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Synthesis, Structure, and Biological Activity of Ferrocenyl Carbohydrate Conjugates

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Seven ferrocenyl carbohydrate conjugates were synthesized. Coupling reactions of monosaccharide derivatives with ferrocene carbonyl chloride produced {6-*N*-(methyl 2,3,4-tri-*O*-acetyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1-ferrocene carboxamide (**3**), {1-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranose)}-1-ferrocene carboxylate (**4**), and {6-*O*-(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose)}-1-ferrocene carboxylate (**5**). Similarly, 1,1′-bis(carbonyl chloride)-ferrocene was coupled with the appropriate sugars to produce the disubstituted analogues bis{6-*N*-(methyl 2,3,4-tri-*O*-acetyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1,1′-ferrocene carboxamide (**8**), bis{1-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranose)}-1,1′-ferrocene carboxylate (**9**), and bis{6-*O*-(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose)}-1,1′-ferrocene carboxylate (**10**). {6-*N*-(Methyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1-ferrocene carboxamide monohydrate (**12**) was synthesized via amide coupling of an activated ferrocenyl ester with the corresponding carbohydrate. All compounds were characterized by elemental analysis, ^{1}H NMR spectroscopy, and mass spectrometry. X-ray crystallography confirmed the solid-state structure of three ferrocenyl carbohydrate conjugates: 2-*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose)-1-ferrocene carboxamide (**1**), 1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-D-glucopyranose)-1-ferrocene carboxylate (**2**), and **12**. The above compounds, along with bis{2-*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose)}-1,1′-ferrocene carboxamide (**6**), bis{1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-deoxy-1-thio-D-glucopyranose)}-1,1′-ferrocene carboxylate (**7**), and 2-*N*-(2-amino-2-deoxy-D-glucopyranose)-1-ferrocene carboxamide (**11**) were examined for cytotoxicity in cell lines (L1210 and HTB-129) and for antimalarial activity in *Plasmodium falciparum* strains (D10, 3D7, and K1, a chloroquine-resistant strain). In general, the compounds were nontoxic in the human cell line tested (HTB-129), and compounds **4**, **7**, and **9** showed moderate antimalarial activity in one or more of the *P. falciparum* strains.

Introduction

Since ferrocene was first prepared and characterized half a century ago, its chemistry and applications have been extensively studied. Ferrocene has a rich chemistry stemming from the accessibility of a large number of derivatives and their facile redox properties; this has led to their use in numerous industries, including petroleum, plastics, textiles, and metallurgy. Current areas of interest in ferrocene chemistry include use in catalysis,[1−6] as sensors, and as immunoassay reagents.[7−14]

Ferrocene has several properties that have facilitated its investigation for potential biological applications.[15,16] Typi-

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cally, organometallics are sensitive to moisture and air, but ferrocene belongs to a unique group thereof whose members are stable under both aqueous and aerobic conditions. The small size, relative lipophilicity, easy chemical modification, and accessible one-electron-oxidation potential of ferrocene make it an attractive reporter moiety[17−23] and an intriguing pharmaceutical vector.[24−32] When these properties are exploited, many ferrocene derivatives, including amino acid,[17] peptide,[18−22,33] protein,[18−22] and DNA[23] conjugates, have been used for the study of electron-transfer processes and in the development of potential biosensors and immunoassay reagents.[7−14] Recently, the redox activity of ferrocene lipid conjugates has been used to modulate cell transfection.[34] Ferrocene itself has been used as both a cytotoxic and an antianemic agent,[24] and its derivatives have shown antitumor[25−29] and antianemic activity.[30−32] Drug conjugates that have been structurally varied to incorporate ferrocene have displayed a wide range of activities,[35,36] examples of which include conjugates with antibiotics (penicillin) and cancer drugs (tamoxifen).[35,36]

Only a handful of ferrocene conjugates with carbohydrates, one of the most ubiquitous biomolecules, have been reported in the literature.[3,28,31,37−43] Several derivatives of common carbohydrates, such as glucose and glucosamine, have been linked to cyclopentadienyl (Cp) rings of ferrocene via carboxylate-,[3,28,37−40] alkyl-,[41] triazole-,[41] benzoic-,[31] ether-,[37] or amino[42,43]-containing side chains. These ferrocene derivatives have been investigated for numerous applications in, among others, enantioselective catalysis[1,3] and medicine.[30,31,40] The numerous stereocenters in carbohydrates make these conjugates attractive chiral auxiliaries for asymmetric synthesis catalyzed by a second metal center.[1,3] Appended carbohydrates increase the water solubility of ferrocene; these conjugates have also been studied for their medicinal applications, for instance, as therapeutics for iron-deficiency disorders.[30,31]

Malaria is a widespread parasitic disease that affects a large population.[44,45] Four species of the human-infecting parasites are known, with *Plasmodium falciparum* being the most lethal of these. It is estimated that 2 billion people have been exposed to the parasite; yearly, 300−500 million new cases are reported, and between 1 and 2.7 million people, mostly children, die from the infection.[44,45] Historically, malaria is found in tropical areas, including the poorest countries; although the majority of cases originate in Africa, southeast Asia, India, and parts of South America, the disease is now threatening to spread into more temperate zones of the world, and global warming would certainly facilitate its spread.

Currently, no commercially available vaccine for malaria exists, and drug resistance is becoming rampant.[44] Since 1942, chloroquine has been widely used as an effective, inexpensive antimalarial agent, but parasite resistance to chloroquine and pyrimethamine, another inexpensive antimalarial agent, has become problematic for treating malaria, especially in poorer countries. In Southeast Asia, there is

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resistance to all of the widely used antimalarial agents, except artemisinins.[44] The need for different categories of antimalarial agents, active against the resistant strains, has risen. Unfortunately, scant funding for a poor person's disease has led to insufficient research for novel drugs and treatments for malaria. Some alternative drugs are being investigated but either are costly or have adverse side effects, such as toxicity.

