels-Alder reactions, due to their electron-poor character and thus high reactivity, as well as their straight forward chemical functionalization, and have been applied for the postsynthetic modification of oligonucleotides,[9c, 9g, 9h] proteins,[9k-m], glycoconjugates[9e, 9f] as well as for the preparation of various synthetic polymer architectures and polymer-bioconjugates.[11] Given the growing research activities in oligopeptides decorated with medicinally active organometallic compounds, we were surprised to notice that orthogonal bioconjugation strategies of water-soluble metal-complexes have received little attention.[12] Note that within the context of peptidic Au(I)-complexes, the Krause lab has reported biotin-[$Au^{I}Cl$(NHC)] conjugates containing hydrophobic ligands.[13] The Metzler-Nolte group has reported a series of amino acid and peptide Au(I)-conjugates based on Au^{I}-NHC,[14], Au(I)-*N*,*S*-heterocyclic carbene (NSHC),[15]

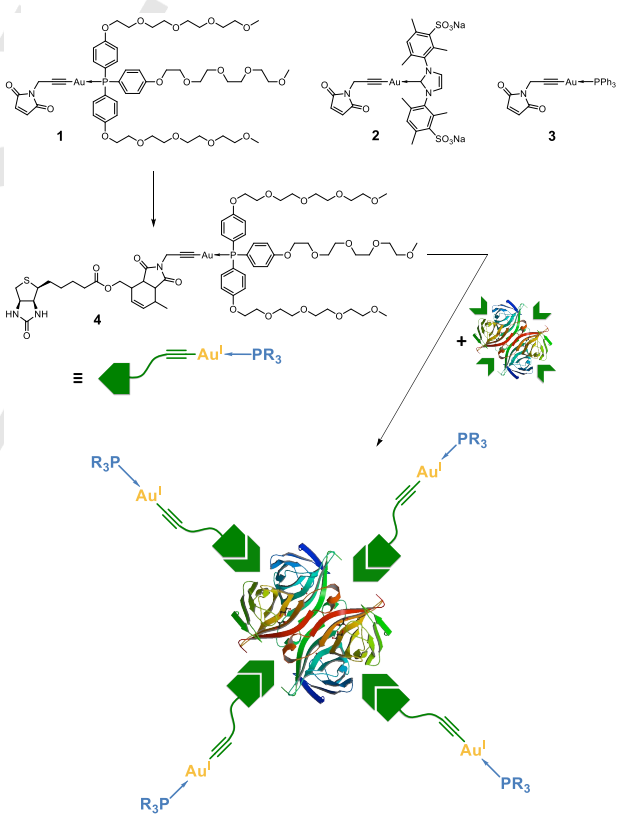

Figure 1. Water-soluble maleimidic Au(I)-alkynyl complexes based on a neutral, dendritic tetraethylene glycol (TEG) functionalized phosphane **1** and a charged, sulfonated NHC **2** as well as the corresponding nonpolar derivative with PPh_3 as neutral ligand **3** (top); Diels-Alder ligation of **1** with biotin (middle); supramolecular complexation of the resulting metalloamphiphilic biotin Au(I)-alkynyl-phosphane conjugate **4** (bottom) with streptavidin in aqueous solution. Image from the RCSB PDB (www.rcsb.org) of PDB ID 2Y3E (C. E. Chivers, A. L. Koner, E. D. Lowe, M. Howarth, *Biochem. J.* 2011, *435*, 55-63).[18]

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Au(I)-triazolyl-phosphane complexes.[16] Laguna and Concepción Gimeno have reported the synthesis of peptidic thiolate Au(I)-phosphane complexes,[17] whereas Amorín and Granja described cyclic peptides linked *via* hydrophobic alkynyl Au(I)-phosphanes.[19] Water-soluble molecular Au(I)-complexes in general[20] and those bearing alkynyl ligands in particular have received increasingly large interest in applications for luminescence,[21] catalysis,[22] and medicinal chemistry.[12, 23]

Most recently, we have reported a synthetic route for the preparation of a self-assembling peptidic Au(I)-metalloamphiphile, *via* coupling of an propargylamine-functionalized Au(I)-phosphane with a *N*-hydroxysuccinimide activated peptide.[24] In order to expand the synthetic toolbox of Au(I)-bioconjugation strategies for the construction of more complex metallo-amphiphile-based nanomaterials and protein conjugates, we hereby report the synthesis of Au(I)-*σ*-alkynyl complexes containing Diels-Alder protected maleimide, equipped with an oligo(ethylene glycol) derived phosphane and a sulfonated NHC ligand (Figure 1). The high stability of these complexes is demonstrated. After deprotection, the former Au(I)-phosphane complex is conjugated with a diene-functionalized biotin derivative. The resulting biotinylated Au(I)-metalloamphiphile is able to self-assemble into larger tetrameric superstructures *via* the supramolecular biotin-streptavidin complexation (Figure 1). We have further assessed the cytotoxicity of the biotinylated Au(I)-complex against a human breast cancer cell line (T47D cells). Our synthetic strategy will facilitate the development of more sophisticated multifunctional Au(I)-bioconjugates.

Results and Discussion

In order to show the versatility of the synthetic route presented herein, we decided to use water-soluble Au(I)-complexes bearing two very different kind of ligands: (1) a neutral, tetraethylene glycol functionalized phosphane[25] and (2) a charged, sulfonated NHC (sIMes)[26] ligand (Schemes 1 and S1). For the preparation of the dendritic Au(I)-phosphane complex **1**, we first exploited a strategy, which was based on a neutral ligand exchange of the corresponding phosphane with tetrahydrothiophene (tht) in [Au^{I}Cl(tht)].[25c] Due to its tendency to oxidize very quickly, the starting tri(4-methoxyphenyl)phosphane was first fully oxidized, followed by deprotection of the methyl ether, before etherification of the phenolic hydroxyl groups with the tosylate of tetraethylene

5 Ligand = P(*p*-C$_6$H$_4$O(TEG))$_3$ (**6**) = sIMes (**7**) = PPh$_3$ (**8**) **9** 18% (after HPLC) **10** 25% (after HPLC) **11** 67%

