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Synthesis and Structure–Activity Relationship Study of Antimicrobial Auranofin against ESKAPE Pathogens

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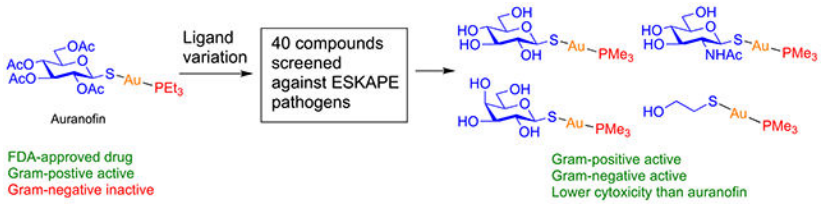
Abstract

Auranofin, an FDA-approved arthritis drug, has recently been repurposed as a potential antimicrobial agent; it performed well against many Gram-positive bacteria, including multidrug resistant strains. It is, however, inactive toward Gramnegative bacteria, for which we are in dire need of new therapies. In this work, 40 auranofin analogues were synthesized by varying the structures of the thiol and phosphine ligands, and their activities were tested against ESKAPE pathogens. The study identified compounds that exhibited bacterial inhibition (MIC) and killing (MBC) activities up to 65 folds higher than that of auranofin, making them effective against Gram-negative pathogens. Both thiol and the phosphine structures influence the activities of the analogues. The trimethylphosphine and triethylphosphine ligands gave the highest activities against Gramnegative and Gram-positive bacteria, respectively. Our SAR study revealed that the thiol ligand is also very important, the structure of which can modulate the activities of the Au^{I} complexes for both Gram-negative and Gram-positive bacteria. Moreover, these analogues had mammalian cell toxicities either similar to or lower than that of auranofin.

Graphical Abstract



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Author Contributions
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Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b00550. Synthesis of **1b–6b**, **7d**, and **8f**; general procedure for qNMR; ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, ^{19}F NMR, and HRMS spectra of intermediates and final products; purity assessments of compounds **1–40** by qHNMR, including methods of determination and calculation; FAAS calibration curves A and B for Au concentration determination; MIC/MBC tables; and cytotoxicity data of auranofin and analogues (PDF)
Molecular formula strings (CSV)

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INTRODUCTION

The increasing prevalence of resistance to the majority of existing antibiotics has generated a pressing global healthcare crisis.[1] To undermine the actions of antibiotics, bacteria have developed powerful resistance mechanisms, including mutational alterations of target proteins, loss of membrane porins,[2] production of enzymes that degrade antibiotics,[3–5] and overexpression of efflux pumps that drive antibiotics out of the bacterium.[6–8] Certain highly resistant bacteria have acquired multiple mechanisms against all available antibiotics.[9–11] The situation is especially dire for Gram-negative bacteria. The recent additions of antibiotics to the clinical pipeline have been limited to treating Gram-positive infections, and there have been no new classes of clinically approved antibiotics for Gram-negative bacteria since the discovery of quinolones in 1968.[12] Untreatable antimicrobial resistance (AMR) is rapidly emerging in, for example, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* strains that are resistant to all commonly used antibiotics, including fluoroquinolones, β-lactams, macrolides, aminoglycosides, and tetracyclines, contributing to the majority of deaths caused by hospital-acquired infections. To combat AMR, new strategies are being developed, such as the use of efflux inhibitors,[13] bacteriophages,[14] and nanoparticles[15–17] as antibacterial agents. However, these endeavors are often associated with lengthy and costly development processes; therefore, other avenues are needed. One such route is drug repurposing, which is the redirecting of known antiinflammatory drugs, anticancer drugs, and other drugs into antimicrobial use, providing viable candidates and potentially low cost ways to fight against AMR.[18–20]

Auranofin is a gold compound consisting of a tetraacetylated thioglucose and a triethylphosphine coordinated to Au^{I} (**1**, Figure 1). The medicinal use of gold compounds can be traced back to the 19th century in treating tuberculosis, syphilis, and inflammatory rheumatoid diseases.[21,22] Early studies showed that coordination of phosphine to Au^{I} gave nonionic complexes that had high lipid solubility.[23] In the search for better antiarthritic drugs, Sutton et al. at Smith Kline and French Laboratories discovered 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*-(triethylphosphine) gold (i.e., auranofin), which gave excellent therapeutic efficacy and high serum gold concentration.[24] Auranofin was subsequently approved by the FDA in 1985 as an oral anti-inflammatory aid in the treatment of rheumatoid arthritis.[25,26] Its safety has been well-documented, with no reported concerns in terms of carcinogenicity, serious side effects, or other long-term safety issues.[27] Auranofin has shown broad activity toward a number of thiol-dependent reductases that are essential for maintaining intracellular levels of free thiols and redox homeostasis. Inhibition of these enzymes by auranofin decreases cellular reducing capacity and weakens the defense against oxidative stress. Its broad activity has sparked increasing interest to repurpose this drug as potential therapy for other diseases.[28] For example, its anticancer activity has been reported for different types of tumors, including melanoma and leukemia,[29,30] and auranofin compares well with the currently marketed drug cisplatin.[31,32] Auranofin has also shown strong activity against parasites that cause malaria[33] and severe diarrhea,[34] as well as invasive fungi that are particularly difficult to target.[35,36] Auranofin is currently undergoing a phase II clinical trial for recurrent epithelial ovarian cancer.[37] The results from an NIH-sponsored phase I trial to characterize the pharmacokinetics and safety of auranofin in

healthy volunteers against the parasites *Entamoeba histolytica* and *Giardia intestinalis* supported the safety of auranofin as a broad-spectrum antiparasitic drug.[25,38]

The precise molecular mechanism of auranofin's mode of action is still unclear. It is generally believed that upon administration of auranofin, the tetraacetylated thioglucose ligand is rapidly displaced by blood thiols.[39] The Au^{I} or $[(PEt_3)Au]^{+}$ species emanating from auranofin then bind to the redox-active selenocysteine or cysteine residues in thioredoxin reductase (TrxR) or other reductases.[40] X-ray structure analyses, however, are inconclusive. In some cases, no gold was found in the active sites of, for example, *E. histolytica* TrxR[41] or *Schistosoma mansoni* thioredoxin-glutathione reductase.[40] In the case of *Leishmania infantum* trypanothione reductase, the tetraacetylated thioglucose was also found at the trypanothione binding site in addition to Au^{I}, hinting at a dual mechanism of inhibition.[42]

Auranofin has also been assessed for its antimicrobial activity against different bacteria, where it showed no activity toward Gram-negative bacteria but performed well compared with current antibiotics against many Gram-positive bacteria, including multidrug resistant strains, and *Mycobacterium tuberculosis*.[18,43–47] The source of this activity was postulated as the inhibition of the bacterial TrxRs, leading to lower intracellular thiol concentrations, disruption of the bacterial thiol-redox homeostasis, and weakening of the bacterial defense against oxidative stress.[43] Other mechanisms have also been proposed, including disruption of selenium metabolism by forming a stable Au–Se adduct in the case of *Clostridium difficile*[45] and inhibition of cell wall, DNA, protein, and toxin syntheses in the case of methicillin-resistant *Staphylococcus aureus* (MRSA).[48] The thiophilic nature of auranofin, which may react with other cysteine-containing enzymes, and the suggested multiple modes of action argue well for the potential of auranofin against drug-resistant bacteria and in preventing the emergence of resistance. In fact, studies have shown the inability of *M. tuberculosis*[43] and *S. aureus*[48] to generate auranofin-resistant mutants.

The ineffectiveness of auranofin toward Gram-negative bacteria was suggested to be the result of their glutathione system, glutathione/glutathione reductase, being able to compensate for the loss of reducing capability in the Trx–TrxR system caused by auranofin.[43] An alternative hypothesis was also proposed, suggesting that the outer membranes of Gram-negative bacteria were an effective barrier to auranofin.[48] This argument was supported by results showing that Gramnegative strains could be made susceptible to auranofin by coadministration with a membrane permeable agent like polymyxin B.[48,49]

There are limited structure–activity relationship (SAR) studies on auranofin.[24,49,50] These and other investigations generally conclude that the thioglucose ligand is not essential, and the pharmacophore is likely either Au^{I} or the $[(PEt_3)Au]^{+}$ in complex with the target. In this study, we synthesized 40 auranofin analogues by varying the structures of the thiol and phosphine ligands and tested their antimicrobial activities against *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*, the six ESKAPE pathogens that have shown growing multidrug resistant virulence and cause two-thirds of all clinical infections,[51] as well as a control strain of Gram-negative *E. coli*. A preliminary *in vitro* toxicity study was also carried

out on A549 cells. Our results demonstrate the following: (1) Auranofin can be made active toward Gramnegative pathogens by manipulating the ligands coordinated to Au^{I}. Although auranofin itself has no activity against Gramnegative pathogens, some analogues become active even for *P. aeruginosa*. (2) The thiol ligand is important for the activity. (3) The analogues showed mammalian cell toxicity either similar to or lower than that of auranofin.

RESULTS

Design and Synthesis of Auranofin Analogues.

Auranofin is a monomeric linear complex consisting of a Au(I) core coordinated with a peracetylated thioglucose and a triethylphosphine ligand (**1**, Figure 1). Gold is a late transition metal with the most common oxidation states of +1 (aurous Au^{I}) and +3 (auric Au^{III}). Approved gold drugs, including sodium aurothiomalate and auranofin, are Au^{I} compounds, probably because of the high toxicity of Au^{III} complexes (e.g., $HAuCl_4$).[27] Au^{I} is a soft acid.[52] Au^{I} salts coordinated with relatively hard bases like chloride are unstable and generally disproportionate into metallic gold and Au^{III} in the presence of water.[27] As such, the most stable Au^{I} complexes and nanoclusters have soft ligands like thiols and phosphines. The roles of the phosphine and thiol ligands were investigated by Sutton et al., who studied the *in vivo* antiarthritic activities of 13 auranofin and analogues prepared by combining different alkylphosphine ligands (Me, Et, *i*-Pr, and *n*-Bu) with either Cl or a thio glucose ligand.[24] It was concluded that the phosphine ligand played a major role in the activity of the gold complex, whereas the nonphosphine ligand had less of an effect. Triethylphosphine (PEt_3), the ligand in auranofin, gave the best therapeutic efficacy and the highest serum gold concentration. The authors also showed that the nonphosphine ligand could modulate the cytotoxicity, such that the acetylated thio glucose ligand rendered auranofin less toxic than triethylphosphine gold chloride (rat acute oral LD_{50} values of 265 and 79.0 mg/kg, respectively), which was further confirmed by other studies.[53]

Here, we conducted an SAR study by varying the structures of the thiol and the phosphine ligands on auranofin. Three groups of compounds were initially synthesized: Group 1 compounds are analogues with different thio sugar structures (**2–14**), Group 2 compounds are analogues with aromatic or aliphatic thiols (**15–23**), and Group 3 compounds are analogues with varying phosphine structures (**24–36**). Subsequent antibacterial activity studies of these compounds led to the identification of structural features that were then applied to the design and synthesis of analogues **37–40**.

In Group 1 (Figure 1), the peracetylated thioglucose ($Glc(Ac)_4$) ligand in auranofin was replaced by peracetylated galactose ($Gal(Ac)_4$, **2**), *N*-acetylglucosamine ($GlcNAc(Ac)_3$; **3, 4,** and **8**), maltose ($Mal(Ac)_7$, **5**), lactose ($Lac(Ac)_7$, **6**), and trehalose ($Tre(Ac)_7$, **7**). Deacetylated analogues of Glc (**9**), Gal (**10**), Man (**11**), GlcNAc (**12**), Lac (**13**), and Mal (**14**) were also included to test the impact of lipophilicity on the activities of the analogues.

Compounds **1–6** were synthesized from β-glycosyl thiols **1c–6c** and Et_3PAuCl according to reported methods (Scheme 1).[54–57] Briefly, α-glycosyl halides **1a–6a** were treated with KSAc to give peracetylated thioglycosides **1b–6b** through a straightforward S_N2 reaction. *S*-Deacetylation of the anomeric thioacetates **1b–6b** by transthioesterification using

dithiothreitol (DTT) afforded the glycosyl thiols **1c–6c**, which were then treated with Et_3PAuCl under mild basic conditions for 1–1.5 h to give auranofin, **1**, and analogues **2–6** in good to excellent yields.

The synthesis of the $Tre(Ac)_7$ analogue, **7**, follows the procedure shown in Scheme 2. Reaction of D-trehalose **7a** with benzaldehyde dimethyl acetal catalyzed by TsOH gave a mixture of trehalose, trehalose benzylidene, and trehalose dibenzylidene, which was purified after acetylation to give peracetylated trehalose benzylidene **7b** in 40% overall yield. The *N*-bromosuccinimide (NBS)-mediated cleavage of benzylidene acetal **7b** gave the 6-bromo trehalose derivative, **7c**, in 89% yield. Bromide **7c** was then converted to thioacetate **7d** through the iodide intermediate in an overall 89% yield. Deprotection of the benzoyl group followed by acetylation afforded **7e** (72%, two steps), which was then S-deacetylated via DTT-mediated transthioesterification to afford the thiol derivative **7f** in 89% yield. Direct coupling of **7f** with Et_3PAuCl gave only the dinuclear gold complex **7**. Even after reduction of the amount of Et_3PAuCl, no mononuclear gold(I) complex was observed. Stronger bases like DBN gave similar results, whereas NaOMe and NaOH removed the acetyl groups instead. The formation of the dinuclear gold structure was supported by NMR and high-resolution mass spectrometry (HRMS). The integrations of peaks at 1.80 and 1.17 ppm in the ^1H NMR (Figure S34), assigned to CH_2 and CH_3 in PEt_3, respectively, corresponded to two PEt_3 ligands in the complex. The ^{31}P NMR gave a single peak at 34.66 ppm (Figure S36), whereas for all other triethylphosphine mononuclear gold complexes synthesized in this work, the chemical shifts of P in PEt_3 appeared at 36–39 ppm instead. The structure was further supported by HRMS, where the highest intensity peak at 1281.2744 corresponded to the molecular ion of **7**. The thiolate in **7f**, formed from a less acidic thiol at C-6, is more nucleophilic than those thiolates formed from more acidic anomeric thiols at C-1 in compounds **1–6**, allowing the C-6 thiolate to react further with Et_3PAuCl to form dinuclear gold complex **7**. There are similar reports in the literature on the formation of dinuclear gold complexes, such as that from the reaction of a mononuclear gold(I) complex bearing a cysteine-containing dipeptide ligand with $[Au(OTf)(PPh_3)]$.[58]

Compound **8** was synthesized from the D-glucosamine hydrochloride **8a** (Scheme 3). Reaction with *p*-anisaldehyde gave the Schiff base glucosamine imine **8b**, which was then peracetylated to afford **8c**. Subsequent hydrolysis in HCl/acetone gave peracetylated glucosamine hydrochloride **8d**, which reacted with chloroacetic anhydride to afford **8e**. A final substitution by 1,4-benzenedithiol followed by coupling with Et_3PAuCl gave gold complex **8** in 96% yield.

The analogues containing unprotected thio sugar ligands (**9–14**) were synthesized by treating the corresponding peracylated thio sugar analogues with NaOMe/MeOH followed by Et_3PAuCl in a one-pot reaction (Scheme 4).

Analogues **15–23** in Group 2 are compounds with the thio sugar replaced by an aromatic or aliphatic thiol ligand (Figure 2). Both electron-donating and electron-withdrawing groups were included to test the electronic effect of the thiol ligand on the antimicrobial activity. For the synthesis of gold(I) complexes with aromatic thiol ligands (**15**, **16**, and **18–20**), the corresponding thiol was treated with Et_3PAuCl in DCM in the presence of DBU, and the

product was obtained after straightforward purification by column chromatography (condition a, Scheme 5). Analogues with aliphatic thiol ligands (**21–23**) were prepared by reacting the corresponding thiol with Et_3PAuCl in the presence of one equivalent of NaOMe in methanol (condition b, Scheme 5). Analogue **17** was also synthesized following this method and was purified by recrystallization.

Analogues **24–36** in Group 3 are compounds with different phosphine structures (Figure 3). Aliphatic phosphines with varying chain lengths (methyl, ethyl, and *n*-butyl) and aromatic phosphines with either electron-donating (OMe) or electron-withdrawing (F or CF_3) groups were prepared as both gold chloride and gold thio sugar complexes. AuCl was generated in situ by reducing $HAuCl_4$ with 2,2′-thiodiethanol, which was treated immediately with a cold solution of 1.05 equiv of the corresponding triphosphine in EtOH/DCM to give triphosphine gold chlorides **24–30** (reaction a, Scheme 6).[24]

Auranofin analogues **31–36** were prepared straightforwardly in high yields by treating the corresponding triphosphine gold chlorides (**24** and **26–30**) with peracetylated thioglucose **1c** (reaction b, Scheme 6)

In Vitro Antimicrobial Activities.