An attractive area of research for new malaria treatments is metal conjugates of known antimalarial drugs.[46−61] These include complexes containing mefloquine, quinine, artemisinin, and chloroquine with metal ions such as $Fe^{II/III}$,[46−53] Ga^{III},[54] Ru^{II},[55,56] Rh,[56] Au^{I},[57,58] Pd^{II},[59] and Pt^{II}.[59] Metal complexes with ligands containing chloroquine, or quinine-type structures, have been shown to retain efficacy in chloroquine-resistant strains[46−48,51,52,55,57,58] including an Au^{I} complex with a carbohydrate, β-D-thioglucopyranoside, co-ligand.[58] The most potent metalloantimalarial agents are chloroquine-based metallocenes of Fe^{II} [47] and Ru^{II}.[55]

Antimalarial activity has also been observed in metal complexes that do not incorporate a known antimalarial drug,[40,61−63] including amine phenol Ga^{III} and Fe^{III} complexes,[62,63] and ferrocenyl carbohydrate derivatives.[40] The amine phenol Ga^{III} and Fe^{III} complexes were found to be inhibitors of hemozoin formation with strong antiplasmodial activity, suggesting a mode of action similar to that of chloroquine. Interestingly, one of these complexes showed efficacy against a chloroquine-resistant strain only.[63] Ferrocenyl ellagitannin derivatives have shown moderate to high antiplasmodial activity, while the ellagitannin derivative without the ferrocene moiety had no activity.[40]

The metalloantimalarial mechanism of enhanced activity in resistant strains is unclear but quite pronounced. For quinine-type antimalarial agents, such as chloroquine, the drug accumulates in the parasite's food vacuoles where it forms a complex with ferric heme (ferriprotoporphyrin IX), a product of the parasite's sequestering and consumption of hemoglobin.[64] The drug-ferric heme complex halts the detoxification of ferric heme to hemozoin, resulting in parasite death. It has been suggested that the resistant parasite accumulates less of the drug in its food vacuoles.[64] Varying the antimalarial drug structure by incorporation of a lipophilic, redox-active metal center should increase membrane permeability and possibly aid in the accumulation of drug in the resistant parasite's food vacuoles, thereby increasing efficacy. A secondary effect might be the toxicity of the metal, or toxicity induced by intracellular oxidation. The metal complex may also have an improved affinity for the drug target.

Ferrocenoyl carbohydrate conjugates have potential as metalloantimalarial agents. Combining the ferrocene moiety with a glucose derivative is a novel approach for developing targeted therapy.[46−53] The ferrocene moiety has proven to be a successful addition to known malaria therapeutics, increasing efficacy toward chloroquine-resistant strains of the parasite.[46,47,50−52,60] As well, glucose uptake and metabolism in infected erythrocytes is elevated at all stages of the parasite's life cycle,[65,66] and glucose consumption has been a target in antimalarial research.[67] The hypothesis of this work is that ferrocenyl carbohydrate conjugates have the potential to retain activity in chloroquine-resistant parasite strains and to have increased efficacy by targeting infected cells. This work studies the cytotoxicity and antiplasmodial activity of several ferrocenyl carbohydrate conjugates and includes the synthesis and characterization of seven new ferrocenyl carbohydrate conjugates.

Experimental Section

Materials and Methods. The starting materials ferrocene carbonyl choride, 1,1′-bis(carbonyl chloride)ferrocene,[68] ferrocenyl benzotriazolate (FcCOOBt),[17] methyl 2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside,[69] and methyl 6-amino-6-deoxy-α-D-glucopyranoside[70] were prepared using previously published methods. The previously published ferrocenoyl carbohydrate compounds **1**,[37] **2**,[37] **6**,[37] **7**,[37] and **11**[38] (Schemes 1−3) were prepared as described in the literature. All other chemicals were purchased from Sigma Aldrich and used without further purification. Water was deionized, purified (Barnstead D9802 and D9804 cartridges), and distilled with a Corning MP-1 Mega-Pure still. All solvents

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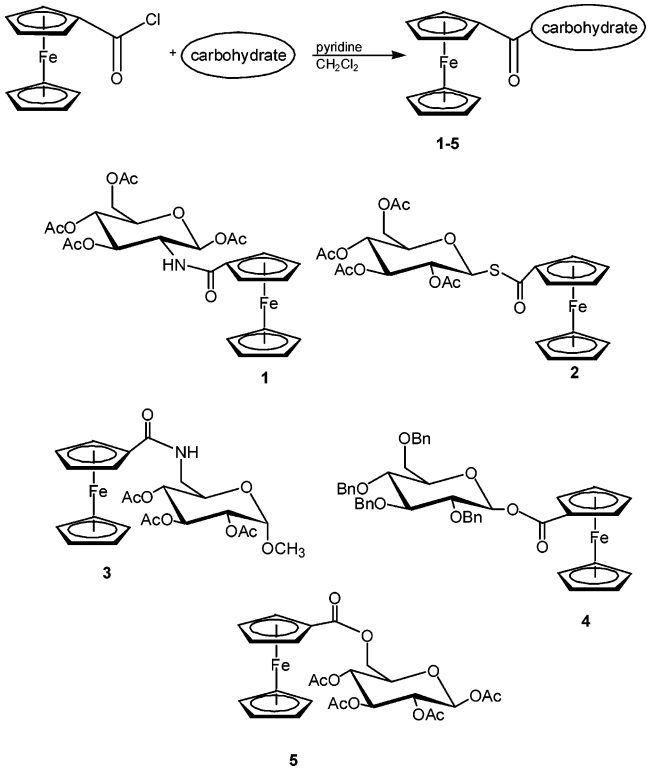
Scheme 1. Compounds **1**−**5**



were high-performance liquid chromatography grade and were purchased from Fisher except dry pyridine (Aldrich; "anhydrous" <0.003% water). Dichloromethane was dried according to standard procedures.[71] N_2 gas was acquired from Praxair. The cell viability assay culture medium reagents were acquired from Gibco products (Grand Island, NY), and well plates were acquired from Beckton Dickinson and Co. (Franklin Lakes, NJ). Glassware used for all reactions was oven dried overnight. Thin-layer chromatography (TLC) was performed using silica TLC plates with aluminum backing (Merck), and silica column chromatography was performed using silica from Silicycle (Quebec City, PQ). All NMR solvents were purchased from Cambridge Isotope Laboratories.

^{1}H NMR spectra were recorded on Bruker AC200-E, AV-300, or AV-400 instruments at 200, 300.13, or 400.21 MHz, respectively, and were calibrated with the deuterated solvent used in each case. ^{1}H−^{1}H COSY 2D NMR spectra were used to aid in the characterization of the compounds. Mass spectra were obtained on either a Kratos Concept II H32Q (Cs^+, LSIMS) or a Macromass LCT (electrospray, ES-MS) instrument. Elemental analyses was performed at the University of British Columbia Chemistry Department by M. Lakha (Carlo Erba analytical instrument) or by Delta Microanalytical Services (Delta, BC).