Scheme 1. Synthesis of *endo*-Diels-Alder protected building blocks **9**-**11** based on water-soluble Au(I)-phosphane **6** and Au(I)-NHC **7** complexes or [Au^{I}Cl(PPh$_3$)] (**8**) *via* anionic ligand exchange: **9**: **6** (1.0 eq.), **5** (1.0 eq.), KOH (1.6 eq.), MeOH, rt, 16 h; **10**: 7 (1.0 eq.), **5** (1.0 eq.), KOH (1.5 eq.), MeOH, 35 °C, 16 h; **11**: **8** (1.0 eq.), **5** (1.0 eq.), KO^{t}Bu (1.5 eq.), EtOH, rt, 18 h.

glycol monomethyl ether took place to yield the precursor ligand **16** (see SI). *In situ* reduction following by addition of [Au^{I}Cl(tht)] led to the desired product **1** in a moderate overall yield of 45% in four steps (Scheme S1). The oxidation/reduction steps on the phosphorous limit the feasibility of this synthetic route. Instead of a late introduction of the phosphane ligand into the Au(I)-complex, we decided to exchange the neutral ligand in the first step before demethylation was performed. Quantitative yields were achieved when tri(4-methoxyphenyl)phosphane was reacted with [Au^{I}Cl(tht)]. Next, deprotection of the aromatic methyl ethers of **18** with BBr_3 followed by hydrolysis gave the phenolic Au(I)-phosphane complex **19** in 99% yield. Note that the Au(I)-phosphane complex remains fully stable in the presence of the strong Lewis acid BBr_3, and furthermore no alkylation of the phosphane was observed during the demethylation reaction. Finally, etherification with tetraethylene glycol monomethyl ether tosylate led to the water-soluble Au(I)-phoshane complex **1** with an improved overall yield of 83% in three steps, on gram scale.

For the functionalization of biomacromolecules with the water-soluble Au(I)-phosphane and NHC complexes **1** and **2**, we decided to link them with maleimides. Maleimide bioconjugation chemistry is widely explored and can be applied on various molecules of biological interest either by Michael-type or Diels-Alder click chemistry. Starting from cheap maleic anhydride and *N*-propargyl amine, *N*-alkynyl-functionalized maleimide **24** was synthesized, as reported in literature[27] (see SI). We decided to protect the maleimide *via* reversible Diels-Alder reaction in order to enable a long-term storage of stable Au(I)-building blocks and further prevent undesired side reactions during the anionic ligand exchange of Au(I)-chlorido complexes. Cycloadditions of electron-rich dienes such as furans with maleimide derivatives have been intensively studied. It is well-known that the unblocking reactions of *endo* protected maleimides proceed at much lower temperatures compared with the corresponding *exo* isomers.[28] By reacting furan with *N*-propargyl maleimide **24** for one week at room temperature, the desired, kinetically favored *endo* isomer **5** was obtained as the major product in 59% yield (see SI). We then proceeded with the anionic ligand exchange of the Au(I)-chlorido complexes **6**-**8** using terminal alkyne of **5** (Scheme 1). Treatment of stoichiometric amounts of the alkyne and the water-soluble Au(I)-complexes **6** and **7**, with potassium hydroxide in degassed methanol led to the formation of the corresponding *σ*-complexes **9** and **10**. In the case of complex **10**, a temperature increase to 35 °C was required to reach full conversion. $^{31}P\{^{1}H\}$ NMR analysis of the reaction mixture of **9** indicated, that the anionic ligand exchange proceeded selectively and without any formation of byproducts.

In view of potential biomedical applications for hybrid bioinorganic metal-complexes, very high purity is mandatory and we decided to show that the metalloamphiphilic Au(I)-complexes **9** and **10** could be purified by preparative RP-HPLC. The chromatograms and full characterization using high-resolution mass spectrometry, ^{1}H, ^{13}C and $^{31}P\{^{1}H\}$ NMR spectroscopy are provided in the supporting information, and confirm the successful synthesis of **9** and **10**. Due to their high amphiphilicity we were unable to grow crystals, and we turned to an apolar model complex Au(I)-alkynyl-triphenylphosphane **11** (Scheme 1 and Figure S1). Instead of

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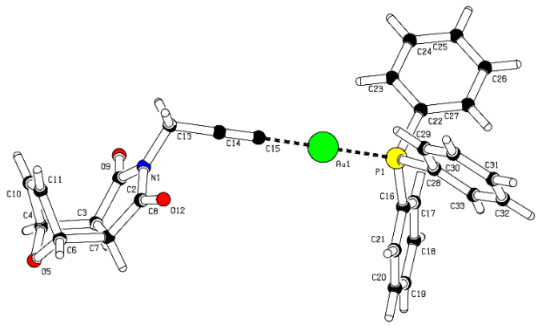

Figure 2. Perspective drawing of the crystal structure of complex **11** with atomic numbering scheme. CCDC reference number XXXXXXX.

using potassium hydroxide, potassium *tert*-butoxide in ethanol was applied in the substitution of the chorido ligand in [Au^{I}Cl(PPh$_3$)] (**8**) with the alkynyl ligand **5**. Isolation and washing of the precipitate afforded the pure, hydrophobic Au(I)-complex **11** in 67% yield. Vapor diffusion of *n*-hexane into a solution of **11** in benzene resulted in colorless crystals. The Au(I)-complex crystallized in the monoclinic space group *P* 2$_1$/*c*. A perspective drawing of the single-crystal X-ray diffraction analysis is shown in Figure 2. The P–Au (2.2697(9) Å), C–Au (1.993(4) Å) and C≡C (1.193(5) Å) bond lengths are comparable to those observed in other Au(I)-alkynyl-phosphane complexes.[29] The P-Au-C angle of 179.73(13)° represents the typical linear geometry and sp-hybridization found in Au(I)-alkynyl complexes. Note that the structure is slightly disordered, showing 70% of the *endo* isomer **11** and 30% of the corresponding *exo* isomer (see SI). Filling almost the same space, the *exo* derivative does not seem to affect the packing of the crystal (Figure S2). Most likely, high summer temperatures during crystal growth are responsible for the observed partial *endo* to *exo* transformation in solution. This is supported by ^{1}H NMR analysis before and after crystal growth, displaying the pure *endo* isomer of the Au(I)-complex **11** before and 30% of the *exo* isomer after crystallization (Figure S1). Importantly, the X-ray diffraction analysis confirms the *σ*-coordinating nature of the hydrophobic Au(I)-complex and the absence of *π*-activating Au(I)-species, which would cause side reactions with nucleophiles in further bioconjugation steps.