The antimicrobial activities of auranofin and its analogues were tested against ESKAPE pathogens, including four Gram-negative strains (*A. baumannii*, *P. aeruginosa*, *E. cloacae*, and *K. pneumonia*) and two Gram-positive strains (*S. aureus* and *E. faecium*). A quality control strain, *E. coli* ATCC 25922, was included in all assays and validated with ciprofloxacin and teicoplanin in every trial. The results are presented in Tables 1–3 (in μM) and in Tables S1–S3 (in μg/mL) as minimal inhibitory concentrations (MICs), the lowest concentration that inhibits the growth of the bacteria, and minimal bactericidal concentrations (MBCs), the lowest concentration that kills the bacteria. Auranofin (**1**) showed potent activities against both Gram-positive strains, *S. aureus* and *E. faecium*, with MICs (MBCs) of 0.03 (0.06) and 0.12 (0.25) μg/mL, respectively (Table S1). It showed weak activity against *A. baumannii* and *E. coli*, with MIC/MBC values of 32 and 16 μg/mL, respectively. Auranofin lacks significant activity against *P. aeruginosa*, *E. cloacae*, and *K. pneumonia*. These results are in agreement with previous reports that consistently showed the anti-Gram-positive-only activity of auranofin.[18,43,44,47,48,59,60]

The results of Group 1 compounds (i.e., analogues with varying thio sugar structures, Figure 1) are shown in Table 1. Changing $Glc(Ac)_4$ in auranofin (**1**) to $Gal(Ac)_4$ (**2**) did not cause much difference in activity against any of the strains. The GlcNAc analogue, **3**, showed 2–4 times increases in activity against all Gram-negative strains and *E. faecium*. Substituting the *N*-acetyl group of GlcNAc with an *N*-trichloroacetyl group (**4**) completely diminished the enhancement in the activities against all Gram-negative strains, and the GlcNAc analogue with an aromatic linker (**8**) showed similar results. However, compound **4** showed excellent activity against *S. aureus*, lowering the MIC/MBC of auranofin by up to 4 times. The disaccharide maltose analogue, **5**, also enhanced the activity against *S. aureus*, *E. faecium*, *P. aeruginosa*, and *E. cloacae* by 2–4 times. This was, however, not observed for the other disaccharide lactose analogue, **6**, which showed no improvement against Gram-positives and

was worse against all Gramnegative strains. The trehalose analogue, **7**, increased the activity against *E. cloacae* by 8-fold and was 15 times better than auranofin against *S. aureus*, with an MIC/MBC of 6 nM, perhaps because of the presence of two $AuPEt_3^+$ in **7**.[58] The deacetylated auranofin analogue, **9**, had similar activity to that of auranofin (**1**) against all strains. Among the other deacetylation analogues, compound **10** showed 3 times enhancement in activity against *E. cloacae*, and compound **12** was 2–3 times better than auranofin against *S. aureus* and *E. coli*.

Table 2 lists the MIC/MBC values of the Group 2 analogues, which contained aromatic and aliphatic thiol ligands (**15–23**, Figure 2). Among the compounds bearing aromatic thiol ligands (**15–20**), those with electron-donating groups on their phenyl rings (**16–18**) were generally more active than auranofin. Compound **17**, with a *p*-aminophenyl group, showed the highest activity enhancement of up to 10 times over that of auranofin against all strains. The electron-withdrawing groups (NO_2 and CF_3), on the other hand, made analogues **19** and **20** completely inactive toward all Gram-negative strains tested. Compound **19** is, however, very active against *S. aureus*, lowering the MIC/MBC of auranofin by up to 10 times. Replacing the thio sugar in auranofin with an aliphatic thiol ligand generally increased the activity (**21–23**). For example, compound **21** had an MIC 19 times lower than that of auranofin against *E. cloacae* as well as against *K. pneumoniae*, and it showed activity against *P. aeruginosa* with a MIC/MBC of 41 μM, which was 9 times better than that of auranofin. For *E. faecium*, the activities of most analogues were similar to that of auranofin, with the exception of compounds **16** and **17**, which had MICs more than 7 times lower than that of auranofin. With the exception of compound **17**, all other analogues showed higher activities than that of auranofin against *S. aureus*.

The compounds in Group 3 are analogues with trialkylphosphines of varying chain lengths (**24–26**, **31**, and **32**) and triarylphosphines with different electronic properties (**27–30** and **33–36**, Figure 3); the MIC/MBC values are shown in Table 3. For the Gram-negative strains, the activity generally decreased with increasing alkyl chain length (**24** > **25** > **26** and **31** > **1** > **32**). Replacing triethylphosphine with trimethylphosphine resulted in a drastic enhancement in activity. For example, the trimethylphosphine analogue **31** improved the activity of auranofin against all Gram-negatives tested. The MICs of **31** against *E. cloacae* and *K. pneumoniae* were 60- and 30-fold lower, making it active against these two strains, whereas auranofin is inactive for both. On the other hand, the tri-*n*-butylphosphine ligand completely diminished the activity of the resulting analogue, **32**, against all Gram-negatives. Aromatic phosphine ligands were also detrimental. Analogues with triarylphosphine ligands having either electron-withdrawing or electron-donating groups (**33–36**) showed almost no activity against the Gram-negative strains.

For the two Gram-positive strains, replacing triethylphosphine in auranofin with trimethylphosphine (**31**) had almost no impact on activity. The longer tri-*n*-butylphosphine ligand decreased the activity by more than an order of magnitude (**32** vs **1** and **26** vs **25**), as did all triarylphosphine ligands (**27–30** and **33–36**).

These results demonstrated that the Gram-negative activity of auranofin can be effectively modulated by altering the thiol and phosphine ligands. The following conclusions can be

drawn from these data with regard to Gram-negative activities: (1) Deprotected thio sugars do not decrease the activity (e.g., **9–14**, Table 1). It was reported that following oral administration, auranofin metabolized to yield deacetylated glucose.[61] (2) Among the nonsugar thiol ligands, mercaptoethanol showed the best activity enhancement against Gram-negatives (**21**, Table 2). (3) Trimethylphosphine is a substantially better ligand than triethylphosphine against Gram-negatives (**24** and **31**, Table 3). On the basis of these observations, analogues **37–40** were designed (Figure 4). Compounds **37–39,** which have trimethylphosphine ligands and deprotected Glc, Gal, and GlcNAc thio sugars, respectively, were prepared following reaction b in Scheme 4, except that Me_3PAuCl was used instead of Et_3PAuCl. All three compounds showed substantial enhancement in activity compared with that of auranofin against almost all Gramnegative strains (Table 4). Aside from the MIC against *P. aeruginosa*, the MICs against *A. baumannii*, *K. pneumoniae*, and *E. coli* were lowered by 3–12-fold, and that against *E. cloacae* was lowered by up to 44-fold. The highest activity was observed for compound **38**, the deacetylated Gal and trimethylphosphine analogue, which reached MIC/MBC values of 4.5 and 34 μM against *E. cloacae* and *K. pneumoniae*, bacteria that are otherwise unaffected by auranofin.

Analogue **40**, with the best phosphine ligand (trimethylphosphine) and the best thiol ligand (mercaptoethanol), was synthesized following condition b in Scheme 5 using Me_3PAuCl instead of Et_3PAuCl. As expected, this compound had the highest activity against all five Gram-negative strains (Table 4). The best activity enhancement was seen for *E. cloacae*, against which it was 65-fold more active than auranofin, with an MIC/MBC of 2.9 μM.

When comparing the activities of all trimethylphosphine analogues and auranofin (Table 4), it can be seen that trimethylphosphine was the best ligand for Gram-negatives. Deacetylation of the thio glucose on **31** lowered the activity of the resulting analogue, **37**, against most Gram-negatives, but the activity could be recovered by using the small polar ligand mercaptoethanol. This was not the case for the Gram-positives, against which **37–40** showed almost no change in activity.

Cytotoxicity.

The cytotoxicity was next tested against A549 human alveolar epithelial cancer cells, and the results are presented in Figure 5 and Table S5. Except for compounds **32–35**, all other analogues had toxicities either similar to or lower than that of auranofin. As anticipated, the deprotected thio sugar analogues **9–14** and **37–39** were 3–10 times less toxic compared with auranofin, **1** (IC_{50} = 11.3 ± 1.9 μM). Analogues **31** and **40**, which showed the largest improvement in activity against Gram-negative strains, had lower toxicity (IC_{50} = 20.6 ± 3.6 and 35.1 ± 8.9 μM, respectively) than auranofin.

DISCUSSION AND CONCLUSIONS

Synthesis of Au^I Complexes.

Auranofin can be straightforwardly synthesized from peracetylated thio glucose and Et_3PAuCl using K_2CO_3 as the base in the biphasic medium DCM/H_2O.[55] Analogues **2–6** were synthesized following the same protocol (Scheme 1). However, this method is not

general. For example, in the synthesis of **32–36**, which contain more nonpolar triphosphines, the reactions required much longer times, and the yields were unsatisfactory, probably because side reaction formed byproducts like phosphine oxide. We then developed a single-phase reaction using DBU as the base in an organic solvent. This method worked well for most analogues (**1–6**, **8**, **15–16**, **18–20**, and **31–36**). The reactions were completed within 2 h, and the products could be purified straightforwardly by column chromatography. For **1–6** and **31–36**, toluene was the preferred solvent instead of DCM (Scheme 6), as it was found that the thio sugars reacted with DCM to form the corresponding glycosylthiomethyl chlorides.[62] Aromatic thiols were inert to DCM under the same conditions; thus analogues **15–16** and **18–20** were prepared in DBU/DCM (condition a, Scheme 5). However, this method failed for compound **17**, for which TLC indicated the formation of an unstable compound. In this case, a strong base, NaOMe, was used, and compound **17** was successfully synthesized in 92% yield. This condition works well for substrates that can tolerate strong bases like NaOMe and are soluble in MeOH, such as for the cases of **9–14** (Scheme 4) and **21–23** (condition b, Scheme 5).

Selection of Bacteria.

ESKAPE pathogens were chosen as they are responsible for the majority of nosocomial infections and also because they provide a good scope of strains including both Gram-negative and Gram-positive species. *A. baumannii* NCTC 13420 was originally identified in 24 hospitals in the United Kingdom during 2000–2003; it produces OXA-51-like β-lactamases and is highly resistant to ampicillin, piperacillin, piperacillin/tazobactam, ceftazidime, cefotaxime, gentamicin, and ciprofloxacin.[63] *P. aeruginosa* NCTC 13437 produces VIM-10 metallo-carbapenemase and Vietnamese extended-spectrum β-lactamase (VEB-1).[64] Wild-type strain *E. cloacae* NCTC 13405 was originally named *E. cloacae* 684 and was isolated from patients at the London Hospital from 1982 to 1983.[65] This strain has inducible AmpC β-lactamase and is resistant to ampiriHin/amoxirillin and cefoxitin. *K. pneumoniae* ATCC 700603 was isolated from the urine of a hospitalized patient in Richmond, Virginia, in 1994, and it produces SHV-18 extended spectrum β-lactamase (ESBL).[66] JE2 (USA300) is a methicillin-resistant *S. aureus* (MRSA) strain that is also resistant to β-lactams, ciprofloxacin, tetracycline, macrolides (erythromycin), lincosamides (clindamycin), streptogramin B, and mupirocin.[67] *E. faecium* ATCC 700221, a positive fecal vancomycin-resistant enterococcus (VRE) isolate, is resistant to several antibiotics, including vancomycin and teicoplanin.[68] The quality control strain, Gram-negative *E. coli* ATCC 25922, was included in all assays and validated with ciprofloxacin and teicoplanin.

In Vitro Antimicrobial Activities.

Several conclusions can be drawn from the SAR studies. (1) Our data are consistent with previous reports that auranofin is active against Grampositive bacteria but inactive toward Gram-negative bacteria. (2) Auranofin can be made active toward Gram-negative bacteria by changing the ligands coordinated to Au^{I}. Although auranofin itself has no activity against Gram-negative pathogens, some analogues become active even for *P. aeruginosa*. (3) The thiol ligand is important for activity in addition to modulating the toxicity. For instance, replacing tetraacetylated thioglucose with 2-mercaptoethanol (**21**) increased the activity of auranofin by 5–19 times for all Gram-negative pathogens while decreasing the cytotoxicity

by 1.6 times. (4) The vast majority of the analogues showed mammalian cell toxicity either similar to or lower than that of auranofin (Figure 5).

Gram-Positive Activities.

Among the thio sugar analogues (Figure 1), the $TreNAc_7$ ligand (**7**) showed the highest activity enhancement of 7-fold and an MIC/MBC value of 6 nM against *S. aureus* (Table 1). Also analogues **16**, **19**, **22**, and **23** showed high activity against *S. aureus*, with MICs in the nanomolar range (Table 2). The best compounds for *E. faecium* are analogues **16–18**, which have electron-donating groups on their benzene thiol ligands; they show 8-fold better activity than auranofin (Table 2). Triethylphosphine appears to be the best phosphine ligand for the two Gram-positive strains. Replacing triethylphosphine with trimethylphosphine (**31**) did not improve the activity (Table 3). Tri-*n*-butylphosphine and all triarylphosphines drastically decreased the activity (Table 3).

Gram-Negative Activities.

For the Gram-negative strains, there was a clear preference for aliphatic over aromatic phosphines. Analogues bearing triarylphosphines with either electron-withdrawing or electron-donating groups showed no activity against Gram-negatives (**33–36**, Table 3). The Au^I chloride complexes (**27–30**) gave similar results. Among the aliphatic phosphine ligands, there was a clear preference for the smallest trimethylphosphine. Analogues prepared by replacing triethylphosphine with trimethylphosphine led to sizable improvements in activity (**31**, **37–39**, and **40**). Increasing the alkyl chain length to *n*-butyl drastically decreased the activity against Gram-negatives, except for that toward *P. aeruginosa* (**26** vs **25** and **31** vs **1**, Table 3). In the work of Sutton et al. that described the discovery of auranofin, a small SAR study was conducted where the alkyl group on trialkylphosphine R_3P was varied to include methyl, ethyl, isopropyl, and *n*-butyl.[24] Unfortunately, the antiarthritic activities and the plasma gold concentrations were only measured on the chloride complexes R_3PAuCl, from which triethylphosphine was identify as the best phosphine ligand.

Auranofin is slightly active against *A. baumannii*, with an MIC/MBC of 47 μM. Changing the thio sugar structure had very little impact on the activity (**2–14**), and the activities of most analogues with aliphatic or aromatic thiols were little changed as well (**15–23**). Analogues with trimethylphosphine ligands showed the best activity against *A. baumannii*, with MIC values of 13 and 5.7 μM for **31** and **40**, respectively.

Lipophilicity appears to play a critical role in the case of *P. aeruginosa*. For example, the trimethylphosphine and deprotected thio sugar analogues **37–39** (log $P = -1.63$ to -2.03, Table 4) improved the activities by 3–44-fold over that of auranofin against all Gram-negative strains except for *P. aeruginosa,* against which the activity was completely lost. Acetyl-protected analogue **31** recovered the activity of **37** against *P. aeruginosa* by 4 times. It appears that *P. aeruginosa* is more sensitive to the thiol than the phosphine ligand. Among the analogues that show activity against *P. aeruginosa* (**21**, **24**, and **40**), none of them bear a thio sugar ligand. The best performing analogue is compound **21**, which has a mercaptoethanol ligand (MIC/MBC at 41 μM) and is 9 times better than auranofin (Table 2).

Increasing the chain length of trialkylphosphine from methyl to ethyl to *n*-butyl led to drastic decreases in activity for all other Gram-negative strains, whereas in the case of *P. aeruginosa*, the changes were minimal (**24–26** and **40** vs **21**). The MIC decreased only 2 times from **24** (methyl) to **25** (ethyl), and the activity increased from **25** (ethyl) to **26** (*n*-butyl, Table 3).

Among the Gram-negative strains tested, *E. cloacae* appears to be the most affected by the structures of the thio sugar and the phosphine ligands. The structure of the phosphine ligand showed the largest impact on the activity toward *E. cloacae*. By replacing triethylphosphine in auranofin with trimethylphosphine, the MIC/MBC of the resulting analogue, **31**, reached 3.1 μM, which is 61-fold lower than that of auranofin. Compound **38**, which combines a trimethylphosphine and a deprotected Gal ligand, has similar activity. Most thiol ligands impact the activity in a positive way, except for aromatic thiols with strong electron-withdrawing groups (**19** and **20**), which completely diminish the activity. The best thiol ligand, mercaptoethanol, combined with the best phosphine ligand, trimethylphosphine, yielded analogue **40**, which lowered the MIC/MBC of auranofin by 65-fold to 2.9, μM.