Methyl 2,3,4-Tri-*O*-acetyl-6-amino-6-deoxy-α-D-glucopyranoside Hydrochloride Salt (13). Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside (760 mg, 2.04 mmol) was dissolved in a mixture of ethyl acetate (20 mL) and methanol (20 mL), and the mixture was transferred into a Parr reactor apparatus fitted with a thick-walled glass insert. To the reaction was added palladium black (23 mg, 0.20 mmol) wetted with water, followed by hydrochloric acid (2 M, 1 mL, 0.2 mmol). The Parr reactor was sealed and pressurized with H_2 gas (100 atm); after rigorously stirring for 24 h, the H_2 gas pressure was released and the reaction

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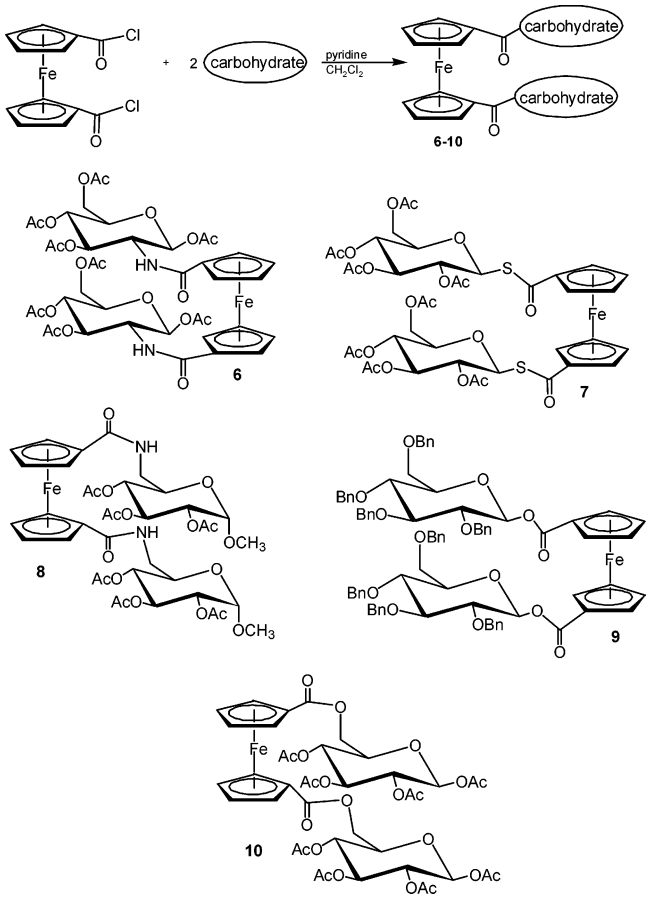
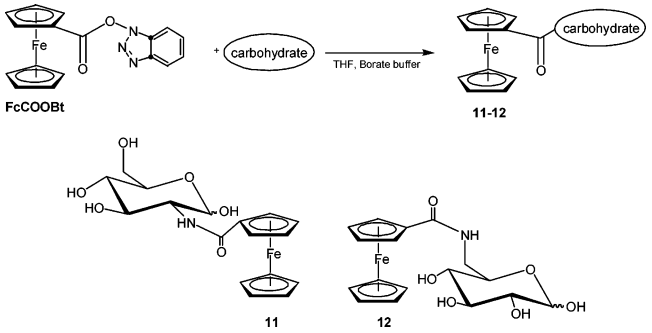
Scheme 2. Compounds **6**−**10**



mixture was filtered through a medium frit to remove the palladium catalyst, which was rinsed with methanol. Rotary evaporation of the filtrate solvent afforded an off-white solid. The solid was dissolved in methanol and precipitated with the addition of ethyl acetate. After cooling of the flask overnight, the precipitate was filtered out and dried in vacuo to yield a white solid, which was used without further purification (394 mg, 54%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.99, 2.03, 2.05 (3s, 9H, CH_3COO), 3.04 (dd, 1H, *H*6a, $^3J_{5,6a}$ = 8.4 Hz, $^2J_{6a,6b}$ = 13.4 Hz), 3.19 (dd, 1H, *H*6b, $^3J_{5,6b}$ = 2.88 Hz, $^2J_{6a,6b}$ = 13.4 Hz), 3.47 (s, 3H, OCH_3), 4.05 (ddd, 1H, *H*5, $^3J_{5,6b}$ = 2.88 Hz, $^3J_{5,6a}$ = 8.36 Hz, $^3J_{4,5}$ = 10.63 Hz), 4.86 (dd, 1H, *H*2, $^3J_{1,2}$ = 3.66 Hz, $^3J_{2,3}$ = 10.05 Hz), 4.91 (t, 1H, *H*4, $^3J_{3,4}$ = 9.75 Hz, $^3J_{4,5}$ = 9.79 Hz), 5.03 (d, 1H, *H*1, $^3J_{1,2}$ = 3.5 Hz), 5.45 (t, 1H, *H*3, $^3J_{3,4}$ = 9.74 Hz, $^3J_{2,3}$ = 10.1 Hz). IR (NaCl, cm^{-1}): 3128−2761 (br, ν_{NH}), 1746 (ν_{CO}), 1608, 1500 (s, ν_{NH}).

General Method for the Preparation of 1−10. A total of 1 or 2 equiv of the appropriate sugar was combined with 1 equiv of either ferrocene carbonyl chloride or 1,1′-bis(carbonyl chloride)-ferrocene. The reagents were dissolved with stirring in dry dichloromethane (10 mL) and placed under N_2. Either 1 or 2 equiv of pyridine was added via a syringe to the reaction mixture, and stirring was continued. For coupling to thio (**2** and **7**) and amino sugars (**1**, **3**, **6**, and **8**), the reaction was quenched with water after stirring at room temperature for 30 min. For coupling involving hydroxyl groups (**4**, **5**, **9**, and **10**), the reaction was stirred while refluxing for 30 h. The reaction was quenched with water (15 mL), washed with 5% bicarbonate (15 mL × 2), and then washed again with water (15 mL × 2). The organic layer was dried with $MgSO_4$, clarified by filtration, and reduced in volume by rotary evaporation. The crude product was purified using column chromatography and/or recrystallization.