The purified and storable Au(I)-building blocks **9**-**11** could be transformed into the corresponding reactive maleimide complexes **1**-**3**, as required. The retro Diels-Alder reaction of the *endo* protected maleimide complexes was performed at slightly increased temperatures. Full conversion was accomplished by heating the protected complexes at 70 °C in non-nucleophilic solvents like dry toluene (**9** and **11**) or dry DMF (**10**) for four to seven hours (Scheme S1). Except for furan, which could be removed under reduced pressure, no other byproduct was detected in the formation of the desired *N*-propargyl maleimide Au(I)-alkynyl complexes **9**-**11**. In this regard, direct maleimide Diels-Alder ligation with biomacromolecules could potentially be applied *in situ* and without further purification.

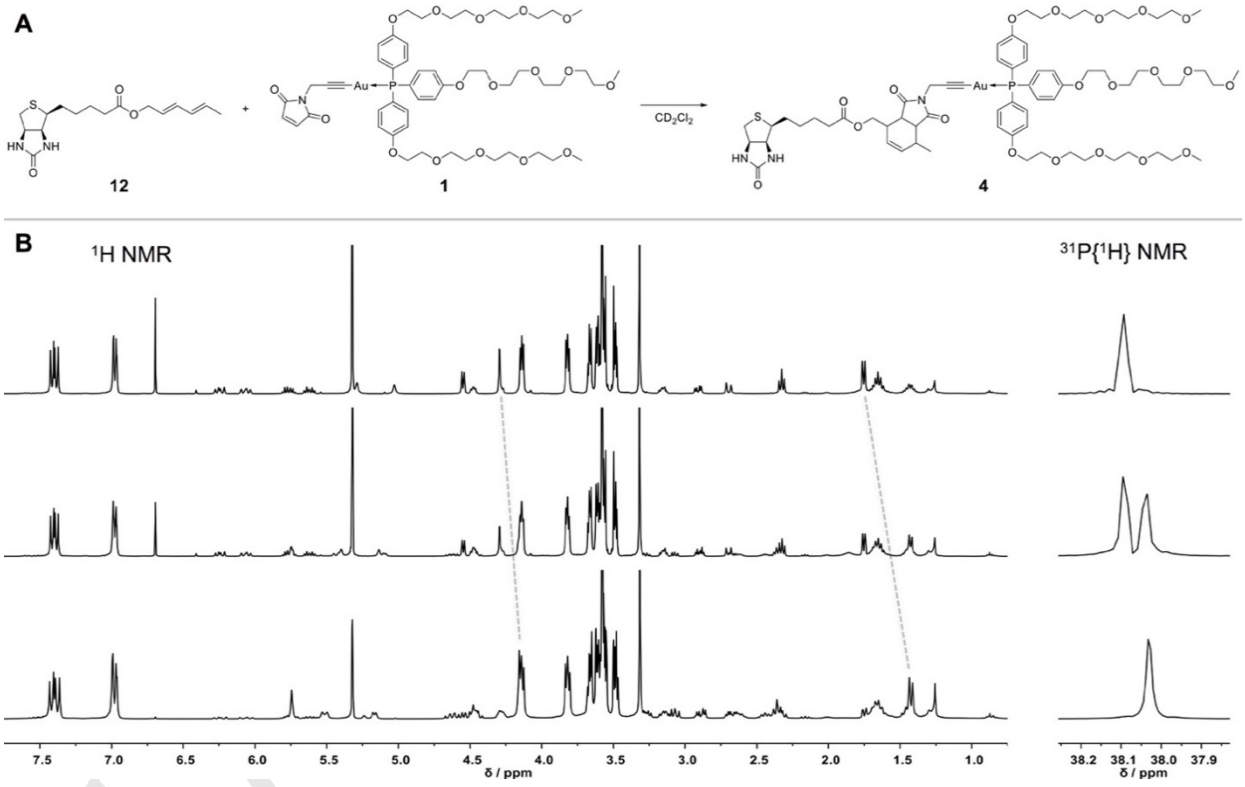

Figure 3. Diels-Alder ligation of diene-functionalized biotin **12** with the metalloamphiphilic Au(I)-alkynyl complex **1** as the malemidic dienophile component in CD_2Cl_2. **(A)**: Reaction scheme; **(B)** Progress of the conjugation followed by ^{1}H NMR (left) and ^{31}P NMR (right) at *t* = 1 d at rt (top), *t* = 6 d at 35 °C (middle) and *t* = 10 d at 50 °C.

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We decided to illustrate the facile bioconjugation of a functional, metalloamphiphilic Au(I)-alkynyl complex by using the established biotin-streptavidin system. Biotin is a co-factor that plays a role in multiple eukaryotic biological processes, and the host-guest interaction between streptavidin and biotin is the strongest known non-covalent interaction of a protein-ligand pair. Whitesides[30] and Ward[31] have reported biotinylated rhodium and ruthenium complexes in the development of artificial metalloenzymes.

