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Disulfide Prodrugs of Albitiazolium (T3/SAR97276): Synthesis and Biological Activities

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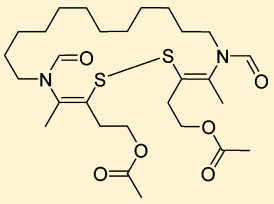
Supporting Information

ABSTRACT: We report herein the design, synthesis, and biological screening of a series of 15 disulfide prodrugs as precursors of albitiazolium bromide (T3/SAR97276, compound **1**), a choline analogue which is currently being evaluated in clinical trials (phase II) for severe malaria. The corresponding prodrugs are expected to revert back to the active bis-thiazolium salt through an enzymatic reduction of the disulfide bond. To enhance aqueous solubility of these prodrugs, an amino acid residue (valine or lysine) or a phosphate group was introduced on the thiazolium side chain. Most of the novel derivatives exhibited potent in vitro antimalarial activity against *P. falciparum*. After oral administration, the cyclic disulfide prodrug **8** showed the best improvement of oral efficacy in comparison to the parent drug.



INTRODUCTION

Malaria is the most prevalent parasite disease, causing each year 250 million cases and 800 000 deaths, mostly in African children, and 80% of these cases are located in sub-Saharan Africa.[1,2] Among the key interventions for controlling this disease, the arsenal of antimalarial drugs is critical, but the current choice of drugs is limited.[3] The discovery and development of the artemisinin derivatives in China have provided a new class of highly effective antimalarials now used as artemisinin-based combination therapy (ACTs) to overcome the chemoresistance problem. However, artemisinin-resistant parasites recently reported in Asia could seriously undermine global malaria control.[4,5] *Plasmodium falciparum*, the most pathogenic human malaria parasite, is becoming pharmacoresistant to conventional as well as newly discovered drugs; thus, the need for new antimalarial strategies involving novel targets is as crucial as ever.[6] Thus, various research groups are developing a new family of derivatives differing in their mechanisms of action.[3,7] A decade ago, some of us contributed to this challenge with a novel class of choline analogues. The structure of these potent antimalarials is based on a long lipophilic chain incorporating two thiazolium cationic heads.[8−12] One lead compound, namely albitiazolium bromide (T3/SAR97276, compound **1**, Figure 1) shows high efficacy in vitro against *P. falciparum* and in vivo against *P. vinckei* in mouse and primate malaria models[8,10,11] and has fulfilled multiple criteria required for its development. Available in a single-step synthesis from commercial reactants, its preparation is therefore adapted for large-scale and low-cost production. Potency and specificity of these antiphospholipid effectors are likely due to their unique property to accumulate in a nonreversible way inside the intraerythrocytic parasite.[11,13,14] The efficiency of albitiazolium comes from its dual mechanism of action that involves, on one hand, the inhibition of the de novo phosphatidylcholine biosynthesis[11] and, on the other hand, an interaction with the ferriprotoporphyrin IX (FPIX) which leads to heme detoxification.[13]

Currently, albitiazolium is undergoing phase II clinical trials to treat severe malaria by parenteral administration due to its poor oral bioavailability. Most of the infections by malaria parasites occur in tropical or subtropical countries where the medical care systems are not always available. Consequently, the way of administration of albitiazolium limits its therapeutic use to the treatment of severe malaria and highlights the need for an oral form to treat uncomplicated malaria on a large scale.

Owing to the presence of two cationic charges, bis-thiazolium derivatives have greater difficulty crossing biological barriers, especially the intestinal epithelium. Thus, we devoted our recent efforts to the design of *S*-acyl prodrug approaches to temporally mask the cationic charges, and it was anticipated that the resulting lipophilic albitiazolium prodrugs would then be able to cross the intestinal epithelium by passive diffusion.[11,15] So far, the best absolute bioavailability obtained for a thioester-type prodrug was 15% in rat,[16] and this modest improvement was attributed to the early conversion of the