Scheme 3. Compounds **11** and **12**



{**6-*N*-(Methyl 2,3,4-Tri-*O*-acetyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1-ferrrocene Carboxamide (3).** Ferrocene carbonyl chloride (129 mg, 0.518 mmol), **13** (164 mg, 0.461 mmol), and anhydrous pyridine (0.08 mL, 1.0 mmol) gave an orange oil. Purification by recrystallization from hot ethanol afforded orange needle crystals (98 mg, 35%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.70, 1.81, 1.86 (3s, 9H, CH_3COO), 3.04 (s, 3H, OCH_3), 3.44 (dt, 2H, *H*6, $^3J_{5,6}$ = 5.79 Hz, $^2J_{6a,6b}$ = 15.03 Hz), 3.88 (m, 2H, *H*5, *H*6), 4.06 (t, 2H, *H*10, *H*10′, $^3J_{9,10}$ = 1.93 Hz), 4.20 (s, 5H, *H*Cp), 4.65, 4.79 (2d, 2H, *H*9, *H*9′, $^3J_{9,10}$ = 1.55 Hz), 5.03 (d, 1H, *H*1, $^3J_{1,2}$ = 3.66 Hz), 5.19 (dd, 1H, *H*2, $^3J_{1,2}$ = 3.66 Hz, $^3J_{2,3}$ = 10.21 Hz), 5.30 (t, 1H, *H*4, $^3J_{3,4}$ = 9.83 Hz), 5.96 (t, 1H, *H*3, $^3J_{3,4}$ = 9.83 Hz), 6.04 (t, 1H, N*H*, $^3J_{2,\ NH}$ = 6.10 Hz). MS (LSIMS$^+$): *m*/*z* 531 ([M + H]$^+$, 100). Anal. Calcd for $C_{17}H_{29}FeNO_9$: C, 54.26; H, 5.50; N, 2.64. Found: C, 54.60; H, 5.45; N, 2.64.

{**1-*O*-(2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose)}-1-ferrocene Carboxylate (4).** Ferrocene carbonyl chloride (193 mg, 0.78 mmol), 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (420 mg, 0.78 mmol), and anhydrous pyridine (0.06 mL, 0.78 mmol) afforded a product that was purified by silica column chromatography, eluting with 1:3 ethyl acetate/hexanes (TLC; 1:1 ethyl acetate/hexanes, R_f = 0.65). After removal of the solvent by rotary evaporation, the resulting dark-orange oil was further purified by recrystallization from hot ethanol to yield dark-orange crystals. ^{1}H NMR (C_6D_6, 300 MHz): δ 3.55−4.02 (m, 6H, *H*2, *H*3, *H*4, *H*5, CH_2), 4.14 (s, 2H, CH_2), 4.18 (s, 5H, *H*Cp), 4.46 (m, 2H, *H*10, *H*10′), 4.70 (d, 1H, *H*6a, $^2J_{6a,\ 6b}$ = 11.4 Hz), 4.87 (dd, 1H, *H*6b, $^3J_{5,6b}$ = 4.35 Hz, $^2J_{6a,\ 6b}$ = 11.4), 4.91−5.05 (m, 6H, *H*9, *H*9′, 2CH_2), 6.22 (d, 1H, *H*1, $^3J_{1,2}$ = 7.90 Hz), 7.16−7.37 (m, 20H, aryl). MS (ESI$^+$): *m*/*z* (relative intensity) 775 ([M + Na]$^+$, 53), 752 (M$^+$, 32). Anal. Calcd for $C_{45}H_{44}FeO_7$: C, 71.81; H, 5.89. Found: C, 71.80; H, 5.91.

{**6-*O*-(1,2,3,4-Tetra-*O*-acetyl-*β*-D-glucopyranose)}-1-ferrocene Carboxylate (5).** Ferrocene carbonyl chloride (231 mg, 0.33 mmol), 1,2,3,4-tetra-*O*-acetyl-D-glucopyranose (110 mg, 0.33 mmol), and anhydrous pyridine (0.03 mL, 0.33 mmol) afforded a product that was purified by silica column chromatography; eluting with 2:1 ethyl acetate/hexanes yielded a yellow-orange oil after solvent removal by rotary evaporation (R_f = 0.32). Recrystallization from hot ethanol yielded a dark-orange powder (60 mg, 32%). ^{1}H NMR (C_6D_6, 300 MHz): δ 1.52, 1.65, 1.67, 1.73 (4s, 12H, CH_3COO), 3.32 (m, 1H, *H*5), 3.95 (d, 1H, *H*6a, *J* = 4.0 Hz), 4.00 (s, 2H, *H*10, *H*10′), 4.10 (s, 5H, *H*Cp), 4.55 (dd, 1H, *H*6b, *J* = 12.6 Hz, *J* = 1.6 Hz), 4.94 (d, 2H, *H*9, *H*9′, *J* = 17.0 Hz), 5.45 (m, 3H, *H*2, *H*3, *H*4), 5.85 (d, 1H, *H*1*β*, *J* = 7.9 Hz). MS (ESI$^+$): *m*/*z* (relative intensity) 599 ([M + K]$^+$, 68), 583 ([M + Na]$^+$, 100). Anal. Calcd for $C_{25}H_{28}FeO_{11}$: C, 53.59; H, 5.04. Found: C, 53.80; H, 5.16.

Bis{6-*N*-(methyl 2,3,4-tri-*O*-acetyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1,1′-ferrrocene Carboxamide (8). 1,1′-Bis(carbonyl chloride)ferrocene (84 mg, 0.27 mmol), **13** (200 mg, 0.56 mmol), and anhydrous pyridine (0.10 mL, 1.2 mmol) yielded a red oil (125 mg, 53%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74, 1.85, 1.96 (3s, 18H, CH_3COO), 3.18 (s, 6H, OCH_3), 3.73 (m, 2H, *H*6), 3.94 (m, 2H, *H*6), 4.08 (m, 2H, *H*5), 4.15, 4.21 (2 br s, 4H, *H*10, *H*10′) 4.80, 4.84 (2 br s, 4H, *H*9, *H*9′), 4.82 (d, 2H, *H*1, $^3J_{1,2}$ = 3.27 Hz), 5.34 (dd, 2H, *H*2, $^3J_{1,2}$ = 3.28 Hz, $^3J_{2,3}$ = 10.21 Hz), 5.49 (t, 2H, *H*4, $^3J_{3,4}$ = 9.83 Hz), 6.01 (t, 2H, *H*3, $^3J_{3,4}$ = 9.83 Hz), 7.16 (br t, 2H, N*H*). MS (LSIMS$^+$): *m*/*z* 877 ([M + H]$^+$, 100). Anal. Calcd for $C_{38}H_{48}FeN_2O_{18}$: C, 52.06; H, 5.52; N, 3.20. Found: C, 52.00; H, 5.26; N, 3.18.