As an appropriate biotin derivative carrying a diene moiety, we prepared substrate **12** (Figure 3A), by facile esterification of commercially available (*E*,*E*)-2,4-hexadien-1-ol with biotin (see SI). With the diene **12** and the maleimidic dienophile embedded in the metalloamphiphilic Au(I)-alkynyl-phosphane complex **1** in hand, we studied the Diels-Alder ligation to form the target molecule **4** (Figure 3). In order to follow the progress of the reaction by NMR analysis, we mixed stoichiometric amounts of **12** and **1** in deuterated dichloromethane. In the $^{31}P\{^{1}H\}$ NMR, the consumption of the dienophilic Au(I)-complex **1** (δ = 38.1 ppm) as well as formation of the desired cycloaddition product **4** (δ = 38.0 ppm) could be easily monitored (Figure 3B, right). These observations are supported by monitoring selected ^{1}H NMR signals (Figure 3B, left). Herein, a significant shift of the diene methyl group as well as of the methylene group of the propargyl moiety is visible (dotted lines, Figure 3B). The disappearance of the signals belonging to the conjugated double bonds of the diene (δ = 6.30–5.56 ppm) and those corresponding to the maleimide double bond (δ = 6.69 ppm) indicate a successful ligation. We found that a stepwise temperature increase from room temperature up to 50 °C accompanied with a slow concentration increase concentration due to partial evaporation of CD_2Cl_2, facilitated full conversion of the Au(I)-complex **1**. In principle, the reaction time and temperature could further be lowered by installation of a furan derivative as the activated diene component[32] or water as a solvent.[33] Concerning the stereochemistry of the cycloaddition, four diastereoisomers are conceivable. Supported by the literature,[9l] our results suggest that the cycloaddition exhibits no face-selectivity and therefore two *endo* isomers are formed.

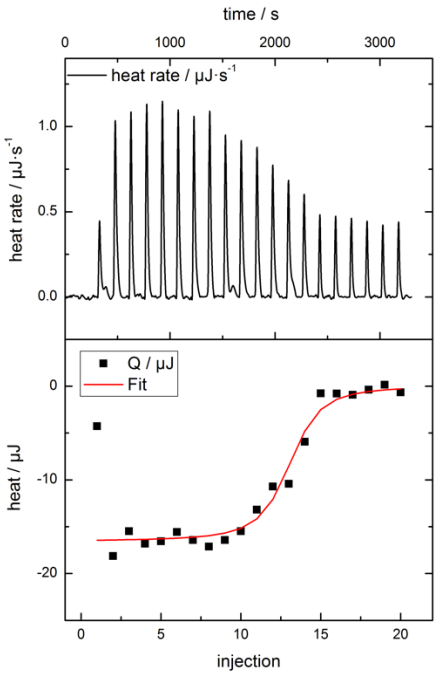

Figure 4. Isothermal titration calorimetry (ITC) of streptavidin (15 µM) with the biotinylated metalloamphiphilic Au(I)-alkynyl-phosphane complex **4** (150 µM) at pH 7.4 (20 mM phosphate buffer, including 10% DMSO). Top row: Change of the heat rate during the titration; Bottom row: Integration of the measured heat.

To demonstrate the strong affinity of the synthesized biotin Au(I)-alkynyl-phosphane conjugate **4** towards streptavidin, ITC measurements were performed (Figure 4 and Figure S3). Despite the high water-solubility of the precursor complex **1**, the ITC experiments with the biotinylated Au(I)-complex **4** had to be performed in an aqueous buffer containing 10% of DMSO, since the incorporation of the hydrophobic biotin moiety impaired the water solubility. Compared to titration experiments of streptavidin with non-functionalized biotin performed in the same aqueous buffer, we were not able to observe any reduction of the binding constant and could confirm the high affinity $K_a > 10^6\ M^{-1}$ for the biotinylated metalloamphiphilic Au(I)-alkynyl-phosphane complex **4**.

We further assessed the toxicity of the Au(I)-complex **4** against a human breast cancer cell line. The T47D cells were cultured for 24 h, 48 h and 72 h in the presence of different concentrations of **4** (Figure 5). The cytotoxicity of complex **4** (IC50 = 26±2 µM after 24 h, 38±2 µM after 48 h and 41±2 µM after 72 h) is higher than for the positive control Cisplatin. This result is consistent with previously disclosed studies for water soluble Au(I)-phosphane complexes, which reported IC50 values between 1 µM and 100 µM, depending on the cell line, incubation time, ligand type and lipophilicity of the Au(I) complexes.[12a, 14-17, 20, 34]

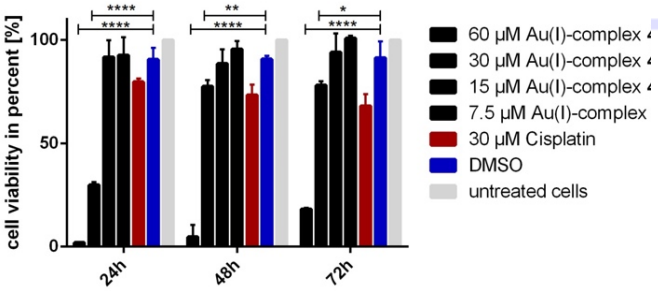

Figure 5. Toxicity assessment of Au(I)-complex **4** against human breast cancer cells. Human breast cancer cells (T47D cells) were cultured for 24h, 48h and 72h in the presence of different concentrations of Au(I)-complex **4**. As positive control the cells were treated with 30 µM Cisplatin. As negative control the cells were treated with 2 vol% DMSO in PBS buffer, as was used within the highest concentration of the Au(I)-complex **4**. T47D cells were stained with a fixable viability dye and cell viability was determined by Flow Cytometry. The value of the untreated cells is considered as 100%. The other values are expressed as percentiles. The mean of three independent experiments (±SD) is shown. A two-tailed unpaired t-test was used to determine the significant effect of the Au(I)-complex on human breast cancer cells in comparison to the negative control. *≤P 0.05; **≤P 0.01; ****≤P 0.0001.

Due to its exceptionally high binding affinity for avidin and streptavidin, biotin has been widely used as a recognition unit for bioassays,[35] and therapeutics[36], for example in the development of supramolecular multicomponent protein based delivery systems[37], antibody-toxin[38], or antibody-siRNA conjugates[39], and cell-surface engineering via oligosaccharide-toxin conjugates.[40] Our synthetic strategy for biotinylated Au(I)-complexes will thus facilitate the preparation of more sophisticated Au(I)-bioconjugates for the development of multifunctional targeted metallodrugs and therapeutics.