Auranofin is ineffective against *K. pneumoniae*, with an MIC/MBC of 377 μM. Changing the thio sugar structure has no impact on the activity (Table 1). Replacing the thio sugar with an aliphatic or an aromatic thiol ligand yielded a number of analogues that showed good activities (Table 2). The *p*-aminobenzene thiol (**17**) lowered the MIC/MBC of auranofin by 10-fold to 36 μM. The mercaptoethanol ligand (**21**) lowered the MIC/MBC further to 20, μM. Trimethylphosphine is again the best phosphine ligand. All trimethylphosphine analogues have good activities against *K. pneumoniae*, with the best candidate, **40**, having an MIC of 11 μ/mL, which is 34-fold lower than that of auranofin.

Lipophilicity.

For Group 1 compounds with thio sugar ligands, the deprotected thio sugar (**9–14**, Table 1) did not impact the activities of the resulting analogues against either Gram-negative or Gram-positive strains, even though the log *P* increased by more than one unit, implying that lipophilicity is unlikely to be the key factor for in vitro activity. Among the Group 2 compounds with aromatic or aliphatic thiol ligands, the analogues with moderate log *P* values (1–2) exhibited better activity, whereas analogues **19** and **20**, with high log *P* values (>3), had no activity against the Gram-negative strains (Table 2). In the trialkyl- and triaryl-phosphine series of Group 3 compounds, the in vitro activity correlated well with the lipophilicity. Analogues with high log *P* values (>4), including the tri-*n*-butylphosphine analogue and all the triarylphosphine analogues, showed either decreased or no activity (Tables 3 and 4). The deprotected thio sugar analogues **37–39** exhibited good activity against all Gram-negative strains except for *P. aeruginosa*. Compounds that are active against *P. aeruginosa* are moderately lipophilic with positive log *P* values of <2.

In summary, repurposing of the arthritis drug auranofin as an antimicrobial has shown that this drug has good activity against Gram-positive but not Gram-negative bacteria, for which we are in dire need of new therapies because of the increasing prevalence of antimicrobial resistance and the lack of new drugs to treat them. In this work, we demonstrated that

antiGram-negative activity can be enabled by modulating the structures of the thiol and phosphine ligands on auranofin. These analogues with a variety of ligand structures were efficiently synthesized under mild conditions in good to high yields. We also demonstrated that both thiol and the phosphine structures influence the activities of the analogues. Trimethylphosphine gives the highest activities against Gramnegative bacteria, whereas triethylphosphine was the best ligand for Gram-positives. Our SAR study revealed that the thiol ligand is very important and that its structure can modulate the activities of the Au^{I} complexes for both Gramnegative and Gram-positive bacteria. Importantly, the active analogues had either similar or lower mammalian cell toxicities compared with that of auranofin, paving the way for clinical translation of this class of compounds.

EXPERIMENTAL SECTION

Materials.

All reagents and solvents were used as received from Sigma-Aldrich or Fisher Scientific. All reactions were monitored by thin layer chromatography (TLC) using TLC plates precoated with TLC silica gel 60 F_{254} (Merck KGaA). Spots on TLC were visualized with ultraviolet light either directly or after staining with 5% H_2SO_4 solution in ethanol. NMR spectra were recorded on a Bruker Avance Spectrospin DRX500 spectrometer (^{1}H and ^{13}C NMR), a Bruker Avance Spectrospin DPX200 spectrometer (^{13}C, ^{19}F and ^{31}P NMR), or a JEOL ECZ 400 MHz NMR (^{31}P NMR). ^{1}H NMR and ^{13}C NMR signals are referenced to either the nondeuterated residual solvent peaks or the tetramethyl silane peak (TMS, δ 0.00 ppm). CF_3COOH (−76.55 ppm) was used as the external standard in ^{19}F NMR. For ^{31}P NMR, a freshly prepared Ph_3P solution (0.1 M in $CDCl_3$, −6.00 ppm) or 85% H_3PO_4 (0.00 ppm) was used as the external standard for samples in $CDCl_3$ or D_2O, respectively. HRMS spectra were collected at the University of Illinois at Urbana–Champaign Mass Spectroscopy Facility. Purities of compounds **1–40** were determined by absolute qNMR following the "general guidelines for quantitative 1D ^{1}H NMR (qHNMR) experiments," provided by the *Journal of Medicinal Chemistry*. The detailed calculations can be found in the Supporting Information. Purities of ≥95% were obtained for all compounds. The purity of each compound is included at the end of the corresponding synthetic procedure, and detailed calculations can be found in the Supporting Information (pp S158–S197).

General Procedure A for the Synthesis of Thioacetates 1b–6b (Step a, Scheme 1).

To a solution of glycosyl halides **1a–6a** (1.0 equiv) in acetone (0.3 M), potassium thioacetate (2.0 equiv) was added. The reaction mixture was stirred at RT for 3—5 h, after which the mixture was concentrated on a rotovap. Water was then added to the residue, and the mixture was extracted with ethyl acetate three times. The combined organic phase was washed with brine and dried over Na_2SO_4. After removal of the solvent under vacuum at RT, the residue was purified by silica gel chromatography to give thioacetates **1b–6b**.

General Procedure B for the Synthesis of 1c–6c (Step b, Scheme 1).

S-Deacetylation was conducted following a reported method.[55] Briefly, DMF (10 mL) was added to a mixture of thioacetates **1b–6b** (1.5 mmol), $NaHCO_3$ (0.15 mmol), and dithiothreitol (DTT, 2.5 mmol). The reaction mixture was stirred at RT until TLC showed

the full consumption of the starting material. The mixture was poured into 100 mL of water and extracted with toluene (80 mL × 3). The combined organic phase was washed with water (100 mL), and the solvent was removed. The crude product was used in the next step without further purification.

General Procedure C for the Synthesis of Auranofin and Analogues 1–6 (Step c, Scheme 1).

To a solution of chloro(triethylphosphine)gold(I) (1.0 mmol) and thiols (1.05 mmol) in DCM (4 mL), a solution of K_2CO_3 (1.2 mmol in 4 mL of water) was added dropwise at 0 °C. The mixture was stirred at RT for 1 h, poured into 30 mL of water, and extracted with DCM (30 mL × 3). The combined organic phase was dried on $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by column chromatography to give **1–6**.

General Procedure D for the Synthesis of Auranofin Analogues 8 (Step f, Scheme 3), 15, 16, 18–20 (Step a, Scheme 5), and 31–36 (Step b, Scheme 6).

To a solution of chloro(triethylphosphine)gold(I) (1.0 mmol) and thiols (1.05 mmol) in toluene or DCM (4 mL), DBU (1.2 mmol in 1 mL of toluene or DCM) was added dropwise. The mixture was stirred at RT for 2 h. The mixture was concentrated, after which the residue was purified by column chromatography to give auranofin analogues **8**, **15**, **16**, **18–20**, and **31–36**.

General Procedure E for the Synthesis of Auranofin Analogues 9–14 (Step b, Scheme 4), 17, 21–23, (Step b, Scheme 5), and 37–40 (Step b, Scheme 6).

To a solution of thioacetate (0.5 mmol) in methanol (12 mL), NaOMe (0.6 mmol in 1 mL of methanol) was added dropwise at 0 °C. The mixture was stirred at RT for 1–4 h, after which chloro(triphosphine)gold(I) (1.0 mmol) was added, and the mixture was stirred for another 1 h. The solvent was removed, and the residue was purified by column chromatography to give auranofin analogues **9–14**, **17**, **21–23**, and **37–40**.

General Procedure F for the Synthesis of Chloro-(triphosphine) Gold(I) 24–30 (Scheme 6).

Thiodiglycol (5 mmol) was added dropwise to a solution of gold acid chloride trihydrate (2.5 mmol, in 6 mL of water). When the bright orange-yellow solution was almost colorless, it was cooled to 0 °C, and triphosphine (2.63 mmol, in DCM/EtOH) was added dropwise. The reaction was stirred at RT for 2 h. DCM (20 mL) and 20 mL of water were added to the mixture, and the aqueous layer was extracted by DCM twice. To remove trace metal impurities in the solution, the combined organic layers were filtered, and 20 mL of EtOH/H_2O (2:1, v/v) was added to this filtrate. The solution was slowly concentrated on a rotovap to remove the DCM. A large amount of precipitate formed, which was collected by filtration, washed with cold EtOH/H_2O (1:2, v/v), air-dried, and vacuum-dried to give the desired product.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)-(triethylphosphine) Gold(I) (1, Auranofin).—This compound was synthesized from compound **1b** (450 mg, 1.11 mmol) according to general procedure B to give 400 mg of **1c**, which was used to directly

prepare compound **1**, according to general procedure C. The crude was purified by flash column chromatography (ethyl acetate/hexanes = 4:3) to give **1** as a colorless, viscous solid (676 mg, 90% over two steps). ^{1}H NMR (500 MHz, $CDDl_3$) δ 5.11–4.99 (m, 3H, H-1, H-3, H-4), 4.94–4.87 (m, 1H, H-2), 4.17 (dd, J = 12.2, 4.8 Hz, 1H, H-6a), 4.3 (dd, J = 12.2, 2.3 Hz, 1H, H-6b), 3.66 (ddd, J = 9.6, 4.7, 2.3 Hz, 1H, H-5), 2.01 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.79 (dq, J = 10.0, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$, 1.15 (dt, J = 18.5, 7.6 Hz, 9H, P(CH$_2$$CH_3$).$_3$ The NMR spectrum was consistent with the published data.[55] Purity 95% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-galacotopyranosato)-(triethylphosphine) Gold(I) (2).—This compound was synthesized from compound **2b** (300 mg, 0.824 mmol) according to general procedure B to give **2c** (quant), which was directly used to prepare compound **2** according to general procedure C. The product was purified by flash column chromatography (ethyl acetate/hexanes = 4:3) to give **1** as a colorless, viscous solid (481 mg, 96% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (dd, J = 3.5, 1.1 Hz, 1H, H-4), 5.16 (t, J = 9.5 Hz, 1H, H-2), 5.11 (d, J = 9.4 Hz, 1H, H-1), 4.94 (dd, J = 9.6, 3.5 Hz, 1H, H-3), 4.11 (d, J = 6.8 Hz, 2H, H-6a, H-6b), 3.92 (td, J = 6.8, 1.1 Hz, 1H, H-5), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.82 (dq, J = 9.9, 7.6 Hz, 6H, CH_2CH$_3$), 1.20 (dt, J = 18.4, 7.6 Hz, 9H, CH$_2$CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.56, 170.31, 170.31, 169.89, 83.79, 74.62, 74.32, 72.23, 68.02, 61.62, 21.33, 20.84, 20.74, 20.74, 18.16 (d, $^{1}J_{CP}$ = 33.4 Hz, P(CH$_2$CH$_3$)$_3$), 9.07 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.57. Purity 95% by qNMR.

(3,4,6-Tri-O-acetyl-2-N-acetyl-1-thio-β-D-glucopyranosato)-(triethylphosphine) Gold(I) (3).—This compound was synthesized from **3b** (203 mg, 0.501 mmol) according to modified general procedure B using ethyl acetate as extraction solvent instead of toluene. The crude product of **3c** was further purified by flash column chromatography (ethyl acetate/hexanes = 4:1) to give **3c** as a colorless, viscous solid (167 mg, 92%). Following general procedure C from **3c** (110 mg, 0.303 mmol) and purification using flash column chromatography (ethyl acetate/acetone = 200:1), product **3** was obtained as a colorless, viscous solid (194 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (d, J = 7.8 Hz, 1H, NH), 5.14 (d, J = 9.8 Hz, 1H, H-1), 5.06 (t, J = 9.5 Hz, 1H, H-3), 5.02 (t, J = 9.5 Hz, 1H, H-4), 4.19 (dd, J = 12.2, 4.9 Hz, 1H, H-6a), 4.06 (dd, J = 12.1, 2.3 Hz, 1H, H-6b), 4.03 (t, J = 9.6 Hz, 1H, H-2), 3.68 (ddd, J = 9.6, 4.8, 2.3 Hz, 1H, H-5), 2.01 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.93 (s, 3H, NHAc), 1.82 (dq, J = 9.9, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.17 (dt, J = 18.4, 7.6 Hz, 9H, P(CH$_2$$CH_3$)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.98, 170.89, 169.80, 169.62, 84.26, 76.04, 74.92, 69.03, 63.05, 60.43, 23.73, 20.87, 20.83, 20.74, 17.91 (d, $^{1}J_{CP}$ = 33.4 Hz, P(CH$_2$CH$_3$)$_3$), 8.97 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 36.93. HRMS (ESI) m/z: [M + Na]$^{+}$ calcd for $C_{20}H_{35}AuNO_8PSNa$ 700.1379; found 700.1380. Purity 95% by qNMR.

(3,4,6-Tri-O-acetyl-2-N-trichloroactyl-1-thio-β-D-glucopyranosato) (triethylphosphine) Gold(I) (4).—Only the procedures for the conversion of **4b** to **4** are described here. The detailed procedures for the synthesis of **4b** and the other intermediates can be found in the Supporting Information. Compound **4b** (260 mg, 0.511 mmol), DTT

(118 mg, 0.767 mmol), and $NaHCO_3$ (5 mg, 0.051 mmol) were added to 3 mL of DMF. The mixture was stirred at RT for 1.5 h and diluted with 30 mL of toluene and 30 mL of water. The aqueous layer was further extracted by toluene twice. The combined organic layer was dried over Na_2SO_4. After removal of the solvent, the crude product was dissolved in 4 mL of DCM; then Et_3PAuCl (162 mg, 0.464 mmol) was added. The reaction was brought to 0 °C; then 2 mL of cold K_2CO_3 solution (35 mg. 0.255 mmol) was added dropwise. The mixture was further stirred at RT for 1.5 h; then it was diluted with 20 mL of DCM and 20 mL of brine. The aqueous layer was further extracted by DCM once more. The combined organic phase was dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (ethyl acetate/hexanes = 1:1) to afford **4** as colorless, viscous solid (242 mg, 61% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, J = 9.3 Hz, 1H, NH), 5.28 (d, J = 9.7 Hz, 1H, H-1), 5.23 (t, J = 9.8 Hz, 1H, H-3), 5.12 (t, J = 9.7 Hz, 1H, H-4), 4.24 (dd, J = 12.3, 5.0 Hz, 1H, H-6a), 4.16–4.03 (m, 2H, H-2, H-6b), 3.79 (ddd, J = 10.1, 5.0, 2.3 Hz, 1H, H-5), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.88 (dq, J = 9.9, 7.7 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.22 (dt, J = 18.3, 7.6 Hz, 9H, P(CH$_2$$CH_3$)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.85, 170.85, 169.61, 161.52, 92.90, 83.85, 76.22, 74.05, 69.12, 63.03, 62.00, 20.91, 20.78, 20.77, 17.92 (d, $^1J_{CP}$ = 33.1 Hz, P(CH$_2$CH$_3$)$_3$), 9.01 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.58. Purity 97% by qNMR.

[2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl(1→4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranosato](triethylphosphine) Gold(I) (5).—This compound was synthesized from **5c** (260 mg, 0.398 mmol) according to general procedure C and purified by flash column chromatography (ethyl acetate/hexanes = 2:3) to give **5** as a colorless, viscous solid (370 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (d, J = 4.0 Hz, 1H, H-1′), 5.31 (dd, J = 10.4,9.6 Hz, 1H, H-3′), 5.13 (t, J = 9.1 Hz, 1H, H-3), 5.12 (d, J = 9.6 Hz, 1H, H-1), 5.00 (t, J = 9.9 Hz, 1H, H-4′), 4.81 (dd, J = 10.5, 4.0 Hz, 1H, H-2′), 4.77 (t, J = 9.4 Hz, 1H, H-2), 4.31 (dd, J = 12.1, 2.7 Hz, 1H, H-6a), 4.20 (dd, J = 12.0, 4.2 Hz, 1H, H-6b), 4.16 (dd, J = 12.4, 3.7 Hz, 1H, H-6'a), 4.00 (dd, J = 12.4, 2.3 Hz, 1H, H-6'b), 3.98–3.93 (t, J = 9.3 Hz, 1H, H-4), 3.91 (ddd, J = 10.3, 3.4, 2.6 Hz, 1H, H-5'), 3.66 (ddd, J = 9.7, 4.2, 2.9 Hz, 1H, H-5), 2.08 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.80 (dq, J = 9.9, 7.6 Hz, 6H, CH_2CH$_3$), 1.16 (dt, J = 18.5, 7.6 Hz, 9H, CH$_2$$CH_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.68, 170.68, 170.57, 170.30, 170.03, 169.92, 169.49, 95.58, 82.76, 78.17, 77.16, 75.96, 73.46, 69.96, 69.44, 68.43, 68.11, 64.06, 61.53, 21.21, 21.05, 21.01, 20.65, 20.65, 20.65, 20.65, 18.02 (d, $^1J_{CP}$ = 33.6 Hz, P(CH$_2$CH$_3$)$_3$)), 8.98 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 36.88. HRMS (ESI) $C_{32}H_{50}AuO_{17}PS$ m/z: $[M + H]^+$ 967.2245; found 967.2233. Purity 96% by qNMR.