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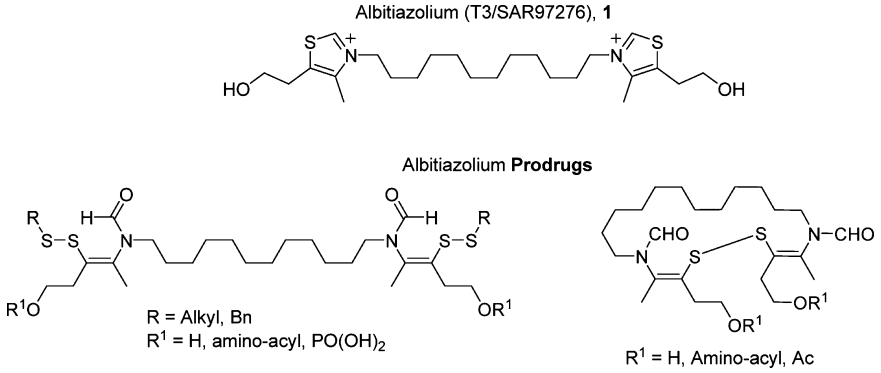
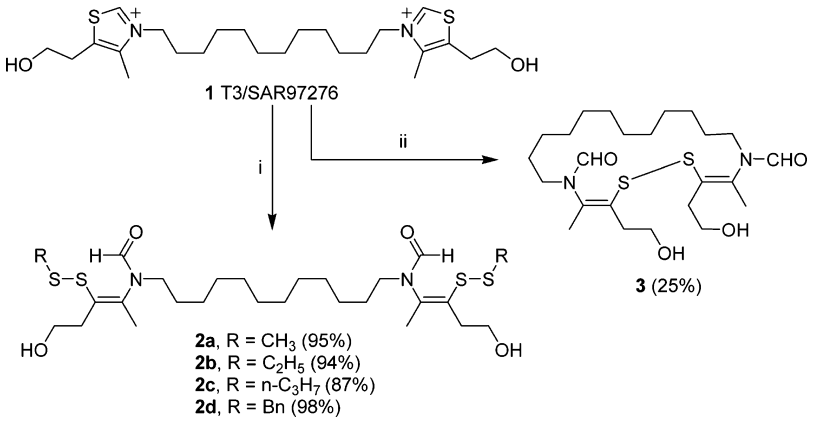


Figure 1. Structures of albitiazolium (T3/SAR97276, compound **1**) and related disulfide prodrugs.

Scheme 1. Synthesis of Disulfide Prodrugs 2a–d and 3[a]



[a]Reagents and conditions: (i) 2 M NaOH, sodium alkyl thiosulfate, $CHCl_3$, 1–2 h; (ii) 2 M NaOH, *tert*-butyl nitrite, CH_2Cl_2, 10 h.

prodrug into the drug, occurring in the gastrointestinal tract before absorption.

Herein, we described a new series of albitiazolium disulfide prodrugs related to the approach previously developed for thiamine (vitamin B1) and which led to orally bioavailable precursors.[17,18] Briefly, it consists of the thiazolium ring-opening and concomitant trapping of the resulting thiol function as a disulfide linkage (Figure 1). The release of the parent drug could involve either an enzymatic reduction of the disulfide bond (for which no evidence is presently available) or a thiol–disulfide exchange reaction.[11]

In addition, to improve the physicochemical properties of the albitiazolium disulfide prodrugs and especially their aqueous solubility, we introduced various polar residues on the hydroxyl function of the side chain of albitiazolium. In this respect, aminoacyl or phosphate moieties were selected, as they have been extensively used for this purpose.[19] Thus, the resulting pro-prodrugs were expected to be converted in vivo in two steps to the active drug. First, the amino acid or phosphate pro-moieties should be hydrolyzed through esterase or alkaline phosphatase activities present in abundance on the brush border surface of the gastrointestinal tract. The resulting lipophilic disulfide prodrug generated nearby is expected to cross the intestinal epithelium more easily than the parent bis-cationic drug. This kind of approach has already proved its efficiency[20,21] and may help us to circumvent the low bioavailability of albitiazolium after oral administration.

RESULTS AND DISCUSSION

Chemistry. *Disulfide Prodrugs of Albitiazolium.* Lipophilic prodrugs of albitiazolium were obtained following a common route used for the synthesis of mixed disulfides and based on the reaction of a Bünte salt (previously prepared from an alkyl halide and sodium thiosulfate) with a mercaptan. The procedure involved a two-step one-pot reaction (Scheme 1): the thiazolium ring is opened in alkaline media, according to the methodology previously reported for the synthesis of thioester prodrugs of bis-thiazolium salts;[15] then addition of the sodium alkyl thiosulfate (Bünte salt) led to the desired disulfide prodrugs **2a–d** in high yields. The different promoieties were chosen to vary the steric and electronic environment in the vicinity of the biolabile linkage, as well as the relative lipophilicity of the resulting prodrugs.

In addition to minimizing the molecular weight and limiting the molecular flexibility, we envisaged the intramolecular cyclization of compound **1**, bridging together the two thiol functions of the molecule. This reaction is based on the peculiarity of thionitrite which spontaneously decomposes in solution into the corresponding disulfide and nitric oxide.[22] Thus, the cyclic disulfide prodrug **3** was prepared in a single step (Scheme 1): after thiazolium ring-opening in alkaline media, the thiolate intermediate was activated by means of *tert*-butyl nitrite, and in situ the thionitrite led to the formation of intra- and intermolecular disulfide bonds. To limit the polymerization of the thionitrite intermediate, the reaction was carried under high dilution conditions (10 mM) and compound **3** was isolated in 25% yield.