Conclusions

In summary, we present a synthetic route for the bioconjugation of metalloamphiphilic Au(I)-alkynyl complexes containing water-soluble phosphane ligands *via* Diels-Alder ligation. In this context, we improved the protocol for the preparation of polar Au(I)-phosphane complexes bearing tetraethylene glycol moieties, relying on the very strong Au-P bond which was stable under demethylation conditions with boron tribromide, etherification reactions and reversible Diels-Alder chemistry. We were able to prepare *σ*-coordinated Au(I)-alkynyl metalloamphiphiles by anionic ligand exchange of Au(I)-chlorido-phosphane and NHC complexes with *endo* furan protected *N*-propargyl maleimide. After RP-HPLC purification, the selective activation of these storable Au(I)-alkynyl complexes *via* retro Diels-Alder reaction at relatively low temperatures yields reactive *N*-propargyl maleimide Au(I)-alkynyl building blocks. In order to demonstrate the feasibility for bioconjugation, the maleimidic Au(I)-alkynyl-phosphane complex **1** was reacted with diene-functionalized biotin by Diels-Alder cycloaddition. The supramolecular function of the resulting biotinylated amphiphilic Au(I)-alkynyl complex remains intact as shown by ITC titration experiments with streptavidin. The cytotoxicity of the biotinylated Au(I)-complex against a T47D human breast cancer cell line was determined and was higher than for cisplatin. The maleimidic and biotinylated Au(I)-complexes will facilitate the preparation of more sophisticated Au(I)-conjugated biomolecules, which could find utilization in the development of multifunctional and targeted therapeutics.

Experimental Section

Detailed synthetic procedures for the remaining compounds, their characterization and full details about the instrumentation and methods, as well as additional experimental data (Figures S1-S3) can be found in the Supporting Information (SI).

Anionic ligand exchange protocol

Au(I)-alkynyl-phosphane complex 9: To a solution of KOH (21.7 mg, 387 µmol, 1.6 eq.) and **5** (49.5 mg, 244 µmol, 1.0 eq.) in dry, degassed MeOH (8 mL), a solution of **6** (267 mg, 240 µmol, 1.0 eq.) in dry, degassed MeOH (6 mL) was added under argon in the dark. The reaction mixture was stirred at room temperature for 16 h in the dark and concentrated *in vacuo*. Purification *via* RP-HPLC afforded the desired product **9** (56.6 mg, 44.2 µmol, 18%) as a yellowish oil.

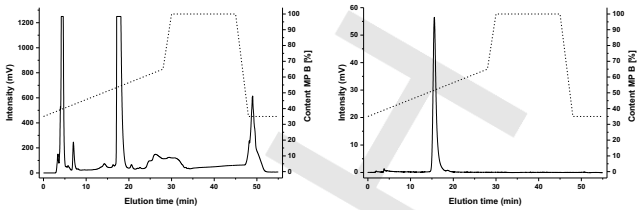

Figure 6. RP-HPLC chromatograms of **9** monitored at λ = 210 nm: a) RP-HPLC separation on a semi-preparative *VariTide RPC* column (flow: 18.9 mL/min), b) RP-HPLC chromatogram (baseline corrected) of the isolated product **9** on an analytical *VariTide RPC* column (flow: 1.0 mL/min) Details about the used gradient (dashed line) are provided in the SI.

^{1}H NMR (600 MHz, MeOD-*d*$_4$, 294 K): δ/ppm = 7.38 (dd, J_{PH} = 12.0 Hz, J = 8.6 Hz, 6H, PCC*H*), 7.04 (dd, J = 8.6 Hz, 1.5 Hz, 6H, C*H*C$_q$O ^{aryl}), 6.40 (t, J = 0.9 Hz 2H, C*H* ^{alkene}), 5.27 (ddd, J = 3.6 Hz, 1.6 Hz, 0.9 Hz, 2H, C*H* ^{furan}), 4.19–4.14 (m, 6H, C$_q$OC*H*$_2$ ^{TEG}), 4.06 (s, 2H, NC*H*$_2$), 3.85–3.82 (m, 6H, C*H*$_2$ ^{TEG}), 3.70–3.65 (m, 6H, C*H*$_2$ ^{TEG}), 3.65–3.57 (m, 24H, C*H*$_2$ ^{TEG}), 3.53 (dd, J = 3.6 Hz, 1.7 Hz, 2H, C*H* ^{maleimide}), 3.51–3.48 (m, 6H, C*H*$_2$ ^{TEG}), 3.32 (s, 9H, C*H*$_3$). **^{13}C NMR (151 MHz, MeOD-*d*$_4$, 296 K):** δ/ppm =175.9 (*C*O), 163.0 (d, J_{PC} = 2.3 Hz, *C*$_q$O ^{aryl}), 136.8 (d, J_{PC} = 15.4 Hz, PC*C*), 135.6 (*C* ^{alkene}), 122.7 (d, J_{PC} = 61.6 Hz, P*C*), 116.6 (d, J_{PC} = 12.3 Hz, *C*C$_q$O ^{aryl}), 110.9 (d, J_{PC} = 154.9 Hz, C*C*Au), 96.8 (d, C*C*Au), 80.7 (*C* ^{furan}), 73.0 (*C*H$_2$), 71.8 (*C*H$_2$), 71.60 (*C*H$_2$), 71.58 (*C*H$_2$), 71.5 (*C*H$_2$), 71.4 (*C*H$_2$), 70.6 (*C*H$_2$), 68.9 (*C*H$_2$), 59.2 (*C*H$_3$), 47.3 (*C* ^{maleimide}), 28.4 (d, N*C*H$_2$). **^{31}P{^{1}H} NMR (162 MHz, MeOD-*d*$_4$, 295 K):** δ/ppm = 37.5 (s). **ESI-HRMS (MeOH) (m/z):** Calculated for [C$_{56}$H$_{77}$AuNO$_{18}$PNa]$^+$: 1302.4436, found: 1302.4410.