[2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranosato](triethylphosphine) Gold(I) (6).—This compound was synthesized from **6c** (300 mg, 0.460 mmol) according to general procedure C and purified by flash column chromatography (ethyl acetate/hexanes = 2:3) to give **6** as a colorless, viscous solid (370 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (dd, J = 3.4, 0.9 Hz, 1H, H-4′), 5.14–5.04 (m, 3H, H-3, H-1, H-2′), 4.92 (dd, J = 10.4, 3.5 Hz, 1H, H-3′), 4.87 (t, J = 9.4 Hz, 1H, H-2), 4.46 (d, J = 7.9 Hz, 1H, H-1′), 4.37 (dd, J = 11.9, 1.6 Hz, 1H, H-6′a),

4.17–4.00 (m, 3H, H-6a, H-6b, H-6′b), 3.84 (t, J = 8.0 Hz, 1H, H-5′), 3.80 (t, J = 9.6 Hz, 1H, H-4), 3.62 (ddd, J = 9.9, 5.1, 1.7 Hz, 1H, H-5), 2.13 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.83 (dq, J = 9.8, 7.6 Hz, 6H, $P(CH_2CH_3)_3$), 1.19 (dt, J = 18.4, 7.6 Hz, 9H, $P(CH_2CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.69, 170.48, 170.30, 170.18, 170.07, 169.97, 169.22, 101.17, 82.94, 77.36, 76.98, 76.80, 74.61, 71.22, 70.66, 69.26, 66.73, 63.35, 60.91, 21.33, 21.09, 21.09, 20.74, 20.74, 20.74, 20.62, 18.10 (d, J = 33.2 Hz), 9.03 (s, $P(CH_2CH_3)_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.35. Purity 96% by qNMR.

Compound 7 (Scheme 2).—Only the procedures for the conversion of **7e** to **7** are described here. The detailed procedures for the synthesis of **7e** and the other intermediates can be found in the Supporting Information. Compound **7f** was prepared from compound **7e** (278 mg, 0.400 mmol) according to general procedure B (24 h) and purified by flash column chromatography (ethyl acetate/hexanes = 2:3) to give **7f** as a viscous solid (260 mg, quant). Compound **7** was from **7f** (260 mg, 0.400 mmol), following general procedure C, and was purified using flash column chromatography (ethyl acetate/hexanes = 3:1) to give compound **7** as a colorless, viscous solid (230 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.68 (d, J = 3.8 Hz, 1H, H-1), 5.51–5.44 (m, 2H, H-3, H-3), 5.25 (d, J = 3.9 Hz, 1H, H-1), 5.15 (dd, J = 10.3, 3.8 Hz, 1H, H-2), 4.99 (m, 3H, H-2, H-4, H-4), 4.16 (dd, J = 12.3, 6.3 Hz, 1H, H-6a), 4.04–3.97 (m, 2H, H-6b, H-5), 3.95–3.88 (m, 1H, H-5), 2.98 (dd, J = 13.7, 2.1 Hz, 1H, H-6a), 2.88 (dd, J = 13.7, 8.5 Hz, 1H, H-6b), 2.21 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.80 (dq, J = 10.0, 7.6 Hz, 12H, $P(CH_2CH_3)_3$), 1.17 (dt, J = 18.5, 7.6 Hz, 18HP$(CH_2CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.60, 170.12, 170.09, 170.00, 170.00, 169.94, 169.75, 91.80, 91.50, 73.07, 72.09, 70.58, 70.34, 69.04, 68.87, 68.34, 62.10, 21.47, 20.91, 20.85, 20.73, 20.73, 20.73, 20.67, 18.19 (d, J = 34.6 Hz), 9.00 (s, $P(CH_2CH_3)_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.66. HRMS (ESI) m/z: $[M - Cl]^+$ calcd for $C_{38}H_{65}Au_2O_{17}P_2S$ 1281.2744; found 1281.2732. Purity 95% by qNMR.

[1,3,4,6-Tetra-O-acetyl-2-((4-sulfanylbenzenethiolato)-(triethylphosphine)gold(I)) Acetamido-2-Deoxy-β-D-glucopyranose (8) (Scheme 3).—Only the last step, **8f → 8,** is described here. The detailed procedures for the synthesis of **8f** and intermediates **8a–8e** can be found in the Supporting Information. To a solution of compound **8f** (110 mg, 0.208 mmol) and Et_3PAuCl (73 mg, 0.208 mmol) in DCM (6 mL), DBU (48 mg, 0312 mmol) was added. The reaction mixture was stirred for 1.5 h. After concentration in a rotovap, the crude was purified by flash column chromatography (ethyl acetate/DCM = 1:1 to 2:1) to give the product as a colorless, viscous solid (169 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46–7.39 (m, 6H, Ar–H), 7.04 (d, J = 9.3 Hz, 1H, NH), 7.01–6.97 (m, 6H. Ar–H), 5.77 (d, J = 8.8 Hz, 1H, H-1), 5.31 (dd, J = 10.6, 9.3 Hz, 1H, H-3), 5.09 (t, J = 9.6 Hz, 1H, H-4), 4.27 (dd, J = 12.5, 4.6 Hz, 1H. H-6a), 4.20 (dt, J = 10.5, 9.1 Hz, 1H, H-2), 4.11 (dd, J = 12.4, 2.2 Hz, 1H, H-6b), 3.85 (ddd, J = 10.0, 4.5, 2.3 Hz, 1H, H-5), 3.49 (q, J = 16.5 Hz, 2H, SCH_2), 2.08 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.87 (dq, J = 9.9, 7.6 Hz, 6H, $P(CH_2CH_3)_3$), 1.22 (dt, J = 18.6, 7.6 Hz, 9H, $P(CH_2CH_3)_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.75, 170.67, 169.43, 169.30, 168.97, 142.54, 133.31 (2C), 128.86 (2C) 127.92, 92.13, 72.72,

72.10, 68.24, 61.77, 53.20, 38.54, 20.83, 20.72, 20.60, 20.57, 18.14 (d, J = 33.9 Hz, 3C, P(CH$_2$CH_3)$_3$)], 9.08 (s, P(CH$_2$CH$_3$)$_3$). Purity 95% by qNMR.

(1-Thio-β-D-glucopyranosato)(triethylphosphine) Gold(I) (9) (Scheme 4).[69]— This compound was prepared from compound **1b** (200 mg, 0.492 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 6:1) to give compound **9** (208 mg, 83%) as a colorless, viscous solid. ^{1}H NMR (500 MHz, D$_2$O) δ 4.87 (d, J = 9.1 Hz, 1H, H-1), 3.81 (dd, J = 12.3, 2.1 Hz, 1H, H-6a), 3.60 (dd, J = 12.3, 6.0 Hz, 1H, H-6b), 3.47–3.28 (m, 3H), 3.20 (t, J = 9.1 Hz, 1H), 1.89 (dq, J = 9.8, 7.7 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.14 (dt, J = 18.5, 7.6 Hz, 9H, P(CH$_2$$CH_3$)$_3$). Purity 98% by qNMR.

(1-Thio-β-D-galatopyranosato)(triethylphosphine) Gold(I) (10) (Scheme 4).— This compound was prepared from compound **1b** (307 mg, 0.755 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 6:1) to give compound **10** as a colorless, viscous solid (284 mg, 74%). ^{1}H NMR (500 MHz, D$_2$O) δ 4.90 (d, J = 9.0 Hz, 1H, H-1), 3.98 (d, J = 3.3 Hz, 1H, H-4), 3.80–3.66 (m, 3H, H-3, H-5, H-6a), 3.62 (dd, J = 9.6, 3.4 Hz, 1H, H-6b), 3.54 (t, J = 9.3 Hz, 1H, H-2), 1.97 (dq, J = 9.8, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.23 (dt, J = 18.7, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (50 MHz, D$_2$O) δ 85.39, 79.42, 76.96, 74.03, 69.54, 61.53, 17.78 (d, J = 33.9 Hz, P(CH$_2$CH$_3$)$_3$), 8.98 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, D$_2$O) δ 37.75. Purity 96% by qNMR.

(1-Thio-D-mannopyranosato)(triethylphosphine) Gold(I) (11) (Scheme 4).—This compound was prepared from 2,3,4,6-tetra-O-acetyl-1-S-acetyl-α-D-mannopyranose[70–73] (283 mg, 0.696 mmol) following general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 6:1) to give compound **11** as a colorless, viscous solid (277 mg, 78%). ^{1}H NMR (400 MHz, D$_2$O, α/β = 7.4:1) δ 5.73 (d, J = 1.2 Hz, 1H, H-1α), 4.72 (d, J = 1.5 Hz, 1H, H-1β), 4.31 (dd, J = 9.6, 3.3 Hz, 1H, H-3α), 4.17 (ddd, J = 9.8, 4.8, 2.7 Hz, 1H, H-5α), 3.99 (dd, J = 3.2, 1.6 Hz, 1H, H-2α), 3.89 (td, J = 3.6, 1.8 Hz, 1H, H-5β), 3.82–3.71 (m, 2H, H-6aα, H-6bα), 3.64 (t, J = 9.8 Hz, 1H, H-4), 1.92 (dq, J = 9.8, 7.6 Hz, 6H), 1.16 (dt, J = 18.4, 7.6 Hz, 9H). ^{13}C NMR (101 MHz, D$_2$O, α-anomer) δ 82.77, 76.37, 72.58, 70.16, 67.30, 60.91, 17.30 (d, J = 33.9 Hz, P(CH$_2$CH$_3$)$_3$), 8.49 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (162 MHz, D$_2$O) δ 37.72. Purity 97% by qNMR.

(2-Acetamido-2-deoxy-1-thio-β-D-glucopyranosato)-(triethylphosphine) Gold(I) (12) (Scheme 4).—This compound was prepared from compound **3b** (310 mg, 0.765 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 5:1) to give compound **12** as a colorless, viscous solid (295 mg, 70%). ^{1}H NMR (500 MHz, D$_2$O) δ 5.10 (d, J = 9.7 Hz, 1H), 3.90 (dd, J = 12.3, 1.8 Hz, 1H), 3.77 (t, J = 9.5 Hz, 1H), 3.71 (dd, J = 12.3, 5.4 Hz, 1H), 3.54–3.40 (m, 2H), 2.08 (s, 3H), 1.98 (dq, J = 15.6, 7.6 Hz, 10H), 1.23 (dt, J = 18.6, 7.6 Hz, 16H). ^{13}C NMR (50 MHz, D$_2$O) δ 173.92, 83.48, 80.51, 76.16, 70.72, 62.18, 61.75, 23.04, 17.75 (d, J = 33.9 Hz, P(CH$_2$CH$_3$)$_3$), 9.07 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, D$_2$O) δ 37.93. Purity 97% by qNMR.

[β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosato]-(triethylphosphine) Gold(I) (13) (Scheme 4).—This compound was prepared from compound **6b** (402 mg, 0.579 mmol) according to general procedure E and purified by silica gel chromatography

(DCM/methanol = 5:1 to 3:1) to give compound **13** as a colorless, viscous solid (243 mg, 62%). ^{1}H NMR (500 MHz, D_2O) δ 5.00 (d, J = 9.2 Hz, 1H), 4.47 (d, J = 7.8 Hz, 1H), 4.02–3.92 (m, 2H), 3.78 (m, 4H), 3.70 (dd, J = 10.0, 3.3 Hz, 1H), 3.67–3.54 (m, 4H), 3.36 (t, J = 8.8 Hz, 1H), 1.98 (dq, J = 9.7, 7.8 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.24 (dt, J = 18.8, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (50 MHz, D_2O) δ 103.36, 84.90, 79.71, 79.42, 79.24, 75.84, 75.78, 73.00, 71.41, 69.02, 61.47, 61.07, 17.82 (d, J = 34.5 Hz, P(CH$_2$CH$_3$)$_3$), 9.06 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, D_2O) δ 39.39. Purity 95% by qNMR.

(1-Thio-β-D-maltosato(triethylphosphine) Gold(I) (14) (Scheme 4).—This compound was prepared from compound **5b** (407 mg, 0.586 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 5:1 to 3:1) to give compound **14** as a colorless, viscous solid (252 mg, 64%). ^{1}H NMR (500 MHz, D_2O) δ 5.41 (d, J = 3.8 Hz, 1H), 4.98 (d, J = 9.2 Hz, 1H), 3.98–3.85 (m, 2H), 3.82–3.66 (m, 5H), 3.65–3.56 (m, 3H), 3.44 (t, J = 9.5 Hz, 1H), 3.34 (t, J = 9.2 Hz, 1H), 1.99 (dq, J = 15.8, 7.7 Hz, 6H), 1.24 (dt, J = 18.7, 7.6 Hz, 9H). ^{13}C NMR (50 MHz, D_2O) δ 100.06, 84.94, 80.0, 79.05, 77.92, 77.63, 73.34, 73.14, 72.18, 69.82, 61.75, 60.99, 17.85 (d, J = 34.0 Hz, P(CH$_2$CH$_3$)$_3$), 9.08 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, D_2O) δ 38.39. Purity 96% by qNMR.

(Benzenethiolato)(triethylphosphine) Gold(I) (15) (Condition a, Scheme 5).—This compound was prepared from thiophenol (35 mg, 0.314 mmol) according to general procedure E and purified by flash column chromatography (ethyl acetate/hexanes = 2:3) to afford the product as a pale yellow oil (104 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 7.8 Hz, 2H), 7.07 (t, J = 7.7 Hz, 2H), 6.96 (t, J = 7.3 Hz, 1H), 1.83 (dq, J = 10.1, 7.7 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.20 (dt, J = 18.5, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.92, 132.44, 127.94, 123.27, 18.10 (d, J = 33.1 Hz, P(CH$_2$CH_3)$_3$), 9.07 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 36.42. Purity 96% by qNMR.

(2-Aminobenzenethiolato)(triethylphosphine) Gold(I) (16) (Condition a, Scheme 5).—This compound was prepared from 2-aminobenzenethiol (36 mg, 0.285 mmol) according to general procedure D using DCM as the solvent, and the crude was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the product as a light orange solid (78 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (dd, J = 7.7, 1.5 Hz, 1H, Ar–H), 6.86 (td, J = 7.7, 1.5 Hz, 1H, Ar–H), 6.66 (dd, J = 7.8, 1.4 Hz, 1H, Ar–H), 6.55 (td, J = 7.5, 1.4 Hz, 1H, Ar–H), 4.09 (bs, 2H, NH_2), 1.80 (dq, J = 9.9, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.17 (dt, J = 18.4, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.03, 135.32, 125.31, 124.15, 117.61, 114.23, 18.13 (d, J = 33.1 Hz, P(CH$_2$CH_3)$_3$), 9.4 (s, P(CH$_2$$CH_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 38.20. Purity 98% by qNMR.

(4-Aminobenzenethiolato)(triethylphosphine) Gold(I) (17) (Condition b, Scheme 5).—To a solution of 4-aminothiophenol (45 mg, 0.359 mmol) and NaOMe (100 μL, 25 wt % in MeOH) in cold MeOH (4 mL), gold triethylphosphine chloride (120 mg, 0.342 mmol) in DCM/MeOH (1:1, v/v) was added dropwise. The reaction was stirred for 1 h. The solvent was removed by rotovap, and water was added to the mixture. The mixture was extracted by DCM twice. The combined organic phase was washed with brine and dried over Na_2SO_4. The solution was concentrated; then toluene (1 mL) was added. The

remaining DCM was removed by rotovap, after which 3 mL of hexanes was added. The solution was kept at −20 °C, after which yellow crystals formed. The supernatant was pipetted out, and the crystals were washed with hexanes and dried in a vacuum to afford the product as yellow crystals (138 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.23 (m, 2H, Ar–H), 6.53–6.44 (m, 2H, Ar–H), 3.49 (s, 2H, NH_2), 1.81 (dq, J = 9.8, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.18 (dt, J = 18.4, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.80, 133.37, 128.36, 115.47, 18.03 (d, J = 32.9 Hz, P(*C*H$_2$CH_3)$_3$), 8.99 (s, P(CH$_2$*CH*$_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.40. Purity 98% by qNMR.

(4-Methoxybenzenethiolato)(triethylphosphine) Gold(I) (18) (Condition a, Scheme 5).—This compound was prepared from 4-methoxybenzenethiol (48 mg, 0.342 mmol) according to general procedure D using DCM as the solvent, and the crude was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the product as a colorless, viscous solid (104 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44–7.38 (m, 2H, Ar–H), 6.71–6.64 (m, 2H, Ar–H), 3.73 (s, 3H, OMe), 1.83 (dq, J = 9.8, 7.6 Hz, 6H, P(*CH*$_2$CH$_3$)$_3$), 1.20 (dt, J = 18.4, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.74, 133.56, 131.72, 113.85, 55.38, 18.18 (d, J = 33.0 Hz, P(*C*H$_2$CH_3)$_3$), 9.09 (s, P(CH$_2$*CH*$_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 38.79. Purity 99% by qNMR.