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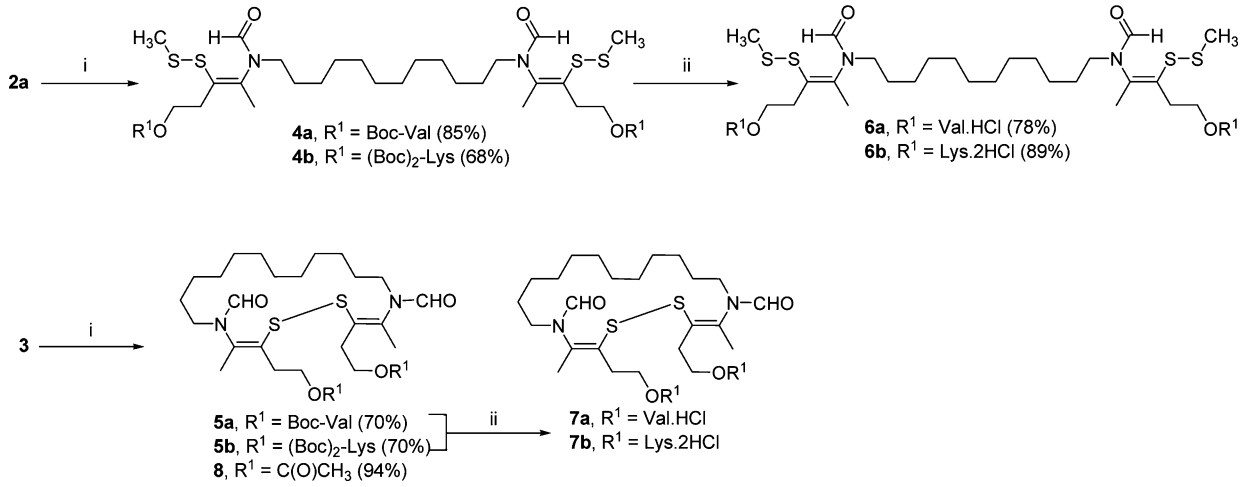
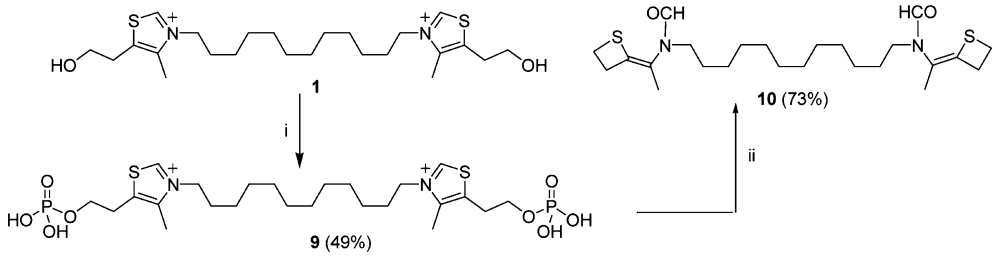
Scheme 2. Synthesis of Aminoacyl 6a,b and 7a,b and Acetyl 8 Disulfide Prodrugs[a]



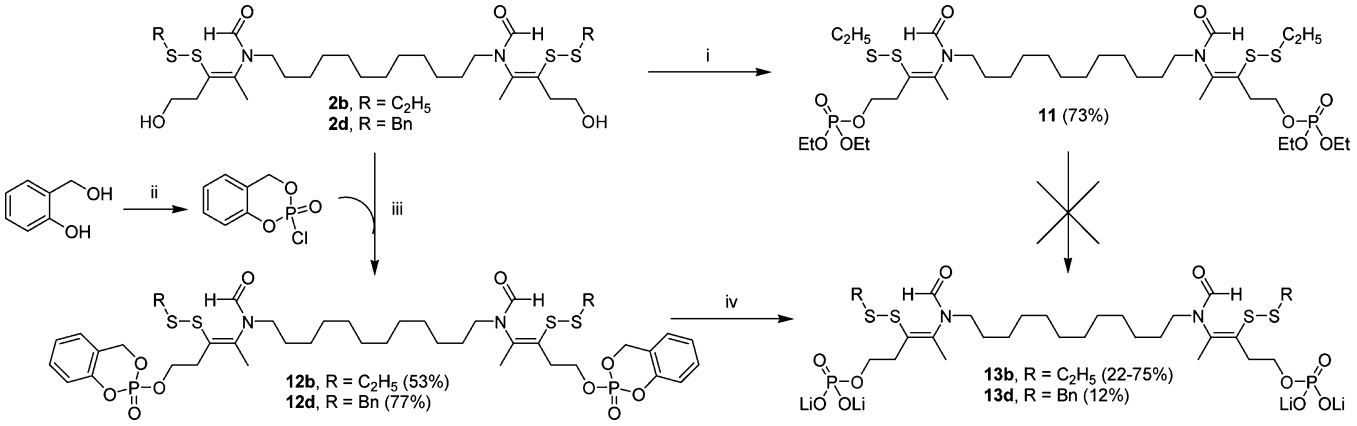
[a]Reagents and conditions: (i) Boc-Val or $(Boc)_2$-Lys, DCC or EDC, DMAP or acetyl chloride, CH_2Cl_2, 2−3 h; (ii) HCl (4 M), 1,4-dioxane, 1 h.