Au(I)-alkynyl-NHC complex 10: To a solution of KOH (6.00 mg, 107 µmol 1.5 eq.) and **5** (14.1 mg, 69.4 µmol, 1.0 eq.) in dry, degassed MeOH (2 mL), Cl-Au-sIMes (51.6 mg, 69.6 µmol, 1.0 eq.) was added under argon in the dark. The reaction mixture was stirred at 35°C for 16 h in the dark and concentrated *in vacuo*. Purification *via* RP-HPLC afforded a mixture of the desired product **10** and **2** in the ratio of 1:0.32 (15.6 mg, 17.1 µmol, 25%) as white solid. Partial retro Diels-Alder reaction seemed to occur during concentration of the HPLC fractions on the rotavap.

^{1}H NMR (600 MHz, MeOD-*d*$_4$, 296 K): δ/ppm = 7.47–7.45 (m, 3.3H, C*H* ^{im}) 7.17–7.14 (m, 3.3H, C*H* ^{aryl}), 6.73 (s, 0.6H, **2**-C*H* ^{alkene}), 6.22 (s, 2H, **10**-C*H* ^{alkene}), 5.23–5.21 (m, 2H, C*H* ^{furan}), 4.07 (s, 0.6H, **2**-C*H*$_2$), 3.86 (s, 2H, **10**-C*H*$_2$), 3.46 (dd, J = 3.7, 1.7 Hz, 2H, C*H* ^{maleimide}), 2.75–2.71 (m, 9.8H, C*H*$_3$), 2.46–2.43 (m, 9.8H, C*H*$_3$), 2.15–2.12 (m, 9.8H, C*H*$_3$). **^{13}C NMR (151 MHz, MeOD-*d*$_4$, 296 K):** δ = 189.7 (N$_2$*C*Au), 175.9 (**10**-*C*O ^{maleimide}), 171.4 (**2**-*C*O ^{maleimide}), 150.87 (*C* ^{aryl}), 143.23 (*C* ^{aryl}), 140.08 (*C* ^{aryl}), 137.93 (*C* ^{aryl}), 137.67 (*C* ^{aryl}), 137.64 (*C* ^{aryl}), 137.61 (*C* ^{aryl}), 136.07 (*C* ^{aryl}), 135.51 (**2**-*C*H ^{alkene}), 135.47 (**10**-*C*H ^{alkene}), 133.20 (*C*H ^{im}), 124.67 (*C*H ^{aryl}), 121.81 (**10**-CCAu), 98.17 (**2**-CCAu), 97.18 (**10**-CCAu), 80.72 (*C*H ^{furan}), 47.18 (*C*H ^{maleimide}), 29.26 (**10**-N*C*H$_2$), 28.28 (**2**-N*C*H$_2$), 23.74 (*C*H$_3$), 23.72 (*C*H$_3$), 18.31 (*C*H$_3$), 18.29 (*C*H$_3$), 16.95 (*C*H$_3$), 16.94 (*C*H$_3$). **ESI-HRMS (MeOH) (m/z):** Calculated for [C$_{32}$H$_{30}$AuN$_3$O$_9$S$_2$Na]$^-$: 884.0987, found: 884.0986.

Au(I)-alkynyl-triphenylphosphane complex 11: To a solution of KO^{t}Bu (17.0 mg, 152 µmol, 1.5 eq.) and **5** (20.7 mg, 102 µmol, 1.0 eq.) in degassed EtOH (11 mL), **8** (50.2 mg, 101 µmol, 1.0 eq.) was added under argon. The resulting suspension was stirred at room temperature for 18 h. The precipitate was isolated *via* centrifugation, followed by washing with EtOH (15 mL). After drying, the desired product **11** (44.8 mg, 67.7 µmol, 67%) was obtained as a colorless solid.
^{1}H NMR (400 MHz, CDCl$_3$, 296 K): δ/ppm = 7.55–7.39 (m, 15H, C*H* ^{aryl}), 6.44 (t, J = 0.9 Hz, 2H, C*H* ^{alkene}), 5.32 (ddd, J = 3.5 Hz, 1.7 Hz, 0.9 Hz, 2H, C*H* ^{furan}), 4.21 (d, J_{PH} = 1.6 Hz, 2H, C*H*$_2$), 3.52 (dd, J = 3.6 Hz, 1.7 Hz, 2H, C*H* ^{maleimide}). **^{13}C NMR (101 MHz, CDCl$_3$, 296 K):** δ/ppm = 173.9 (*C*O), 134.6 (*C* ^{alkene}), 134.4 (d, J_{PC} = 13.8 Hz, C_{ortho}), 131.7 (d, J_{PC} = 2.5 Hz, C_{para}), 129.8 (d, J_{PC} = 56.0 Hz, C_{ipso}), 129.3 (d, J_{PC} = 11.2 Hz, C_{meta}), 124.5 (d, J_{PC} = 142.4 Hz, C*C*Au), 95.3 (d, J_{PC} = 27.1 Hz, *C*CAu), 79.7 (*C* ^{furan}),

46.2 ($C^{maleimide}$), 28.9 (d, J_{PC} = 2.6 Hz, CH_2). **$^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$, 296 K):** δ/ppm = 41.8 (s). **ESI-HRMS (CH_2Cl_2:MeOH = 1:1) (m/z):** Calculated for $[C_{29}H_{23}AuNO_3PNa]^+$: 684.0973, found: 684.0967.