(4-Nitrobenzenethiolato)(triethylphosphine) Gold(I) (19) (Condition a, Scheme 5).—This compound was prepared from 4-nitrobenzenethiol (56 mg, 0.359 mmol) according to general procedure D using DCM as the solvent, and the crude was purified by flash column chromatography (ethyl acetate/hexanes = 1:4 to 1:2) to afford the product as a yellow solid (151 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95–7.88 (m, 2H, Ar–H), 7.63–7.57 (m, 2H, Ar–H), 1.92 (dq, J = 9.9, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.24 (dt, J = 18.7, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.67, 143.86, 131.94, 122.99, 18.07 (d, J = 33.5 Hz, P(*C*H$_2$CH_3)$_3$), 9.12 (s, P(CH$_2$*CH*$_3$)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.77. Purity 99% by qNMR.

(4-(Trifluoromethyl)benzenethiolato)(triethylphosphine) Gold(I) (20) (Condition a, Scheme 5).—This compound was prepared from 4-(trifluoromethyl)benzenethiol (61 mg, 0.342 mmol) according to general procedure D using DCM as the solvent, and the crude was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to give the product as a colorless, viscous solid (126 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63–7.58 (m, 2H, Ar–H), 7.31–7.26 (d, J = 8.1 Hz, 2H, Ar–H), 1.87 (dq, J = 9.9, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.22 (dt, J = 18.6, 7.7 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.93, 132.22, 125.18 (q, $^{2}J_{CF}$ = 32.4 Hz), 124.88 (q, $^{1}J_{CF}$ = 270.72 Hz, CF_3), 124.63 (q, $^{3}J_{CF}$ = 3.6 Hz), 18.18 (d, $^{1}J_{CP}$ = 33.4 Hz, P(*C*H$_2$CH_3)$_3$), 9.13 (s, P(CH$_2$*CH*$_3$)$_3$). ^{19}F NMR (188 MHz, $CDCl_3$) δ −60.60. ^{31}P NMR (81 MHz, $CDCl_3$) δ 39.08. Purity 101% by qNMR.

(2-Mercaptoethanolato)(triethylphosphine) Gold(I) (21) (Condition b, Scheme 5).—To a solution of 2-sulfanylethanol (24 μL, 0.342 mmol) in methanol (4 mL), NaOMe (20 mg, 25 wt % in methanol) was added. The solution was brought to 0 °C. A solution of Et_3PAuCl (120 mg, 0.342 mmol) in methanol (2 mL) was added dropwise, and the reaction mixture was stirred for 1 h. The solution was concentrated, water was added, and the

mixture was extracted by DCM twice. The combined organic phase was washed with brine and dried over Na_2SO_4 to give the product as a pale-yellow oil (134 mg, quant). ^{1}H NMR (500 MHz, CD_3OD) δ 3.68–3.59 (m, 2H, CH_2OH), 2.98 (t, J = 7.5 Hz, 2H, CH_2S), 1.94 (dq, J = 10.2, 7.6 Hz, 6H, P(CH_2CH_3)$_3$), 1.23 (dt, J = 18.4, 7.6 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, CD_3OD) δ 68.04, 31.24, 18.95 (d, $^1J_{CP}$ = 33.4 Hz, P(*C*H$_2$CH$_2$*C*H$_3$)$_3$), 9.50 (s, P(CH$_2$*C*H$_3$)$_3$). ^{31}P NMR (81 MHz, CD_3OD) δ 38.71. Purity 98% by qNMR.

(Methyl Mercaptoacetatato)(triethylphosphine) Gold(I) (22) (Condition b, Scheme 5).—This compound was prepared from methyl thioglycolate (38 mg, 0.359 mmol) according to general procedure E. After the reaction was completed, the mixture was concentrated. Water was added, and the mixture was extracted by DCM twice. The combined organic phase was washed with brine and dried over Na_2SO_4. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 2:3) to afford the product as a colorless oil (85 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.70 (s, 3H, OMe), 3.56 (s, 2H, MeOCOCH_2), 1.84 (dq, J = 9.8, 7.7 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.22 (dt, J = 18.4, 7.7 Hz, 9H, P(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.69, 52.16, 29.15, 18.16 (d, $^1J_{CP}$ = 32.8 Hz, P(CH_2CH$_3$)$_3$), 9.03 (s, P(CH$_2$CH_3)$_3$). ^{31}P NMR (81 MHz, $CDCl_3$) δ 37.57. Purity 99% by qNMR.

(2,2,2-Trifluoroethanethiolato)(triethylphosphine) Gold(I) (23) (Condition b, Scheme 5).—This compound was prepared from (triethylphosphine)gold chloride (120 mg, 0.342 mmol) and 2,2,2-trifluoroethanethiol (44 mg, 0.377 mmol) according to general procedure C. The product was obtained as a colorless oil (135 mg, 92%) with satisfactory purity without column chromatography purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.43 (q, J = 10.1 Hz, 2H, CF_3CH_2), 1.85 (dq, J = 9.8, 7.6 Hz, 6H, P(CH_2CH$_3$)$_3$), 1.21 (dt, J = 18.4, 7.6 Hz, 9H, P(CH_2CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 127.32 (d, J = 274.7 Hz, CF_3), 30.33 (q, J = 32.2 Hz, CF_3CH_2), 18.13 (d, $^1J_{CP}$ = 33.2 Hz, P(CH$_2$CH_3)$_3$), 9.00 (s, P(CH$_2$*C*H$_3$)$_3$). ^{19}F NMR (188 MHz, $CDCl_3$) δ −65.55 (t, J = 10.0 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 38.87. Purity 102% by qNMR.

(Trimethylphosphine)gold Chloride (24) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (1.05 g, 2.62 mmol) and PMe_3 (2.86 mL, 1 M in THF) according to general procedure F. The product was obtained as a white, amorphous solid (580 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.63 (d, J = 11.3 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 16.25 (d, $^1J_{CP}$ = 40.3 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ −9.94. The NMR spectra were in agreement with the published data.[74] Purity 100% by qNMR.

(Triethylphosphine)gold Chloride (25) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (1.57 g, 3.99 mmol) and PEt_3 (0.500 g, 4.25 mmol) according to general procedure F. The product was obtained as a white, amorphous solid (1.41 g, quant). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87 (dq, J = 10.3, 7.6 Hz, 6H), 1.21 (dt, J = 19.0, 7.7 Hz, 9H). ^{1}P NMR (81 MHz, $CDCl_3$) δ 33.15. The NMR spectra were consistent with the published data.[55] Purity 97% by qNMR.

(Tri-n-butylphosphine)gold Chloride (26) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (525 mg, 1.31 mmol) and tri-*n*-butylphosphine

(287 mg, 1.40 mmol) according to general procedure F, with a slight modification. After extraction, the solvent was removed, and the residue was purified by column chromatography (ethyl acetate/hexanes = 1:3) to afford the product as a colorless oil (530 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85–1.74 (m, 2H), 1.63–1.50 (m, 2H), 1.46 (q, J = 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 27.45, 25.77 (d, $^{1}J_{CP}$ = 36.1 Hz), 24.19 (d, $^{2}J_{CP}$ = 15.0 Hz), 13.75. ^{31}P NMR (81 MHz, $CDCl_3$) δ 22.38. The NMR spectra were consistent with the published data.[75] Purity 100% by qNMR.

(Triphenylphosphine)gold Chloride (27) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (0.785 g, 1.99 mmol) and PPh_3 (556 mg, 2.12 mmol) according to general procedure F. The product was obtained as a white, amorphous solid (960 mg, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57–7.50 (m, 9H, Ar–H), 7.43–7.49 (m, 6H, Ar–H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.30 (d, J = 13.8 Hz), 132.00 (d, J = 2.4 Hz), 129.35 (d, J = 11.8 Hz), 129.21 (d, J = 59.9 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 33.35. The NMR spectra were consistent with the published data.[76] Purity 99% by qNMR.

(Tris(4-methoxyphenyl)phosphine)gold Chloride (28) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (525 mg, 1.31 mmol) and tris(4-methoxyphenyl)phosphine (500 mg, 1.35 mmol) according to general procedure F with slight modification. The precipitate was washed with EtOH instead of EtOH/H_2O solution. The product was obtained as a white, amorphous solid (716 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57–7.35 (m, 6H, Ar–H), 7.04–6.86 (m, 6H, Ar–H), 3.84 (s, 9H, OMe). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.49 (d, J = 2.5 Hz), 135.71 (d, J = 15.3 Hz), 120.55 (d, J = 68.2 Hz), 114.88 (d, J = 13.0 Hz), 55.60. ^{31}P NMR (81 MHz, $CDCl_3$) δ 29.49. The NMR spectra were consistent with the published data.[77] Purity 98% by qNMR.

(Tris(4-fluorophenyl)phosphine)gold Chloride (29) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (525 mg, 1.31 mmol) and tris(4-fluorophenyl)phosphine (443 mg, 1.37 mmol) according to general procedure F with slight modification. The precipitate was washed with EtOH instead of EtOH/H_2O solution. The product was obtained as a white, amorphous solid (672 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63–7.48 (m, 6H, Ar–H), 7.21 (td, J = 8.5, 1.8 Hz, 6H, Ar–H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.18 (dd, J = 255.7, 2.6 Hz), 136.34 (dd, J = 15.8, 8.9 Hz), 124.29 (dd, J = 65.2, 3.3 Hz), 117.04 (dd, J = 21.7, 13.3 Hz). ^{19}F NMR (188 MHz, $CDCl_3$) δ −102.96 (m). ^{31}P NMR (81 MHz, $CDCl_3$) δ 30.14 (s). The NMR spectra were consistent with the published data.[78] Purity 97% by qNMR.

(Tris(4-trifluoromethylphenyl)phosphine)gold Chloride (30) (Scheme 6).—This compound was prepared from gold acid chloride trihydrate (525 mg, 1.31 mmol) and tris(4-trifluoromethylphenyl)-phosphine (647 mg, 1.37 mmol) according to general procedure F with slight modification. The precipitate was washed with EtOH instead of EtOH/H_2O solution. The product was obtained as a white, amorphous solid (857 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (dd, J = 8.5, 2.1 Hz, 6H, Ar–H), 7.69 (dd, J = 12.8, 8.1 Hz, 6H, Ar–H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.71 (qd, J = 33.4, 2.6 Hz), 134.69 (d, J = 14.7 Hz), 132.25 (d, J = 57.7 Hz), 126.63 (dq, J = 11.8, 3.6 Hz), 123.25 (q, J = 273.1 Hz, CF_3).

^{19}F NMR (188 MHz, $CDCl_3$) δ −60.82. ^{31}P NMR (81 MHz, $CDCl_3$) δ 31.86. The ^{1}H NMR, ^{19}F NMR, and ^{31}P NMR spectra were consistent with the published data.[79] Purity 101% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)-(trimethylphosphine) Gold(I) (31) (Scheme 6).—This compound was synthesized from trimethylphosphine gold chloride (85 mg, 0.274 mmol) and compound **1c** (105 mg, 0.274 mmol) according to general procedure D using toluene as the solvent and purified by flash column chromatography (ethyl acetate/hexanes = 5:1) to afford the product as a colorless, viscous solid (145 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.19–5.08 (m, 3H, H-1, H-3 and H-4), 4.98 (t, J = 9.3 Hz, 1H, H-2), 4.25 (dd, J = 12.2, 4.8 Hz, 1H, H-6a), 4.12 (dd, J = 12.1, 2.4 Hz, 1H, H-6b), 3.74 (ddd, J = 9.5, 4.7, 2.2 Hz, 1H, H-5), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.61 (d, J = 10.8 Hz, 9H, PMe_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.76, 170.24, 169.72, 169.65, 83.21, 77.30, 75.76, 74.40, 68.87, 62.83, 21.26, 20.91, 20.72, 20.69, 15.96 (d, J = 37.1 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ −3.47. Purity 95% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)(tri-n-butylphosphine) Gold(I) (32) (Scheme 6).—This compound was synthesized from tri-*n*-butylphosphine gold chloride (143 mg, 0.329 mmol) and compound **1c** (126 mg, 0.345 mmol) according to general procedure D using toluene as the solvent and purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the product as a colorless, viscous solid (225 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.18–5.06 (m, 3H, H-1, H-3 and H-4), 4.99 (t, J = 9.0 Hz, 1H, H-2), 4.24 (dd, J = 12.3, 4.9 Hz, 1H, H-6a), 4.10 (dd, J = 12.2, 2.3 Hz, 1H, H-6b), 3.72 (ddd, J = 8.6, 4.6, 2.0 Hz, 1H, H-5), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.85–1.75 (m, 6H, $P(CH_2CH_2CH_2CH_3)_3$), 1.65–1.53 (m, 6H, $P(CH_2CH_2CH_2CH_3)_3$), 1.48 (h, J = 7.2 Hz, 6H, $P\text{-}(CH_2CH_2CH_2CH_3)_3$), 0.96 (t, J = 7.2 Hz, 9H, $P\text{-}(CH_2CH_2CH_2CH_3)_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.72, 170.25, 169.53, 169.51, 83.34, 77.53, 75.73, 74.31, 69.00, 62.94, 27.23, 25.58 (d, J = 32.6 Hz), 24.16 (d, J = 14.4 Hz), 21.15, 20.83, 20.67, 20.64, 13.68. ^{31}P NMR (81 MHz, $CDCl_3$) δ 27.02. Purity 96% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)-(triphenylphosphine) Gold(I) (33) (Scheme 6).—This compound was synthesized from triphenylphosphine gold chloride (258 mg, 0.521 mmol) and compound **1c** (200 mg, 0.548 mmol) according to general procedure D using toluene as the solvent and purified by flash column chromatography (ethyl acetate/hexanes = 2:3 to 1:1) to afford the product as a colorless, viscous solid (377 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62–7.44 (m, 15H, Ar–H), 5.23–5.01 (m, 4H, H-1, H-2, H-3 and H-4), 4.22 (dd, J = 12.2, 4.8 Hz, 1H, H-6a), 4.12 (dd, J = 12.2, 2.4 Hz, 1H, H-6b), 3.76 (ddd, J = 9.8, 4.8, 2.4 Hz, 1H, H-5), 2.5 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.89 (s, 3H, OAc). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.88, 170.43, 169.70, 169.70, 134.36 (d, J = 14.2 Hz), 131.71 (d, J = 2.4Hz), 129.84 (d, J = 55.4 Hz), 129.28 (d, J = 11.5 Hz), 83.20, 77.73, 75.82, 74.34, 69.08, 62.97, 21.23, 20.83, 20.83, 20.78. ^{31}P NMR (81 MHz, $CDCl_3$) δ 36.93. The NMR spectra were consistent with the published data.[80] Purity 96% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)(tris(4-methoxyphenyl)phosphine) Gold(I) (34) (Scheme 6).—This compound was synthesized from tris(4-methoxyphenyl)phosphine gold chloride (160 mg, 0.274 mol) and compound **1c** (105 mg, 0.288 mmol) according to general procedure D using toluene as the solvent and purified by column chromatography (ethyl acetate/hexanes = 1:1) to afford the product as a colorless, viscous solid (224 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.44 (m, 6H, Ar–H), 7.01–6.95 (m, 6H, Ar–H), 5.20–5.10 (m, 3H, H-1, H-3, H-4), 5.06 (t, J = 9.3 Hz, 1H, H-2), 4.22 (dd, J = 12.2, 4.8 Hz, 1H, H-6a), 4.13 (dd, J = 12.1, 2.5 Hz, 1H, H-6b), 3.84 (s, 9H, 3 × OMe), 3.77 (ddd, J = 9.7, 4.7 2.5 Hz, 2H, H-5), 2.05 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.91 (s, 3H, OAc). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.84, 170.34, 169.64, 169.58, 162.22, 135.70 (d, J = 15.1 Hz), 121.27 (d, J = 62.8 Hz), 114.79 (d, J = 12.5 Hz), 83.19, 77.41, 75.70, 74.32, 69.09, 62.99, 55.49, 55.49, 55.49, 21.23, 20.79, 20.79, 20.79. ^{31}P NMR (81 MHz, $CDCl_3$) δ 33.82. Purity 96% by qNMR.