Bis{1-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranose)}-1,1′-ferrocene Carboxylate (9). 1,1′-Bis(carbonyl chloride)ferrocene (100 mg, 0.32 mmol), 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside (348 mg, 0.64 mmol), and anhydrous pyridine (0.05 mL, 0.64 mmol) yielded a red oil. Purification was done by silica column chromatography; eluting with 1:1 ethyl acetate/dichloromethane followed by recrystallization from hot ethanol gave a dark-orange powder (35 mg, 25%). ^{1}H NMR (C_6D_6, 300 MHz): δ 3.54−4.02 (m, 6H, *H*2, *H*3, *H*4, *H*5, CH_2), 4.37−4.50 (m, 4H, *H*10, *H*10′, CH_2), 4.69 (d, 1H, *H*6a, $^2J_{6a,\ 6b}$ = 11.2 Hz), 4.89 (dd, 1H, *H*6b, $^3J_{5,\ 6b}$ = 4.95 Hz, $^2J_{6a,\ 6b}$ = 11.1 Hz), 4.97−5.09 (m, 6H, *H*9, *H*9′, 2CH_2), 6.17 (d, 1H, *H*1, $^3J_{1,2}$ = 7.90 Hz), 7.11−7.36 (m, 20H, aryl). MS (ESI$^+$): *m*/*z* (relative intensity) 1341 ([M + Na]$^+$, 100). Anal. Calcd for $C_{80}H_{78}FeO_{14}$: C, 72.83; H, 5.96. Found: C, 72.82; H, 5.93.

Bis{6-*O*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose)}-1,1′-ferrocene Carboxylate (10). 1,1′-Bis(carbonyl chloride)ferrocene (100 mg, 0.32 mmol), 1,2,3,4-tetra-*O*-acetyl-D-glucopyranose (153 mg, 0.44 mmol), and anhydrous pyridine (0.06 mL, 0.64 mmol) yielded a crude red oil, which was purified by silica column chromatography, eluting with 1:1 ethyl acetate/dichloromethane (R_f = 0.56). After removal of the solvent by rotary evaporation, recrystallization from hot ethanol yielded a dark-orange powder (62 mg, 15%). ^{1}H NMR (C_6D_6, 300 MHz): δ 1.58, 1.69, 1.79, 1.85 (4s, 24H, CH_3-COO) 3.52 (d, 2H, *H*5, *J* = 8.7 Hz), 4.05 (dd, 4H, *H*10, *H*10′, *J* = 24.3 Hz, *J* = 1.2 Hz), 4.22 (dd, 2H, *H*6a, *J* = 12.3 Hz, *J* = 3.7 Hz), 4.52 (dd, 2H, *H*6b, *J* = 12.3 Hz, *J* = 1.7 Hz), 5.03 (dd, 4H, *H*9, *H*9′, *J* = 30.6 Hz, *J* = 1.2 Hz), 5.54 (m, 6H, *H*2, *H*3, *H*4), 5.93 (d, 2H, *H*1, *J* = 8.1 Hz). MS (ESI$^+$): *m*/*z* (relative intensity) 957 ([M + Na]$^+$, 100). Anal. Calcd for $C_{40}H_{46}FeO_{22}$: C, 51.40; H, 4.96. Found: C, 51.65; H, 5.08.

{**6-*N*-(Methyl-6-amino-6-deoxy-α-D-glucopyranoside)}-1-ferrocene Carboxamide Monohydrate (12).** Methyl 6-amino-6-deoxy-α-D-glucopyranoside (71 mg, 0.37 mmol) was dissolved in 5 mL of borate buffer (20 mM, pH 9). Fc(COOBt) (190 mg, 0.55 mmol) was dissolved in 5 mL of tetrahydrofuran, and the solution was slowly added to the borate buffer solution with stirring. The resulting solution was adjusted to pH 9 using 0.3 N NaOH and stirred overnight. The solvent was removed using a rotary evaporator, and the product was purified by silica column chromatography, eluting with 8:2 ethyl acetate/methanol. Fractions containing the product (R_f = 0.5) were reduced in volume on a rotary evaporator, and the residue was vacuum-dried to yield an orange solid (83 mg, 56%). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.27 (t, 1H, *H*4, ^3J = 9.06 Hz), 3.41 (s, 3H, OCH_3), 3.51 (dd, 1H, *H*2, $^3J_{1,2}$ = 3.66 Hz, $^3J_{2,3}$ = 9.72 Hz), 3.53 (d, 1H, *H*6b, *J* = 3.53 Hz) 3.66 (t, 1H, *H*3, ^3J = 9.24 Hz), 3.70 (m, 1H, *H*5, ^3J = 5.97 Hz, ^3J = 4.02 Hz), 3.75 (d, 1H, *H*6a, *J* = 2.49 Hz), 4.26 (s, 5H, *H*Cp), 4.48 (t, 2H, *H*10, *H*10′, $^3J_{9,10}$ = 1.92 Hz), 4.69 (d, 1H, *H*1, $^3J_{1,2}$ = 3.45 Hz), 4.80 (t, 2H, *H*9, *H*9′, $^3J_{9,10}$ = 1.92 Hz). MS (LSIMS$^+$): 406 ([M + H]$^+$, 100). Anal. Calcd for $C_{18}H_{23}FeNO_6 \cdot H_2O$: C, 51.09; H, 5.65; N, 3.31. Found: C, 51.00; H, 5.95; N, 3.63.

X-ray Crystallographic Analyses of 1, 2, and 12. Orange crystals were grown after several days by cooling concentrated ethanol solutions of the respective compounds. Crystals were

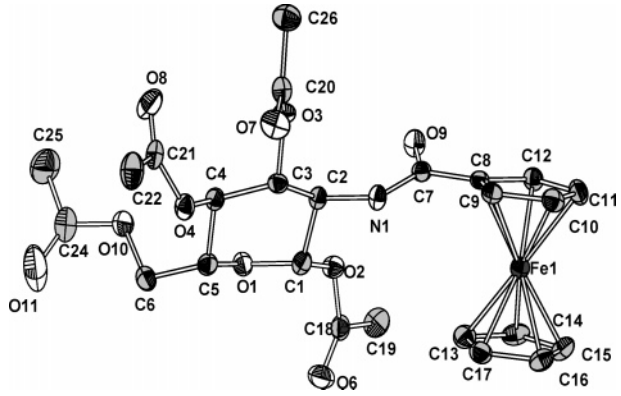


Figure 1. Structure of **1** showing an atom-labeling scheme (50% thermal ellipsoids).

Table 1. Selected Bond Lengths (Å) and Angles (deg) in **1**, **2**, and **12**

	1	**2**	**12**
Fe−C_{mean}	2.046	2.039	
Fe−C_{mean} (substituted)	2.050	2.040	2.039
C7−C8	1.492(3)	1.460(5)	1.473(9)
C7−O9/O6/O1	1.226(2)	1.217(4)	1.241(7)
C7−N1/S1	1.361(2)	1.797(4)	1.353(8)
C8−C7−O9/O6/O1	122.49(18)°	124.5(3)°	121.0(6)°
C8−C7−N1/S1	114.20(16)°	113.2(3)°	116.9(5)°
O9/O6/O1−C7−N1/S1	123.32(18)°	122.3(3)°	122.0(6)°
tilt between Cp rings	0.56°	2.76°	

mounted on glass fibers and cooled to 173 K. Data sets were collected on either a Bruker X8 APEX or a Rigaku/ADSC CCD diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). Data were collected and integrated using the Bruker *SAINT*[72] software package and corrected for Lorentz and polarization effects as well as for absorption (*SADABS*[73]). The structures were solved by direct methods (*SIR92*[74]). Non-hydrogen atoms were refined anisotropically, while hydrogen atoms were added but not refined. Final refinement was completed using *SHELXL-97*.[75] Selected bond distances and angles are found in Table 1, structure diagrams in Figures 1−3, and CIFs in the Supporting Information.