Retro Diels-Alder reaction

Au(I)-alkynyl-phosphane complex 1: A solution of **9** (26.9 mg, 21.0 µmol) in dry toluene (5.0 mL) was stirred at 70 °C for 4 h under argon. Concentration *in vacuo* and drying afforded the desired product **1** (25.3 mg, 20.9 µmol, 99%) as a yellowish oil.
^{1}H NMR (400 MHz, MeOD-d_4, 296 K): δ/ppm = 7.36 (dd, J_{PH} = 12.1 Hz, J = 8.8 Hz, 6H, PCC*H*), 7.02 (dd, J = 8.6 Hz, 1.8 Hz, 6H, C*H*C_qO^{aryl}), 6.81 (s, 2H, C*H*^{alkene}), 4.25 (s, 2H, NCH_2), 4.18–4.13 (m, 6H, $C_qOCH_2^{TEG}$), 3.85–3.80 (m, 6H, CH_2^{TEG}), 3.69–3.65 (m, 6H, CH_2^{TEG}), 3.65–3.55 (m, 24H, CH_2^{TEG}), 3.51–3.47 (m, 6H, CH_2^{TEG}), 3.32 (s, 9H, CH_3). **^{13}C NMR (101 MHz, MeOD-d_4, 296 K):** δ/ppm = 171.4 (*C*O), 163.0 (d, J_{PC} = 2.2 Hz, C_qO^{aryl}), 136.8 (d, J_{PC} = 15.4 Hz, PC*C*), 135.7 (C^{alkene}), 122.7 (d, J_{PC} = 61.8 Hz, P*C*), 116.7 (d, J_{PC} = 12.4 Hz, CC_qO^{aryl}), 111.4 (C*C*Au), 97.9 (*C*Au), 73.0 (CH_2), 71.8 (CH_2), 71.60 (CH_2), 71.58 (CH_2), 71.5 (CH_2), 71.4 (CH_2), 70.6 (CH_2), 68.9 (CH_2), 59.2 (CH_3), 28.6 (NCH_2). **$^{31}P\{^1H\}$ NMR (162 MHz, MeOD-d_4, 296 K):** δ/ppm = 37.4 (s). **^{1}H NMR (400 MHz, CD_2Cl_2, 296 K):** δ/ppm = 7.40 (ddd, J_{PH} = 12.1 Hz, J = 8.8 Hz, 1.7 Hz, 6H, PCC*H*), 6.98 (dd, J = 8.8 Hz, 1.8 Hz, 6H, C*H*C_qO^{aryl})), 6.70 (s, 2H, C*H*^{alkene}), 4.29 (d, J_{PH} = 1.7 Hz, 2H, NCH_2), 4.17–4.11 (m, 6H, $C_qOCH_2^{TEG}$), 3.85–3.79 (m, 6H, CH_2^{TEG}), 3.69–3.64 (m, 6H, CH_2^{TEG}), 3.64–3.53 (m, 24H, CH_2^{TEG}), 3.51–3.47 (m, 6H, CH_2^{TEG}), 3.32 (s, 9H, CH_3). **$^{31}P\{^1H\}$ NMR (162 MHz, CD_2Cl_2, 296 K):** δ/ppm = 38.1 (s). **ESI-HRMS (MeOH) (m/z):** Calculated for $[C_{52}H_{73}AuNO_{17}PNa]^+$: 1234.4174, found: 1234.4153.

Au(I)-alkynyl-NHC complex 2: A solution of **10** (4.64 mg, 5.11 µmol) in DMF (750 µL) was stirred at 70 °C for 4 h under argon. Concentration *in vacuo* and drying afforded the desired product **2** (4.4 mg, 5.2 µmol, quant.) as off-white solid.
^{1}H NMR (600 MHz, MeOD-d_4, 296 K): δ/ppm = 7.46 (s, 2H, C*H*^{imidazole}), 7.16 (s, 2H, C*H*^{aryl}), 6.73 (s, 2H, C*H*^{alkene}), 4.07 (s, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.13 (s, 3H, CH_3). **ESI-MS (MeOH) (m/z):** Calculated for $[C_{28}H_{26}AuN_3O_8S_2Na]^-$: 816.0725, found: 816.5405.

Au(I)-alkynyl- triphenylphosphane complex 3: A solution of **11** (14.6 mg, 22.1 µmol) in dry toluene (2.5 mL) was stirred at 70 °C for 7.5 h under argon. Concentration *in vacuo* and drying afforded the desired product **3** (13.1 mg, 22.1 µmol, quant.) as a colorless solid.
^{1}H NMR (400 MHz, $CDCl_3$, 296 K): δ/ppm = 7.55–7.39 (m, 15H, C*H*^{aryl}), 6.79 (s, 2H, C*H*^{alkene}), 4.43 (d, J_{PH} = 1.4 Hz, 2H, CH_2). **^{13}C NMR (101 MHz, $CDCl_3$, 296 K):** δ/ppm = 169.8 (*C*O), 134.5 (C^{alkene}), 134.4 (d, J_{PC} = 13.7 Hz, C_{ortho}), 131.7 (d, J_{PC} = 2.2 Hz, C_{para}), 129.8 (d, J_{PC} = 55.8 Hz, C_{ipso}), 129.3 (d, J_{PC} = 11.2 Hz, C_{meta}), 124.3 (d, J_{PC} = 142.3 Hz, C*C*Au), 96.3 (d, J_{PC} = 27.0 Hz, *C*CAu), 28.2 (d, J_{PC} = 2.3 Hz, CH_2). **$^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$, 296 K):** δ/ppm = 41.9 (s). **ESI-HRMS (CH_2Cl_2:MeOH = 1:1) (m/z):** Calculated for $[C_{25}H_{19}AuNO_2PH]^+$: 594.0892, found: 594.0916.

Diels-Alder ligation

Biotinylated amphiphilic Au(I)-alkynyl-phosphane complex 4: A solution of **1** (25.3 mg, 20.9 µmol, 1.1 eq.) in CD_2Cl_2 (0.6 mL) was treated with **12** (6.4 mg, 19.4 µmol, 1.0 eq.) in a NMR tube under argon in the dark. The progress of the reaction was followed by ^{1}H and $^{31}P\{^1H\}$ NMR analysis while stepwise heat acceleration from room temperature up to 50 °C over a period of ten days was performed until full conversion was observed. Concentration *in vacuo* and drying afforded the desired product **4** as a yellowish oil, which contained a small amount of unreacted **12** (20%, estimated *via* ^{1}H NMR analysis). For toxicity studies a small amount of **4** was purified *via* RP-HPLC.