(2,3,4,6-Tetra-O-acety/-1-thio-β-D-glucopyranosato)(tris(4-fluorophenyl)phosphine) Gold(I) (35) (Scheme 6).—This compound was synthesized from tris(4-fluorophenyl)phosphine gold chloride (144 mg, 0.262 mmol) and compound **1c** (100 mg, 0.276 mmol) according to general procedure D using toluene as the solvent and purified by column chromatography (ethyl acetate/hexanes = 1:2) to afford the product as a colorless, viscous solid (198 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64–7.50 (m, 6H, Ar–H), 7.25–7.18 (m, 6H, Ar–H), 5.16 (d, J = 9.5 Hz, 1H, H-1), 5.15 (t, J = 9.3 Hz, 1H, H-3), 5.08 (dd, J = 9.9, 9.4 Hz, 1H, H-4), 5.03 (t, J = 9.3 Hz, 1H, H-2), 4.24 (dd, J = 12.2, 5.0 Hz, 1H, H-6a), 4.11 (dd, J = 12.2, 2.4 Hz, 1H, H-6b), 3.79–3.74 (m, 1H, H-5), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.94 (s, 3H, OAc). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.69, 170.26, 169.77, 169.71, 163.99 (d, J = 2.4 Hz), 136.43 (dd, J = 16.0, 8.8 Hz), 125.37 (dd, J = 57.3, 3.5 Hz), 116.92 (dd, J = 21.6, 12.7 Hz), 83.31, 78.01, 75.83, 74.15, 69.02, 62.96, 21.19, 20.79, 20.73, 20.69. ^{19}F NMR (188 MHz, $CDCl_3$) δ −103.75. ^{31}P NMR (81 MHz, $CDCl_3$) δ 35.38. Purity 95% by qNMR.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato)(tris(4-(trifluoromethyl)phenylphosphine)) Gold(I) (36) (Scheme 6).—This compound was synthesized from tris(4-trifluoromethyl)-phenylphosphine gold chloride (183 mg, 0.262 mmol) and compound **1c** (100 mg, 0.275 mmol) according to general procedure D using toluene as the solvent and purified by column chromatography (ethylacetate/hexanes = 1:3) to afford the product as a colorless, viscous solid (69 mg, 26%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 6.6 Hz, 6H, Ar–H), 7.71 (dd, J = 11.9, 8.1 Hz, 6H, Ar–H), 5.18 (d, J = 9.4 Hz, 1H, H-1), 5.15 (t, J = 9.3 Hz, 1H, H-3), 5.08 (t, J = 9.8 Hz, 1H, H-4), 5.04 (t, J = 9.3 Hz, 1H, H-2), 4.27 (dd, J = 12.3, 5.1 Hz, 1H, H-6a), 4.11 (dd, J = 12.3, 2.3 Hz, 1H, H-6b), 3.78 (ddd, J = 9.9, 5.0, 2.4 Hz, 1H, H-5), 2.08 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.94 (s, 3H, OAc). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.70, 170.29, 169.99, 169.75, 134.77 (d, J = 15.3 Hz), 134.52–132.25 (m), 126.49 (dq, J = 11.0, 3.5 Hz), 123.42 (q, J = 272.7 Hz, CF3), 83.50, 78.27, 76.06, 74.16, 69.02, 62.99, 21.27, 20.83, 20.73, 20.73. ^{19}F NMR (188 MHz, $CDCl_3$) δ –60.82. ^{31}P NMR (81 MHz, $CDCl_3$) δ 32.62. Purity 95% by qNMR.

(1-Thio-β-D-glucopyranosato)(trimethylphosphine) Gold(I) (37).—This compound was prepared from compound **1b** (120 mg, 0.295 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 5:1) to give the product as a colorless, viscous solid (70 mg, 57%). ^{1}H NMR (500 MHz, D_2O) δ 4.94 (d, J = 9.1 Hz, 1H), 3.93 (dd, J = 12.3, 1.7 Hz, 1H), 3.73 (dd, J = 12.3, 5.9 Hz, 1H), 3.53–3.42 (m, 3H), 3.28 (t, J = 8.9 Hz, 1H), 1.69 (d, J = 10.9 Hz, 9H, PMe_3). ^{13}C NMR (126 MHz, D_2O) δ 84.54, 80.7, 79.30, 76.90, 70.08, 61.22, 15.11 (d, J = 37.7 Hz, PMe_3). ^{31}P NMR (162 MHz, D_2O) δ −1.06. Purity 95% by qNMR.

(1-Thioβ-D-galatopyranosato)(trimethylphosphine) Gold(I) (38).—This compound was prepared from compound **2b** (120 mg, 0.295 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 5:1) to give compound 38 as a colorless, viscous solid (72 mg, 59%). ^{1}H NMR (500 MHz, D_2O) δ 4.91 (d, J = 9.1 Hz, 1H), 4.01 (d, J = 3.4 Hz, 1H), 3.83–3.70 (m, 3H), 3.65 (dd, J = 9.6, 3.4 Hz, 1H), 3.53 (t, J = 9.3 Hz, 1H), 1.69 (d, J = 10.0 Hz, 9H). ^{13}C NMR (126 MHz, D_2O) δ 85.15, 79.12, 76.53, 73.69, 69.16, 61.17, 15.10 (d, J = 38.3 Hz, PMe_3). ^{31}P NMR (162 MHz, D_2O) δ −0.87. Purity 96% by qNMR.

(2-Acetamido-2-deoxy-1-thio-β-D-glucopyranosato)-(trimethylphosphine) Gold(I) (39).—This compound was prepared from compound **3b** (150 mg, 0.370 mmol) according to general procedure E and purified by silica gel chromatography (DCM/methanol = 10:1 to 5:1) to give compound **39** as a colorless, viscous solid (126 mg, 67%). ^{1}H NMR (500 MHz, D_2O) δ 5.09 (d, J = 8.6 Hz, 1H), 3.92 (d, J = 12.1 Hz, 1H), 3.86–3.66 (m, 2H), 3.60–3.35 (m, 3H), 2.10 (s, 3H), 1.67 (d, J = 11.1 Hz, 9H, PMe_3). ^{13}C NMR (101 MHz, D_2O) δ 173.62, 83.19, 80.24, 75.69, 70.09, 61.44, 61.14, 22.69, 15.06 (d, J = 37.7 Hz, PMe_3). ^{31}P NMR (162 MHz, D_2O) δ −1.98. Purity 95% by qNMR.

(2-Mercaptoethanolato)(trimethylphosphine) Gold(I) (40).—To a solution of 2-sulfanylethanol (40 mg, 0.512 mmol) and Me_3PAuCl (0.538 mmol, 166 mg) in MeOH, NaOMe (121 mg, 25 wt %, 0.563 mmol) was added. After being stirred at RT for 1.5 h, the solvent was removed. DCM (5 mL) and water (5 mL) were added, and the mixture was extracted by DCM twice. The combined organic phase was dried over Na_2SO_4 and filtered. After removal of the solvent, the desired product was obtained as a white solid (179 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73–3.62 (m, 2H, $HOCH_2CH_2$), 3.18–3.07 (m, 3H, $HOCH_2CH_2$ and OH), 1.60 (d, J = 10.5 Hz, 9H, PMe_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 65.98, 32.24, 16.03 (d, $^{1}J_{CP}$ = 35.7 Hz, PMe3). ^{31}P NMR (162 MHz, $CDCl_3$) δ −0.13. Purity 100% by qNMR.

Antibacterial Assay.—The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) were determined by the broth microdilution method, as described in the Clinical & Laboratory Standards Institute (CLSI) guidelines.[81] All bacterial strains were cultured in cation-adjusted Muller–Hinton broth (CAMHB) up to log phase and then diluted to give a concentration of 7.5×10^5 CFU/mL. The gold complexes were dissolved in DMSO or water as stock solutions, which were then serially diluted 2-fold in a 96-well plate. Aliquots (100 μL) of the dilutions were mixed with equal volumes of the

bacterial suspension in a 96-well plate (in triplicates). The plates were incubated at 37 °C for 18 h without shaking. Negative and positive controls were included in the same well plate, with wells having culture medium only and wells having bacterial solution only, respectively. The sample concentrations that gave inhibition values above 90% were determined as MICs, as calculated from absorbance values at 600 nm (OD600) obtained using a Tecan SPARK 10 M plate reader. To determine minimum bactericidal concentrations (MBCs), agar plates were treated with 5 μL of sample solutions from each well, and then incubated at 37 °C for 18 h without shaking. The sample concentrations that resulted in no colony formation on agar plates were determined as MBCs.

Cytotoxicity Assay.

Sample Preparation.: The compounds were dissolved in DMSO at 12.8 mg/mL as the stock solutions, except for compounds **9–14** and **37–39**, which were directly dissolved in the culture medium at 12.8 mg/mL. The stock solutions were diluted with the culture medium to the testing concentration at 128 μg/mL (compounds **9–14** and **37–39** were diluted to 1280 μg/mL). The solutions were then serially diluted 2-fold in a 96-well plate and used in the cytotoxicity assay immediately.

Cell Culture and Cytotoxicity Assay.: A549 cells (ATCC CCL-185) were cultured in high glucose DMEM (Sigma-Aldrich, D6429) supplemented with 10% FBS, 1% penicillin–streptomycin solution (Sigma-Aldrich, P4333), 1% gentamicin solution (Sigma-Aldrich, G1272), and 1% amphotericin B solution (Sigma-Aldrich, A2942) at 37 °C in a humidified atmosphere containing 5% CO_2. After cells reached 80–90% confluency, they were detached and counted by an Invitrogen Countess automated cell counter. Cells with viability over 90% were seeded at a density of 5×10^3 cells per well in 96-well cell culture plates. After overnight culture, the culture medium in each well was pipetted out; 80 μL of the compound solutions from each well of the 96-well plate prepared above were transferred to this 96-well plate containing the cells, and the plates were incubated for 20 h. After the addition of 8 μL of 110 μg/mL resazurin, the plates were incubated for a further 3 h at 37 °C in 5% CO_2. The fluorescence intensity was measured using a Tecan M200 Infinite Pro microplate reader at an excitation wavelength of 560 nm and an emission wavelength of 590 nm. IC_{50} were calculated by curve fitting of the inhibition values versus the log of the concentration in GraphPad's Prism. The cytotoxicity assays (in triplicate) were repeated twice using freshly prepared cells and compound solutions.

Log *P* Determination.

Calibration Curves.: Milli-Q Water (150 mL) was added to *n*-octanol (150 mL), and the mixture was stirred for 3 days to allow both phases to reach saturation. The gold standard solution (1000 ppm Au in hydrochloric acid, Fisher Scientific) was diluted to 1, 2, 3, 4, and 5 ppm with *n*-octanol-saturated water, and the absorbances of the resulting solutions were measured by flame atomic absorption spectroscopy (FAAS, Agilent Technology 200 Series AA, equipped with a gold HC Lamp-Au coded). The absorbance data were plotted against Au concentration, and the resulting calibration curve (curve A, Figure S142) was used for determining the concentrations of the analogues in the aqueous phase.

To construct the standard calibration curve of the Au concentration in the *n*-octanol phase, compound **40** was used. The Au concentration in compound **40** was first calibrated against the aqueous gold standard. For this, 0.440 mg of compound **40** was weighted on a Sartorius MC5 microbalance and was then added to 22 mL of *n*-octanol-saturated water. After the compound was fully dissolved, the absorbance of the solution was measured by FAAS, and the Au concentration was determined from calibration curve A to be 9.88 ppm. This data was then used to construct the standard calibration curve of the Au concentration in the *n*-octanol phase. For this, solutions of varying concentrations (0.99, 2.47, 4.94, 5.93, and 9.88 ppm) of compound **40** in water-saturated *n*-octanol were prepared. The absorbance was measured by FAAS, and the data were plotted against the Au concentration to give calibration curve B (Figure S143), which was used for determining the Au concentrations of the analogues in the *n*-octanol phase.

Determination of Au Concentrations in Auranofin and Analogues: Method 1.— Compound (0.5–1 mg) was weighed into a 2 mL Eppendorf tube containing 2 mL of *n*-octanol-saturated water. After being shaken for about 4 h, it was centrifuged at 6000 rpm for 3 min. The supernatant (1 mL) was transferred to a new tube containing 1 mL of water-saturated *n*-octanol, and the mixture was shaken for 1 h. After centrifugation at 6000 rpm for 5 min, the two layers were collected, and the gold concentration in each layer was determined by FAAS by comparing the absorbance to calibration curve A or B.

Determination of Au Concentrations in Auranofin and Analogues: Method 2.— This method was used for compounds **19**, **20**, **26**–**30**, and **32**–**36**, which gave very low absorbance reading on FAAS, likely because of the low solubilities of these compounds in *n*-octanol-saturated water. For Method 2, 0.6–1 mg of compound was weighed into a 2 mL Eppendorf tube containing 1 mL of *n*-octanol-saturated water and 1 mL of water-saturated *n*-octanol. After being shaken for 3–4 h, the mixture was centrifuged, and all the liquid was transferred to a 15 mL tube. The liquid was then diluted with 2 mL of *n*-octanol-saturated water and 2 mL of water-saturated *n*-octanol, and the mixture was shaken for another hour. After centrifugation at 6000 rpm for 5 min, the two layers were collected, and the gold concentration in each layer was determined by FAAS by comparing the absorbance to calibration curve A or B.

The log *P* value was calculated as follows: log P = log-$[\text{compound}]_{oct}/[\text{compound}]_{water}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

AMR	antimicrobial resistance

MIC	minimal inhibitory concentration
MBC	minimal bactericidal concentration
SAR	structure–activity relationship
Trx	thioredoxin
TrxR	thioredoxin reductase
DCM	dichloromethane
DMF	dimethylformamide
DTT	dithiothreitol
DBU	1,8-diazabicyclo-[5.4.0]undec-7-ene
Et_2O	diethyl ether
RT	room temperature
ATCC	American Type Culture Collection
NCTC	National Collection of Type Cultures
MRSA	methicillin-resistant *Staphylococcus aureus*
M. tuberculosis	Mycobacterium tuberculosis
A. baumannii	Acinetobacter baumannii
P. aeruginosa	Pseudomonas aeruginosa
E. cloacae	Enterobacter cloacae
K. pneumonia	Klebsiella pneumonia
S. aureus	Staphylococcus aureus
E. coli	Escherichia coli
E. faecium	Enterococcus faecium
OXA	oxacillinases
VEB	Vietnamese extended-spectrum β-lactamase
VIM	Verona integron-mediated metallo-μ-lactamase
AmpC	ampicillin class C
ESBL	extended spectrum β-lactamase
VRE	vancomycin-resistant enterococcus
HRMS	high-resolution mass spectrometry

Glc	D-glucose
Gal	D-galactose
GlcNAc	*N*-acetylglucosamine
TLC	thin layer chromatography
HRMS	high-resolution mass spectrometry
TMS	tetramethylsilane
CAMHB	cation-adjusted Muller–Hinton broth
CFU	colony-forming unit

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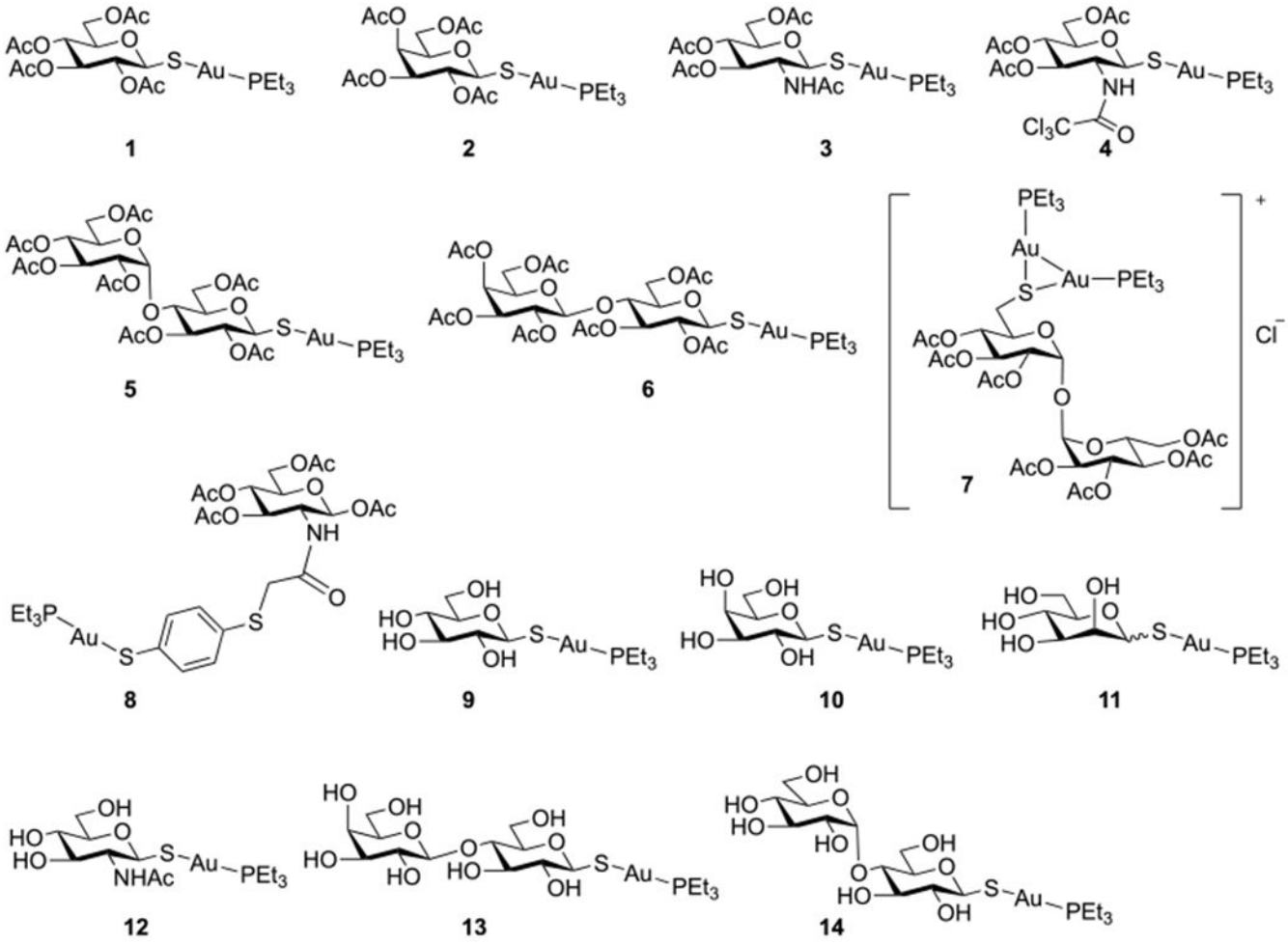


Figure 1.