In Vitro Antiplasmodial Activity Studies. All samples were tested in duplicate on a single occasion against the D10 strain. Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[76] Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase (pLDH) assay using a modified method described by Makler et al.[77] All compounds were dissolved in 2 mg/mL of 10% methanol/dimethyl sulfoxide (DMSO). The samples were then diluted with water to 25, 12.5, and 6.25 μg/mL and stored at 20 °C until use. Chloroquine was used as the positive control in all experiments and was tested at concentrations of 30, 15, and 7.5 ng/mL.

All samples were tested in triplicate against the 3D7 and K1 strains. The cultures were maintained in continuous log phase growth in a RPMI1640 medium supplemented with 5% wash human A+ erythrocytes, 25 mM HEPES, 32 nM $NaHCO_3$, and AlbuMAXII (lipid-rich bovine serum albumin). All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Stock compound solutions were prepared in 100% DMSO at 5 mg/mL. The compounds were further diluted using a complete RPMI1640 medium supplemented with cold hypoxanthine and AlbuMAXII. Assays were preformed on sterile 96-well microtiter plates, with each plate containing 100 μL of the parasite culture (1% parasitemia, 2.5% hemacrit). Each compound was tested at 30, 10, 3, 1, 0.3, and 0.1 μg/mL. After 24 h of incubation at 37 °C, 3.7 Bq of [^{3}H]hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filter mats, and the radioactivity was counted using a Wallac Microbeta 1450 scintillation counter.[78]

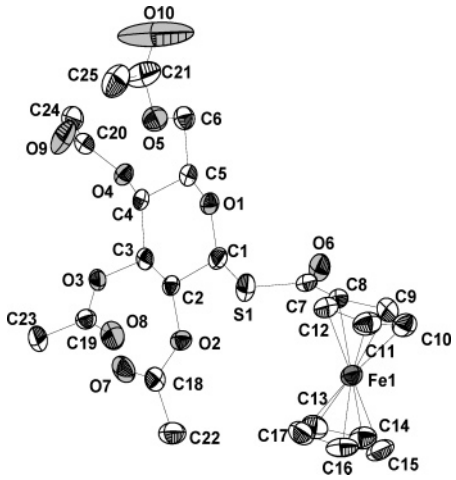


Figure 2. Structure of **2** showing an atom-labeling scheme (50% thermal ellipsoids).

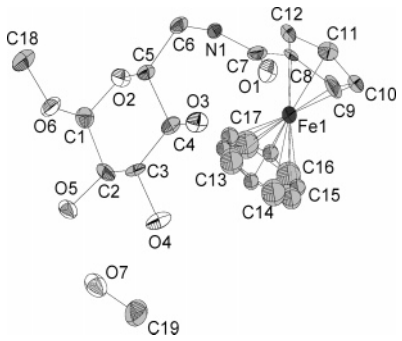


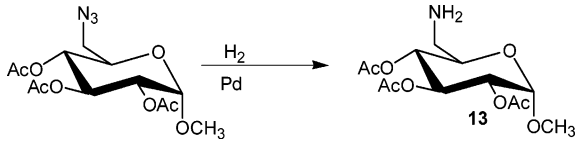
Figure 3. Structure of **12** showing an atom-labeling scheme and the disorder in the unsubstituted Cp ring (50% thermal ellipsoids).

Cell Viability Assays. HTB-129 cells were detached from culture flasks with 0.25% trypsin and 0.03% EDTA (Sigma) and resuspended in a fresh culture medium (90% Leibovitz's L-15 medium with 2 mM L-glutamine, 0.01 mg/mL bovine insulin, and 10% fetal bovine serum) at a density of 1×10^5 cells/mL. Using a Falcon 96-well, flat-bottom plate, 100 μL of the cell suspension was added to the wells. Sterile, distilled water (200 μL) was added to the plate's outer wells to prevent evaporation. The cells were incubated for 24 h at 37 °C under 5% CO_2(g).

Using another Falcon 96-well plate, 100 μL of a L1210 cell suspension in a RPMI1640 medium (200 μL) supplemented with 10% heat-inactivated horse serum, 50 IU/mL penicillin, 50 μg/mL streptomycin, 0.5 mM sodium pyruvate, 0.05% (w/v) pluronic acid F68, and 0.01 M HEPES (1×10^5 cells/mL) was added to the wells. The cells were incubated for 24 h at 37 °C under 5% CO_2(g).

(72) *SAINT*, version 6.02; Bruker AXS Inc.: Madison, WI, 1999.
(73) *SADABS*, version 2.05; Bruker AXS Inc.: Madison, WI, 1999.
(74) Altomare, A.; Cascarano, M.; Giaconazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1994**, *26*, 343.
(75) *SHELXL*, version 5.1; Bruker AXS Inc.: Madison, WI, 1997.
(76) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 635−637.
(77) Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739−741.
(78) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.

Scheme 4. Sugar Derivative **13**



For both cell lines, compounds were tested in triplicate. The compounds were dissolved in DMSO and diluted in culture medium to concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.13μg/mL. Aliquots (100μL) were then transferred to the cells in plate columns 5 through 11, making final experimental compound concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.56μg/mL. A DMSO control was also done adding 100μL 2% DMSO. The cells were then incubated for 72 h under the same conditions described above.

Measurement of the cell viability was done with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (50 μL, 2.5 mg/mL), which was added to each of the plates' experimental wells, and the cells were incubated for a further 3 h. The plates were then spun on an IEC HN-SII centrifuge for 5 min at low speed. The supernatant was carefully aspirated from each experimental well and DMSO (150 μL/well) was added to each experimental well to dissolve the tetrazolium salts. The plates were read on a Beckman-Coulter DTX800 plate reader (Fullerton, CA) at 570 nm.