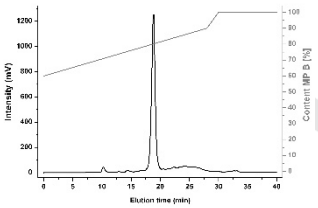

Figure 7. RP-HPLC chromatogram of **4** monitored at λ = 254 nm on a semi-preparative *Luna RP-C18(2)* column (flow: 20.0 mL/min). Details about the used gradient (dashed line) are provided in the SI.

^{1}H NMR (600 MHz, CD_2Cl_2, 294 K, mixture of isomers): δ/ppm = 7.40 (dd, J_{PH} = 12.0 Hz, J = 8.8 Hz, 6H, PCC*H*), 6.98 (dd, J = 8.7 Hz, 1.5 Hz, 6H, C*H*C_qO^{aryl}), 5.78–5.71 (m, 2H, C*H*^{alkene}), 5.15 *and* 5.12 (s, 1H, N*H*), 4.87 *and* 4.85 (s, 1H, N*H*), 4.67–4.58 (m, 1H, $CO_2CH^ACH^BCH$), 4.51–4.42 (m, 2H, C*H*CH_2S, $CO_2CH^ACH^BCH$), 4.33–4.26 (m, 2H, C*H*CHS), 4.18–4.11 (m, 8H, NCH_2, $C_qOCH_2^{TEG}$), 3.85–3.79 (m, 6H, CH_2^{TEG}), 3.68–3.65 (m, 6H, CH_2^{TEG}), 3.63–3.54 (m, 24H, CH_2^{TEG}), 3.50–3.47 (m, 6H, CH_2^{TEG}), 3.32 (s, 9H, OCH_3), 3.29–3.24 (m, 1H, $CH_2CHCH^{maleimide}$), 3.18–3.13 (m, 1H, C*H*S), 3.09–3.05 (m, 1H, $CH_3CHCH^{maleimide}$), 2.92–2.87 (m, 1H, CH^AH^BS), 2.70–2.64 (m, 1H, CH^AH^BS), 2.64–2.59 (m, 1H, CO_2CH_2CH), 2.47–2.41 (m, 1H, C*H*CH_3), 2.38–2.31 (m, 1H, $CH_2CH_2CO_2$), 1.77–1.58 (m, 4H, CH_2^{alkyl}), 1.48–1.39 (m, 5H, CH_2^{alkyl}, CHCH_3), **^{13}C NMR (151 MHz, CD_2Cl_2, 294 K, mixture of isomers):** δ/ppm = 176.84 *and* 176.82 (*C*O^{maleimide}), 176.64 *and* 176.60 (*C*O^{maleimide}), 173.81 *and* 173.80 (*C*O^{ester}) 163.4 (*C*O^{urea}), 161.9 (d, J_{PC} = 2.2 Hz, C_qO^{aryl}), 136.2 (d, J_{PC} = 15.5 Hz, PC*C*), 135.1 (C^{alkene}), 129.32 *and* 129.26 (C^{alkene}), 125.8 (d, J_{PC} = 143.3 Hz, C*C*Au), 122.1 (d, J_{PC} = 61.4 Hz, P*C*), 115.7 (d, J_{PC} = 12.3 Hz, CC_qO^{aryl}), 95.9 (d, J_{PC} = 27.3 Hz, *C*CAu), 72.4 (CH_2^{TEG}), 71.3 (CH_2^{TEG}), 71.1 (CH_2^{TEG}), 71.01 (CH_2^{TEG}), 70.99 (CH_2^{TEG}), 70.9 (CH_2^{TEG}), 69.9 (CH_2^{TEG}), 68.2 (CH_2^{TEG}), 64.75 *and* 64.72 (CO_2CH_2CH), 62.23 *and* 62.23 (*C*HCHS), 60.61 *and* 60.57 ($CHCH_2S$), 59.2 (OCH_3), 55.91 *and* 55.89 (*C*HS), 45.5 ($CH_3CHCH^{maleimide}$), 43.1 *and* 43.0 ($CH_2CHCH^{maleimide}$), 41.16 *and* 41.13 (CH_2S), 36.21 *and* 36.17 (CO_2CH_2CH), 34.38 *and* 34.35 ($CH_2CH_2CO_2$), 31.7 ($CHCH_3$), 29.2 (NCH_2), 28.82 *and* 28.80 (CH_2^{alkyl}), 28.77 *and* 28.75 (CH_2^{alkyl}), 25.38 *and* 25.34 (CH_2^{alkyl}), 17.0 ($CHCH_3$). **$^{31}P\{^1H\}$ NMR (162 MHz, CD_2Cl_2, 296 K):** δ/ppm = 38.0 (s). **ESI-HRMS (MeOH) (m/z):** Calculated for $[C_{68}H_{97}AuN_3O_{20}PSNa]^+$: 1558.5681, found: 1558.5712.

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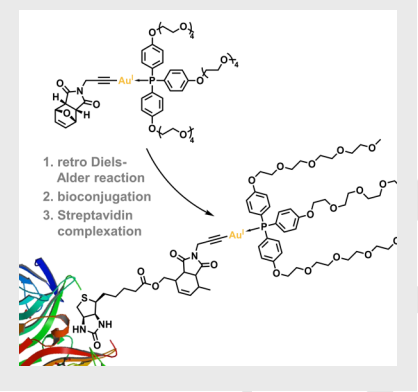
The ligation of Au(I)-metalloamphiphiles with biomolecules is reported. Two different polar ligands were applied: a dendritic tetraethylene glycol functionalized phosphane and a sulfonated *N*-heterocyclic carbene. A biotinylated Au(I)-alkynyl-phosphane complex was obtained by retro Diels-Alder reaction of a furan protected maleimide-Au(I)-precursor complex, followed by Diels-Alder cycloaddition with a diene functionalized biotin under mild conditions.



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Reversible covalent and supramolecular functionalization of water-soluble Au(I)-complexes