Auranofin (**1**) and Group 1 analogues with protected and unprotected thio sugar ligands (**2–14**).

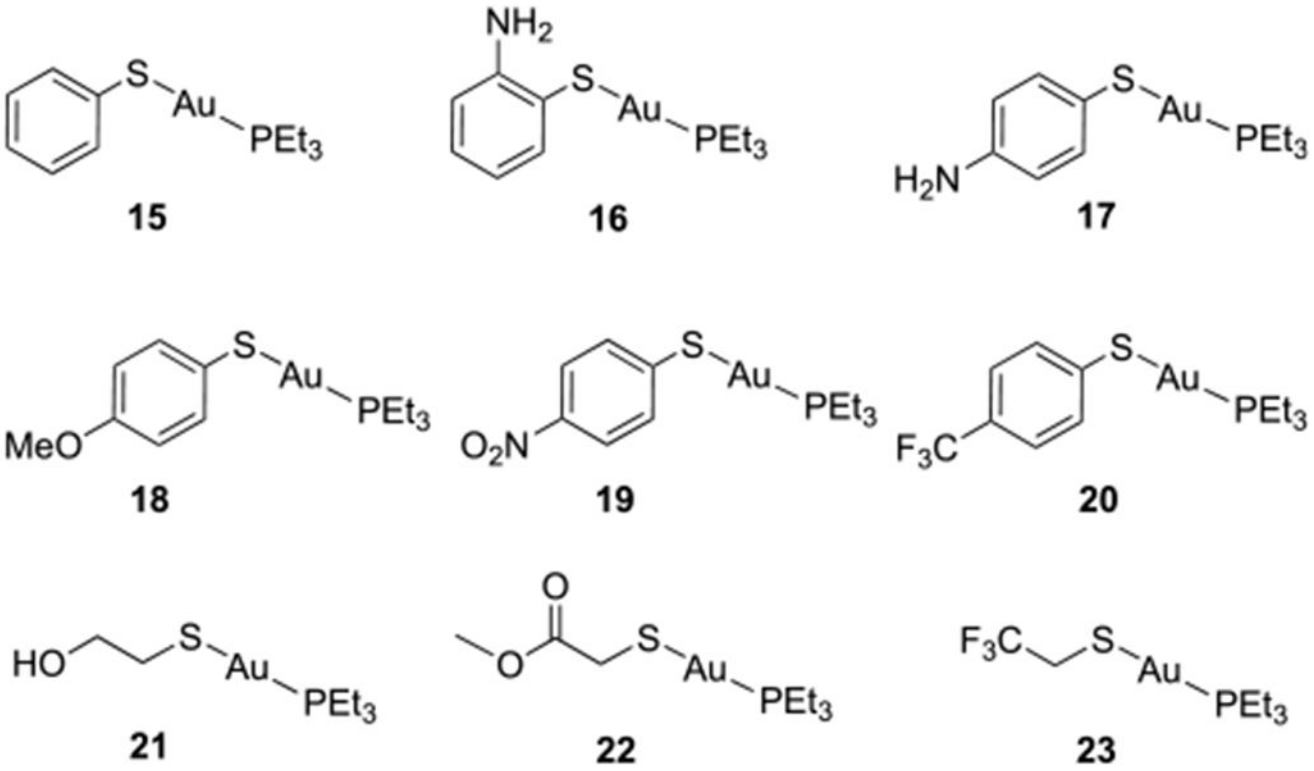


Figure 2.
Group 2 analogues with aromatic and aliphatic thiol ligands.

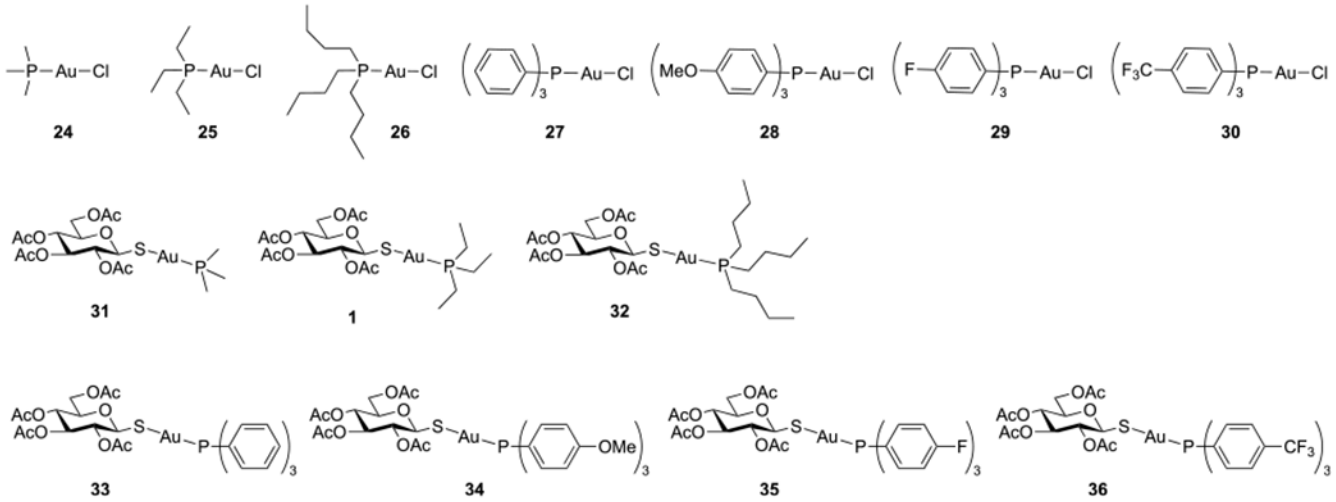
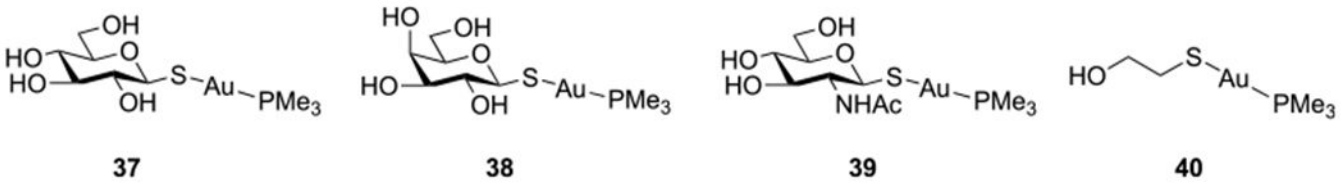


Figure 3.
Group 3 analogues with different phosphine ligand structures.



37 38 39 40

Figure 4.

Auranofin analogues with trimethylphosphines and deprotected thio sugars (**37–39**) or mercaptoethanol ligands (**40**).

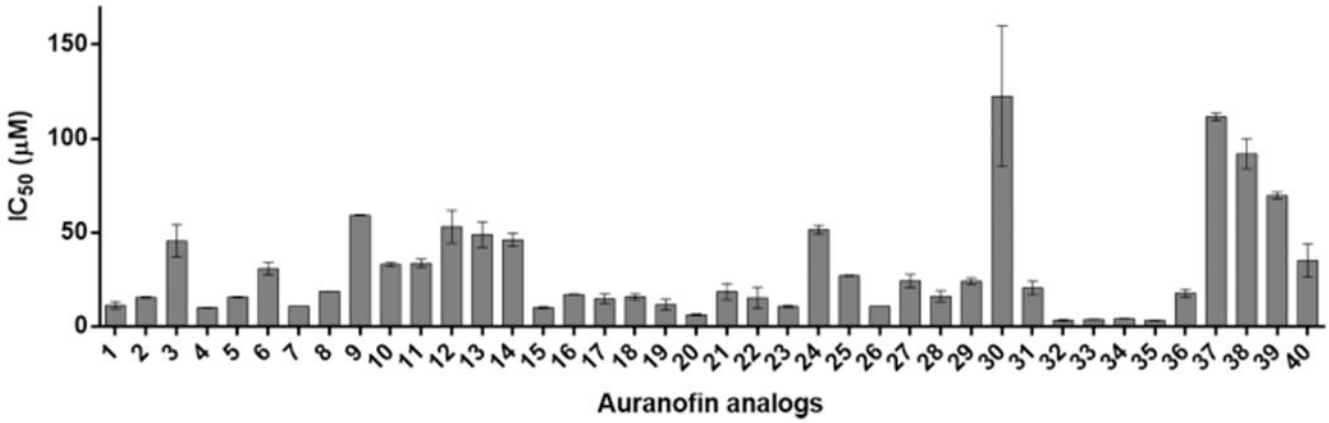
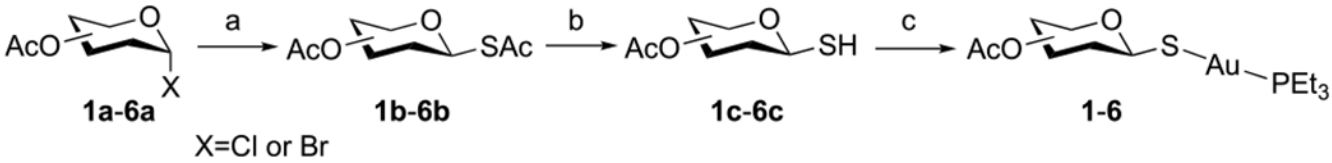


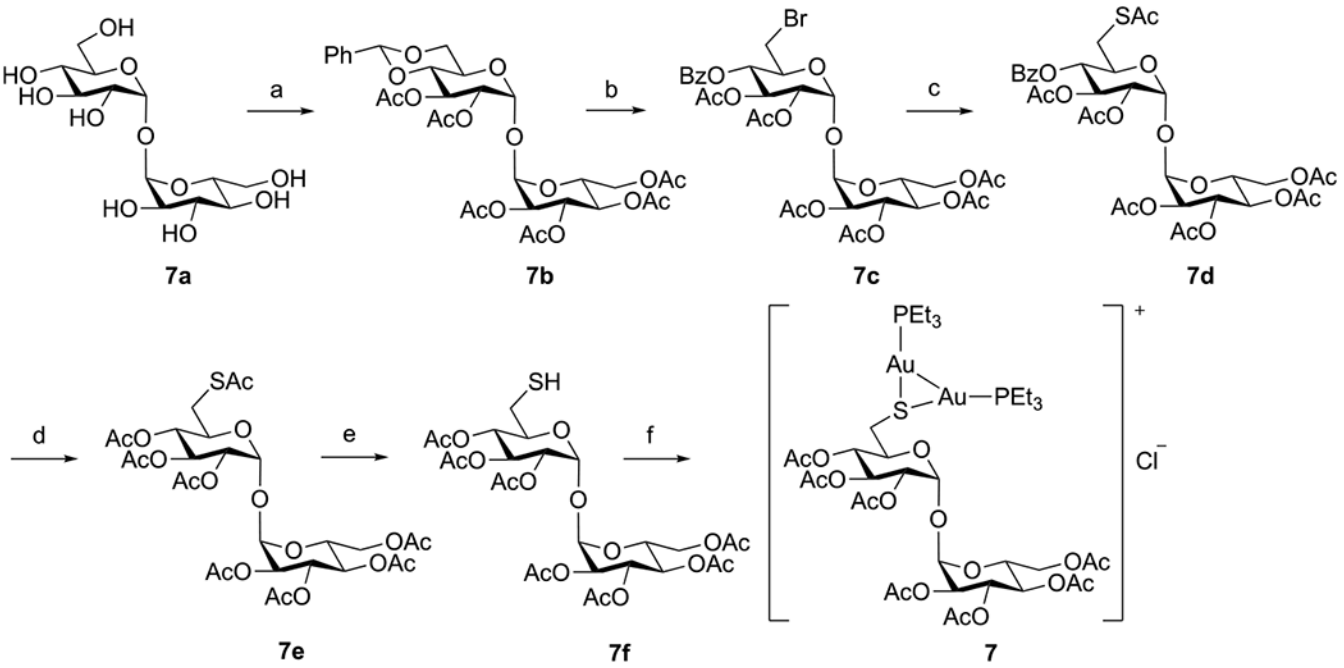
Figure 5.

IC_{50} (μM) values of auranofin and analogues against A549 cells. The data are presented as means ± SEM from two independent experiments.



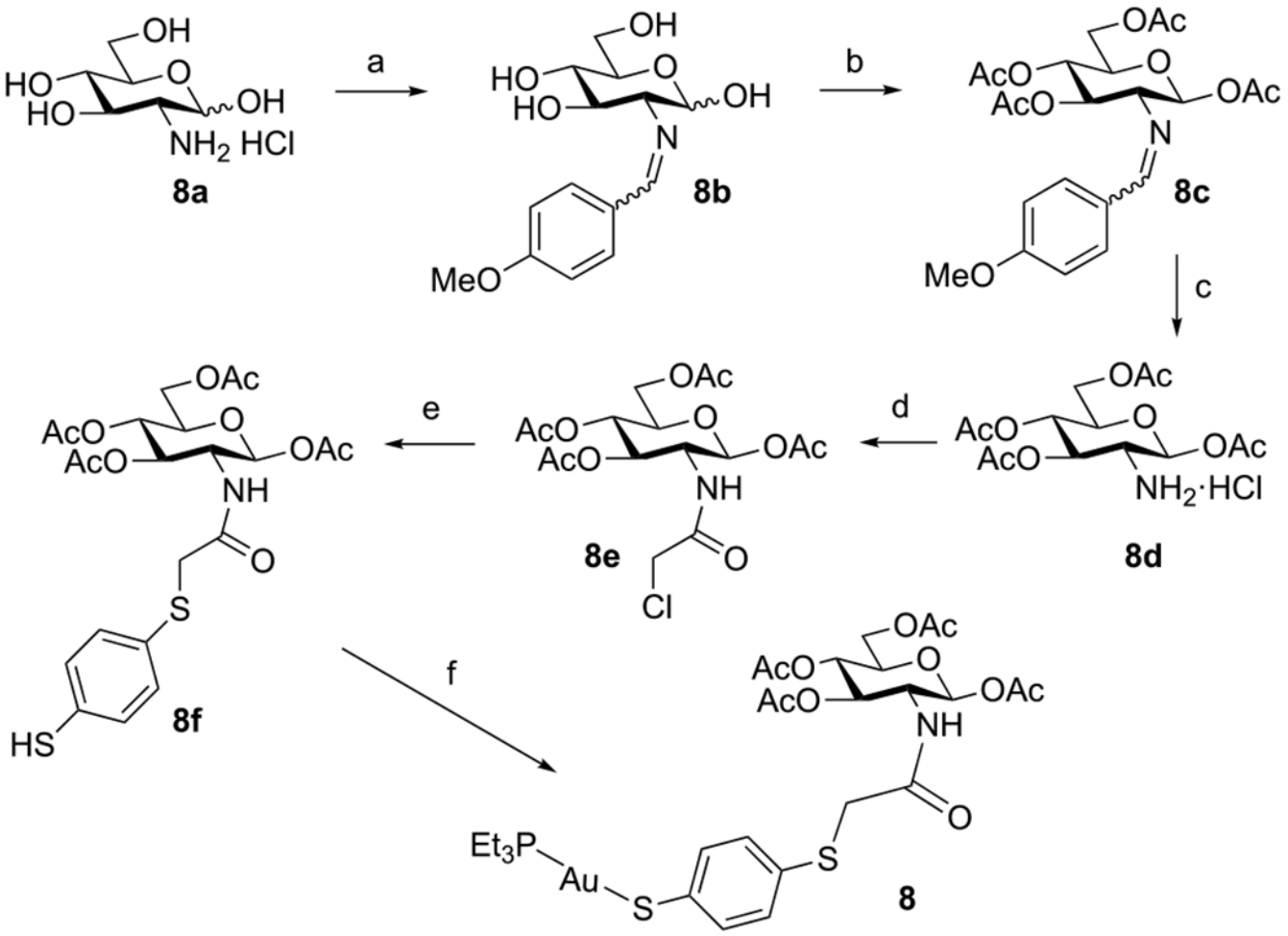
Scheme 1. Synthesis of Auranofin, 1, and Analogues 2–6[a]

[a]Reagents and conditions: (a) KSAc, acetone, RT, 3–5 h (82–90%). (b) DTT, $NaHCO_3$, DMF, RT, 1–1.5 h (90–95%). (c) Et_3PAuCl, K_2CO_3, DCM/H_2O, 0 °C to RT, 1–1.5 h (61–94%).