Results and Discussion

Both novel and previously reported ferrocenyl carbohydrate conjugates, with slight variations in structure, were prepared (Schemes 1−3). Some compounds had only one Cp ring substituted (**1**−**5**, **11**, and **12**) with various glucose derivatives, while others had both Cp rings substituted with identical derivatives (**6**−**10**). The carbohydrate moieties in these compounds were attached to the ferrocene via amide (**1**, **3**, **6**, **8**, **11**, and **12)**, ester (**4**, **5**, **9**, and **10**), or thioester (**2** and **7**) linkages. The ester or amide bond was linked at the C-1 (**2**, **4**, **7**, and **9**), C-2 (**1**, **6**, and **11**), or C-6 (**3**, **5**, **8**, **10**, and **12**) position of the sugar. Protecting groups, including methoxy, benzyl, and acetyl groups, were present on the other positions of the sugar.

Compounds **1**, **2**, **6**, **7**, and **11** (Schemes 1−3) have been previously reported[37,38] and were prepared by those literature methods. Following purification, their structures were verified by ^{1}H NMR spectroscopy.

The sugar derivative **13** (Scheme 4) is closely related to known compounds[69,70] and was prepared using similar methods. Conversion of the azide in methyl 2,3,4-tris-*O*-acetyl-6-azido-6-deoxyglucopyranoside to the amino compound **13** was achieved by palladium-metal-catalyzed hydrogenation. A small amount of hydrochloric acid was added to the hydrogenation to form a hydrochloride salt with the newly formed amine, thereby obviating intermolecular transamidation. After precipitation as a white solid, compound **13** was verified by ^{1}H NMR and IR spectroscopy and then used without further purification; the ^{1}H NMR spectrum did confirm that the product was pure. The three acetyl groups were confirmed by three singlets between 1.99 and 2.05 ppm, and the presence of only one anomer was confirmed by the one doublet observed at 5.03 ppm. The IR spectrum confirmed that the azido group had been converted to an amine group and that the amine group was present as an ammonium salt. The NH stretch at 3128−2761 cm^{-1} and the NH bend at 1608 and 1500 cm^{-1} were at lower frequencies than the analogous free primary amine values, as expected for an ammonium salt.[79]

Compounds **3**−**5** and **8**−**10** (Schemes 1 and 2) were all prepared by methods similar to those for compounds **1**, **2**, **6**, and **7**. As expected, because of the better nucleophilicity of nitrogen compared to that of oxygen, the formation of the amide bond in the synthesis of **3** and **8** proceeded rapidly, with yields similar to those for other ferrocenyl carbohydrate conjugates of this nature.[37,40] In contrast, the ester linkage in **4**, **5**, **9**, and **10** required longer reactions times and refluxing to obtain moderate to low yields. The complexes were purified by either recrystallization from ethanol or column chromatography followed by recrystallization.

Compound **12** was prepared in a manner similar to that for **11** (Scheme 3). The appropriate sugar was reacted with FcCOOBt in a buffered mixed-solvent system. Despite the aqueous reaction conditions, the activated ester was not converted back to carboxylic acid but selectively reacted to form an amide in moderate yields; **12** was purified by column chromatography.

All new compounds were fully characterized by elemental analysis, mass spectrometry, and ^{1}H NMR spectroscopy. Elemental analyses of the bulk samples were consistent with the proposed molecular formulas and evince the purity of the compounds. In the case of **12**, which was synthesized under aqueous conditions, one water molecule was associated with the compound, typical of unprotected carbohydrate derivatives, even when dried in vacuo for extended periods of time, with or without heating. The mass spectra of the compounds showed the parent ion, $[M^+]$ or $[M + Na]^+$, with typically 100% relative intensity. Other peaks in the mass spectra included fragments with protected groups or complete carbohydrate moieties removed from the ferrocene core. Assignments in the ^{1}H NMR spectra were facilitated by 2D ^{1}H−^{1}H COSY experiments and support the proposed structures.

The ^{1}H NMR resonances assigned to the sugar moieties were typically shifted downfield relative to the free sugar ^{1}H NMR resonances, with additional hydrogen resonances observed for the hydrogen atoms associated with the amide groups. The largest shifts were observed for the hydrogen atoms on the sugar carbon directly connected to the amide or ester linkage. For example, in the ^{1}H NMR spectrum of **12**, the resonances of the sugar C-6 hydrogen atoms were shifted downfield by 0.41 and 0.52 ppm, while the other hydrogen atoms associated with the sugar shifted ≤0.1 ppm. Although the amine hydrogen atoms in **13** were not observed, the amide hydrogen atoms were observed for **3** and **8**, likely because of the slower deuterium exchange for the amide versus the primary amine. In contrast, the amide hydrogen atoms in **12** were not observed because the ^{1}H NMR spectrum of **12** was run in methanol-d_4, a protic solvent

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where deuterium easily exchanges with hydrogen atoms such as those in amine or amide groups. Conversely, the ^{1}H NMR spectra of **3** and **8** were run in aprotic solvents; deuterium exchange with the amide protons did not occur (or was significantly slower), and the amide hydrogen resonances were observed.

The Cp ring hydrogen ^{1}H NMR resonances are indicative of both the structure and the symmetry of the compounds. For **3**−**5**, which are substituted on one Cp ring only, three different resonances are observed. The unsubstituted Cp ring resonance was a singlet between 4.0 and 4.3 ppm. The substituted Cp had resonances for the hydrogen atoms on the carbons adjacent, or α (between 4.80 and 4.95 ppm), and the hydrogen atoms on the carbons β (between 4.00 and 4.48 ppm) to the carbonyl linker. It is expected in a symmetric compound that the two α hydrogen atoms should give one doublet and the two β hydrogen atoms should give a doublet of doublets. Because of the stereocenters of the glucose moiety, all hydrogen atoms on the substituted Cp ring are inequivalent, and two sets of the above-described resonances are expected. For example, in the ^{1}H NMR spectrum of **12**, the α hydrogen atoms appeared as two doublets, as predicted for an asymmetric structure. The β hydrogen atoms appeared as a triplet, which may have been an overlapped doublet of doublets. For **8**−**10**, where both Cp rings are substituted, only one set of resonances for each of the sugar hydrogens and for each of the four hydrogen atoms on the substituted Cp ring was observed. Each of the substituted Cp rings and the respective linked sugar moieties are equivalent. Like the monosubstituted compounds, the two α protons of the Cp ring are rendered inequivalent by the stereocenters of the sugar and appeared as two doublets instead of one.

Solid-state structures of the monosubstituted ferrocenoyl carbohydrate conjugates **1**, **2**, and **12** were determined by X-ray crystallography and bear many structural similarities (Figures 1−3 and Table 1). (Few crystal structures of ferrocene carbohydrate conjugates have been reported.)[1,3,28,42] In each structure reported herein, the unit cell is monoclinic containing two molecules in the asymmetric unit. In the structures of **1** and **2**, the two Cp rings are eclipsed and are very close to parallel. In the structure of **12**, the nonsubstituted Cp ring is rotationally disordered, equally occupying the eclipsed and staggered orientations, relative to the other Cp ring. The Fe−C bond lengths are similar to those reported elsewhere for ferrocene derivatives.[1,22,28,29] Slightly shorter Fe−C bond lengths are observed in the nonsubstituted Cp ring than in the substituted ring, while **2** and **12** have shorter Fe−C bond lengths than does **1**. The C−C bonds of the Cp ring are shorter in the nonsubstituted ring and in the Cp rings of **2**. The slight differences in the Fe−C and Cp C−C bond lengths can be attributed to the electron-withdrawing nature of the amide and thioether linkages, reducing the electron density in the substituted ring with concomitant longer bond lengths. Many ferrocenes substituted by an amide have been structurally analyzed, and the bond lengths and angles are similar to those reported here.[20,29,80] The geometry around the carbonyl carbon is distorted trigonal planar, with the C8−C7−N1/S1 angle being slightly less than, and the other two angles slightly greater than, 120°. As expected, only the β anomers were isolated for **1** and **2** and only the α anomer was isolated for **12**, which agrees with the single anomeric forms observed in the respective ^{1}H NMR spectra. In the structure of **2**, O10 has a relatively large anisotropic thermal ellipsoid, due to the significant motion consistent with an alkyl chain. All structures confirm that the respective carbohydrates are pendant and do not interact in any way with the iron metal center.

Table 2. Cytotoxicity and Antiplasmodial Activity of Ferrocenyl Carbohydrate Complexes

	cytotoxicity IC_{50} (μM)		antiplasmodial activity ED_{50} for *P. falciparum* strains (μM)		
compd	mouse lymphoma (L1210)	human breast cancer (HTB-129)	D10	3D7	K1 (chloroquine-resistant)
1	17	>321	32	33	>54
2	25	87	>43	33	40
3	55	>325	>47	>56	52
5	ND[a]	180	12.5	>54	>54
6	15.5	>115	ND[a]	>32	>32
7	13.5	ND[a]	>26	4.9	6.1
10	ND[a]	>121	<6.7	16.4	15.5
11	67	>468	56	>77	61
12	ND[a]	>474	ND[a]	>74	>74

[a] ND = not determined.

To show that the compounds would not be toxic to the infected host, the cytotoxicity of each of the ferrocenoyl carbohydrate complexes was studied in two cell lines (Table 2). Cytotoxicity was determined via the MTT assay.[81] All compounds showed toxicity values similar to one another, within each cell line. In the human cell line, only two compounds, **2** and **5**, had IC_{50} values in the range of concentrations tested. Other ferrocenoyl carbohydrates have also been reported to show only low levels of toxicity, even at high concentrations.[28,40]

Antimalarial activity was assessed by the inhibition of three strains of *P. falciparum*, two chloroquine-sensitive strains and one chloroquine-resistant strain. Compounds **4** and **9** were tested only in the D10 strain (ED_{50} > 25 μM). Several other compounds showed promising antimalarial activity (Table 2). In the D10 strain, two compounds, **5** and **10**, showed moderate to potentially high activity, respectively. Compound **5** had an ED_{50} value of 12.5 μM. Compound **10**, which is the disubstituted analogue of **5**, displayed 78% inhibition at the lowest concentration tested, 6.7 μM. In the 3D7 and K1 (chloroquine-resistant) strains, **5** showed no activity while **10** showed only moderate activity with ED_{50} values of 16.4 and 15.5 μM in the two strains, respectively. Compound **7**, which did not show activity in the D10 strains, was moderately active in the 3D7 and K1 strains with EC_{50} values of 4.9 and 6.1 μM, respectively. The difference in the antiplasmodial activities between the two nonresistant strains, although mainly due to the differ-

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ences in the strains themselves, may be partially attributed to the differences in the assay methods. For the nonresistant D10 strain, parasite viability was determined by measuring the enzymatic activity of parasite lactate dehydrogenase (pLDH). For the 3D7 and the chloroquine-resistant K1 strains, incorporation of tritium-labeled hypoxanthine was employed to measure parasite growth. The latter is considered to be the more sensitive of the two assays.[82]

Because most the compounds showed similar toxicity and antiplasmodial activity, only a minimal structure activity relationship can be determined. Notably, for the active compounds, those substituted on both Cp rings were more active than their monosubstituted analogues. For example, compound **7** was more than 6-fold more active than its monosubstituted analogue, **2**, in both the 3D7 and K1 strains. The most active compounds all contained acetyl protecting groups on the carbohydrate moiety and were linked to the ferrocene via either a thioester or ester rather than an amide functional group.

Although none of the compounds proved to be highly effective inhibitors of *P. falciparum* in vitro, carbohydrate functionalization is an attractive approach to novel antimalarial agents. Addition of the carbohydrate substituent(s) yielded ferrocene compounds with low toxicity, even for the compounds with the best antimalarial activity. This suggests that the cytotoxic and antimalarial activities are not correlated and that the concentrations needed for antimalarial activity would likely be nontoxic toward the host. As well, the antimalarial activity is similar in both chloroquine-resistant and nonresistant strains. Because the malaria parasite sequesters many nutrients, such as hemoglobin and glucose, it has developed a higher affinity for these substrates than the host.[65] Cell permeability for carbohydrates is increased and the rate of glucose consumption is elevated 50−100-fold in infected erythrocytes.[65,66] This higher affinity for glucose may result in targeting of the parasite by glucose derivatives, such as ferrocenyl carbohydrate conjugates, in vivo. Targeting would result in lower dose amounts and higher efficacy than suggested by the in vitro studies.

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Conclusions

Ferrocenyl carbohydrate conjugates were easily synthesized via amide or thioester linkages between ferrocene carbonyl chloride and a respective amine or thiol carbohydrate derivative. The esterification of a carbohydrate hydroxyl required more aggressive conditions. The structures of the compounds were characterized by elemental analysis, mass spectrometry, and NMR spectroscopy and showed low levels of symmetry due to the carbohydrate stereocenters. Three of the compounds were also analyzed in the solid state by X-ray crystallography, which confirmed the pendant nature of the carbohydrate substituents and the anomeric conformation of the carbohydrates. The compounds were nontoxic at high levels in the human breast cancer HTB129 cell line, while compounds **7** and **10**, both disubstituted compounds with acetyl-protected glucose moieties, showed moderate antimalarial activity in vitro. Further experiments are needed to determine if these complexes have higher in vivo activity attributable to targeting of the parasites' elevated level of glucose consumption.

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Supporting Information Available: X-ray crystallography data (CIF and PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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