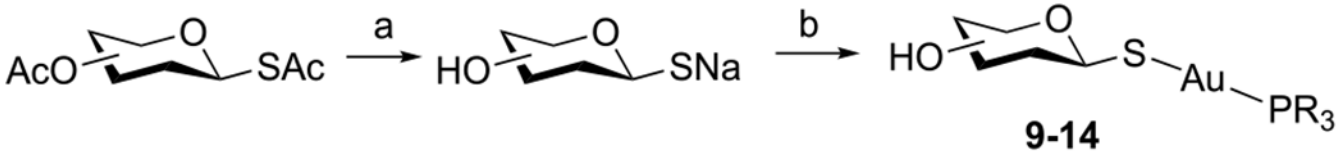
Scheme 2. Synthesis of Analogue 7[a]

[a]Reagents and conditions: (a) (i) $PhCH(OMe)_2$, TsOH, DMF; (ii) Ac_2O, Et_3N, DMF (40% over two steps). (b) NBS, $CaCO_3$, CCl^4 (89%). (c) (i) KI, DMF, 60 °C; (ii) KSAc, DMF, RT, 8 h (87%). (d) (i) NaOMe, MeOH, RT; (ii) Ac_2O, pyridine, 0 °C to RT (72%). (e) DTT, $NaHCO_3$, DMF, RT, 24 h (93%). (f) Et_3PAuCl, DBU, DCM, RT (62%).



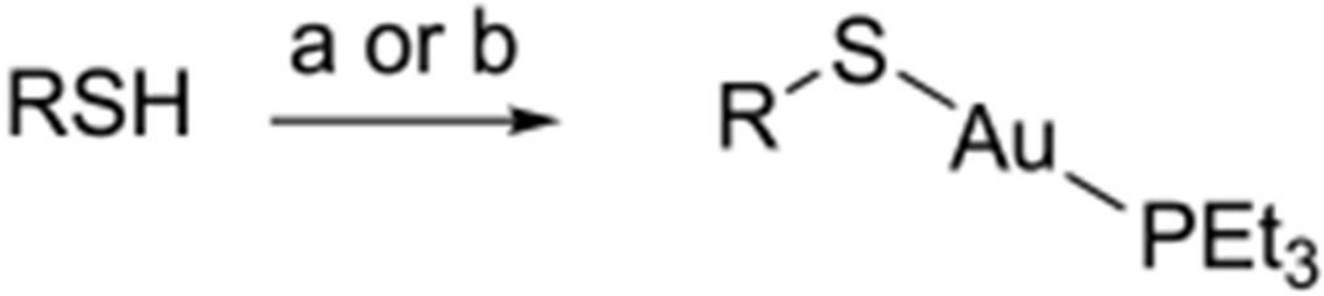
Scheme 3. Synthesis of Analogue 8[a]

[a]Reagents and conditions: (a) *p*-Anisaldehyde, NaOH, H_2O, RT, 1 h (80%). (b) Ac_2O, pyridine, RT, overnight (81%). (c) 5 M HCl, acetone, reflux, 30 min (86%). (d) Chloroacetic anhydride, pyridine, DCM, 0 °C to RT (89%). (e) 1,4-Benzenedithiol, TEA, DCM, 16 h (91%). (f) Et_3PAuCl, DBU, DCM, RT, 1.5 h (96%).



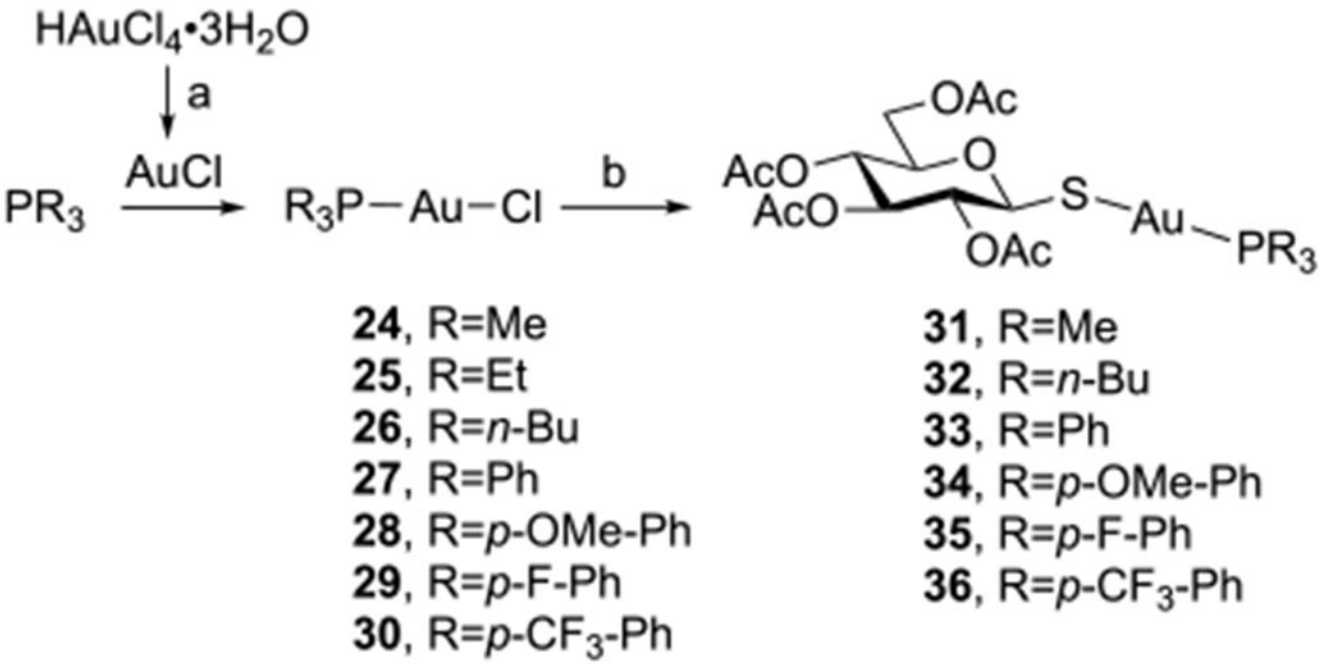
Scheme 4. Synthesis of Analogues 9–14[a]

[a]Reagents and conditions: (a) NaOMe, MeOH, RT, 2–4 h. (b) Et_3PAuCl, MeOH, 1–2 h, 57–83% over two steps.



Scheme 5. Synthesis of Group 2 Analogues 15–23 with Aromatic and Aliphatic Thiol Ligands[a]

[a]Reagents and conditions: (a) For compounds **15**, **16**, **18–20**: Et_3PAuCl, DBU, DCM, RT, 1–2 h (62–91%). (b) For compounds **17**, **21–23**: Et_3PAuCl, NaOMe, MeOH, RT, 1–2 h (59–92%).



Scheme 6. Synthesis of Auranofin Analogues with Different Phosphine Ligand Structures[a]

[a]Reagents and conditions: (a) 2,2′-Thiodiethanol, H_2O/EtOH/DCM, 0 °C to RT, 2 h (72–94%). (b) **1c**, DBU, toluene, RT, 2 h (83–90%).

Table 1.

MIC/MBC (μM) Values[a] of Group 1 Analogues with Varying Thio Sugar Structures

	***A. baumannii* NCTC 13420**	***P. aeruginosa* NCTC 13437**	***E. cloacae* NCTC 13405**	***K. pneumoniae* ATCC 700603**	***S. aureus* JE2 (USA300)**	***E.faecium* ATCC 700221**	***E. coli* ATCC 25922**	**log *P***
1	47 (47)	377 (377)	189 (189)	377 (377)	0.04 (0.09)	0.2/0.09 (0.4)	24 (24)	0.56
2	47 (94)	377 (377)	377 (377)	>377	0.04 (0.09)	0.2/0.4 (0.4)	24 (24)	0.38
3	24 (47)	189 (189)	47 (94)	189 (189)	0.04/0.09 (0.09)	0.09 (0.4)	12 (12)	0.26
4[f]	82 (164)	>328	>328	>328	0.01 (0.01)	0.08 (0.2)	21 (21)	1.01
5[f]	33 (33)	132 (132)	66 (66)	>265	0.008 (0.06)	0.03/0.06 (0.1)	17 (17)	0.45
6[g]	66 (132)	132 (132)	265 (265)	>265	0.03 (0.1)	0.1 (0.3)	33 (33)	1.04
7[c]	12 (12)	>194	24 (24)	>194	0.003/0.006 (0.006)	0.05 (0.09)	24 (24)	−0.28
8[f]	76/19 (19)	>303	152 (303)	>303	0.04/0.009 (0.1)	0.04/0.07 (0.3)	152/323 (152)	−0.36
9	63/16 (251)	502 (502)	125 (125)	502 (502)	0.06/0.1 (0.5)	0.1 (2)	31 (31)	−0.81
10	31 (31)	251 (251)	63 (63)	251 (251)	0.02 (0.1)	0.1/0.2 (0.5)	16 (16)	−0.73
11	31 (63)	502 (502)	125 (251)	502 (502)	0.02/0.06 (0.1)	0.1/0.06 (0.5)	16 (16)	−0.59
12	15/29 (116)	464 (464)	116 (232)	464 (464)	0.02 (0.05)	0.05/0.1 (0.5)	7/4 (7)	−0.89
13	24/48 (190)	381 (381)	190 (190)	381 (381)	0.02/0.05 (0.09)	0.09 (0.4)	24 (24)	−1.81
14	24 (24)	381 (381)	190 (190)	>381	0.09/0.04 (0.09)	0.09/0.2 (0.4)	12 (12)	−1.69

[a]Assays were repeated twice. Only one value is presented unless both data are shown.

[b]Lowest precipitation concentration at 4 μg/mL.

[c]Lowest precipitation concentration at 8 μg/mL.

[d]Lowest precipitation concentration at 16 μg/mL.

[e]Lowest precipitation concentration at 32 μg/mL.

[f]Lowest precipitation concentration at 64 μg/mL.

[g]Lowest precipitation concentration at 128 μg/mL.

Table 2.

MIC/MBC (μM) Values[a] of Group 2 Analogues with Aromatic or Aliphatic Thiol Ligands

	***A. baumannii* NCTC 13420**	***P. aeruginosa* NCTC 13437**	***E. cloacae* NCTC 13405**	***K. pneumoniae* ATCC 700603**	***S. aureus* JE2 (USA300)**	***E. faecium* ATCC 700221**	***E. coli* ATCC 25922**	**log *P***
1	47 (47)	377 (377)	189 (189)	377 (377)	0.04 (0.09)	0.2/0.09 (0.4)	24 (24)	0.56
15[f]	19/75 (>603)	302 (302)	75 (603)	>603	0.02 (0.6)	0.6 (1)	151 (151)	2.20
16[g]	18/36 (18)	291 (291)	18/73 (36/73)	146 (146)	0.009 (0.6)	0.02 (0.1)	36 (36)	0.61
17[d]	18 (18)	146 (146)	36 (36)	36 (36)	0.07/0.02 (0.07)	0.02 (0.1)	9 (9)	1.36
18[f]	35 (35)	282 (282)	141 (141)	141 (141)	0.02 (0.3)	0.02/0.1 (0.3)	35 (35)	2.01
19[e]	>546	>546	>546	>546	0.0004/0.02 (0.02)	0.06/0.1 (0.5)	>546	>3.28
20[e]	520 (>520)	>520	>520	>520	0.02/0.1 (0.2)	0.02 (0.2)	>520	>3.18
21[e]	10 (82)	41 (41)	5/10 (82)	20 (20)	0.03 (0.03)	0.2/0.3 (0.6)	10 (10)	1.19
22[d]	19 (76)	305 (305)	38 (152)	76 (76)	0.005/0.02 (0.02)	0.1/0.3 (0.6)	76 (76)	1.75
23[g]	37/74 (149)	149 (149)	37 (149)	149 (149)	0.009 (0.1)	0.6 (1)	74 (74)	2.08

[a] Assays were repeated twice. Only one value is presented, unless both data are shown.

[b] Lowest precipitation concentration at 4 μg/mL.

[c] Lowest precipitation concentration at 8 μg/mL.

[d] Lowest precipitation concentration at 16 μg/mL.

[e] Lowest precipitation concentration at 32 μg/mL.

[f] Lowest precipitation concentration at 64 μg/mL.

[g] Lowest precipitation concentration at 128 μg/mL.

Table 3.

MIC/MBC (μM) Values[a] of Group 3 Analogues with Trialkyl- or Triaryl-phosphine Ligands

	A. baumannii NCTC 13420	*P. aeruginosa* NCTC 13437	*E. cloacae* NCTC 13405	*K. pneumoniae* ATCC 700603	*S. aureus* JE2 (USA300)	*E. faecium* ATCC 700221	*E. coli* ATCC 25922	log *P*
24	7/13 (13)	52 (52)	3 (3)	7/13 (13)	0.1/0.2 (1.6)	0.4 (3)	7 (7)	0.16
25	11/23 (23)	92 (183)	23 (23)	46 (46)	0.001/0.02 (0.2)	0.09/0.2 (0.7)	23 (23)	1.74
26[e]	74/147 (147)	74 (74)	>589	147 (147)	2 (4)	5 (5)	>589	>3.99
27[d]	16/129 (518)	>518	>518	>518	1/2 (4)	4 (4)	>518	>3.94
28[c]	>438	>438	>438	>438	2 (3)	3 (3)	>438	>4.09
29[d]	29/58 (233)	>467	>467	>467	1/ (4)	2/4 (4)	>467	>4.04
30[e]	>366	>366	>366	>366	3/1 (11)	>366	>366	>3.94
31[g]	6/13 (101)	101 (101)	3 (3)	13 (13)	0.1 (0.2)	0.2/0.4 (0.8)	6 (6)	0.35
1	47 (47)	377 (377)	189 (189)	377 (377)	0.04 (0.09)	0.2/0.09 (0.4)	24 (24)	0.56
32[e]	84 (>336)	>336	>336	>336	3 (3)	3/5 (3)	>336	>4.32
33[c]	>311	>311	>311	>311	1/2 (2)	5 (5)	>311	>3.87
34[c]	>281	>281	>281	>281	1 (2)	2/4 (4)	>281	>3.03
35[c]	>292	>292	>292	>292	1 (2)	2/5 (5)	>292	>3.04
36[e]	>249	>249	>249	>249	4 (8)	31 (62)	>249	>3.55

[a] Assays were repeated twice. Only one value is presented, unless both data are shown.

[b] Lowest precipitation concentration at 4 μg/mL.

[c] Lowest precipitation concentration at 8 μg/mL.

[d] Lowest precipitation concentration at 16 μg/mL.

[e] Lowest precipitation concentration at 32 μg/mL.

[f] Lowest precipitation concentration at 64 μg/mL.

[g] Lowest precipitation concentration at 128 μg/mL.

Table 4.

MIC/MBC (μM) Values[a] of Analogues with Trimethylphosphine Ligands

	A. baumannii NCTC 13420	*P. aeruginosa* NCTC 13437	*E. cloacae* NCTC 13405	*K. pneumoniae* ATCC 700603	*S. aureus* JE2 (USA300)	*E. faecium* ATCC 700221	*E. coli* ATCC 25922	log *P*
1	47 (47)	377 (377)	189 (189)	377 (377)	0.04 (0.09)	0.2/0.09 (0.4)	24 (24)	0.56
31[g]	6/13 (101)	101 (101)	3 (3)	13 (13)	0.09 (0.2)	0.2/0.4(0.8)	6(6)	0.35
37	17/4 (17)	>547	4/9 (17)	34 (34)	0.3/0.5 (1)	0.3/0.5 (0.5)	9 (9)	−1.63
38	17/9 (17)	>547	4(4)	34 (34)	0.3 (0.3)	0.3/0.5 (0.5)	9 (9)	−1.88
39	16/8 (16)	>503	8/16 (16)	31 (126)	0.5 (0.5)	0.2/0.5 (0.5)	8/31 (31)	−2.03
40[f]	6/3 (23)	23/91 (91)	3 (3)	11 (23)	0.3 (0.7)	0.3 (0.3)	1/6 (11)	−0.15

[a]Assays were repeated twice. Only one value is presented, unless both data points are shown.

[b]Lowest precipitation concentration at 4 μg/mL.

[c]Lowest precipitation concentration at 8 μg/mL.

[d]Lowest precipitation concentration at 16 μg/mL.

[e]Lowest precipitation concentration at 32 μg/mL.

[f]Lowest precipitation concentration at 64 μg/mL.

[g]Lowest precipitation concentration at 128 μg/mL.