Scheme 3. Side Reaction Observed during the Synthesis of Monophosphate Prodrug of 1[a]



[a]Reagents and conditions: (i) $POCl_3$, $(EtO)_3PO$, 15 h; then TEAB, 0 °C; (ii) NaOH, H_2O/CH_2Cl_2, 0 °C, 30 min.

Scheme 4. Synthesis of Phosphorylated Disulfide Prodrugs 13b,d[a]



[a]Reagents and conditions: (i) Diethyl chlorophosphate, NEt_3, DMAP (cat.), 1 h; (ii) $POCl_3$, NEt_3, CH_2Cl_2, −60 °C to room temperature, 1 h; (iii) NEt_3, DMAP, CH_2Cl_2, −60 °C; (iv) LiOH, CH_3CN/H_2O (1:1 v/v), 24 h.

Aminoacyl Disulfide Prodrugs. Between the different disulfide prodrugs previously obtained, we selected the ones with the lower molecular weights to prepare the pro-prodrugs, i.e., compounds **2a** and **3**, respectively. Thus, the aminoacyl-prodrugs were synthesized (Scheme 2) by coupling Boc-Val or $(Boc)_2$-Lys in the presence of DCC or EDC and a catalytic amount of DMAP. Then removal of the Boc protecting groups from intermediates **4a,b** and **5a,b** afforded compounds **6a,b** and **7a,b** as hydrochloride salts, respectively.

Finally, an O-acylated prodrug (compound **8**, Scheme 2) was prepared by treatment of derivative **3** with acetyl chloride. This last prodrug was designed to evaluate the effect of masking the two hydroxyl functions of prodrug **3** with the smallest (size and molecular weight) esterase labile promoiety.

Phosphorylated Disulfide Prodrugs. To obtain the disulfide prodrugs bearing a phosphate moiety, we first adapted the synthetic pathway already described in the literature for the preparation of benfotiamine (*S*-benzoylthiamine *O*-monophosphate).[23] Thus, preparation of the O-phosphorylated

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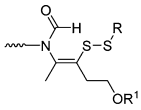
Table 1. In Vitro and in Vivo Antimalarial Activities and Oral Absorption Index after Administration in the Mouse[a]

Drugs or Prodrugs			clogP[b]	*In vitro* IC_{50} (nM) (*P. f.*)	*In vivo* ED_{50} (mg/kg) (*P. vinckei*)		*ip/po* ratio[e] (%)
	R^1	R			ip	po	
Chloroquine			5.0	23	1.1	3.4	
1 (T3)	H	-	-4.8	2.25	0.2[c]	13[c]	1.5
2a	H	Me	5.3	6	0.4	15	2.7
6a	Valine	Me	7.6	160	1.0	58	1.7
6b	Lysine	Me	5.3	120	1.0[c]	44[c]	2.3
2b	H	Et	6.3	5.4	1.1	44	2.5
2c	H	Pr	7.4	1.3	0.5	>54	
2d	H	Bn	7.7	14.4	1.3	80	1.6
3	H	cyclic	4.4	65	0.9[d]	22[d]	
7a	Valine	cyclic	4.5	590	1.0	37	4.1
7b	Lysine	cyclic	6.9	130	0.9[c]	50[c]	1.8
8	Ac	cyclic	6.2	24	1.0	7.3	13.7
9	$P(O)OH_2$	-	-5.9	6.65	0.34	34	1
11	$P(O)OEt_2$	Et	7.6	61.7	>1.0	>90	
12b	CycloSal	Et	5.5	520	5.0	>90	
13b	$P(O)OH_2$	Et	5.5	5700	1.1	60	1.8
12d	CycloSal	Bn	6.9	865	>5	>>90	
13d	$P(O)OH_2$	Bn	6.9	4400	3	>90	

[a] IC_{50} is the concentration to inhibit by 50% in vitro *P. falciparum* growth, ED_{50} is the efficient dose to inhibit by 50% in vivo *P. vinckei* growth according to a 4-day suppressive test, ip means intraperitoneal administration, and po means per-os administration. [b] clogP values were calculated with Chemdraw. Compounds were administered in vivo in DMSO for both ip and po routes except for compounds noted (footnotes *c* and *d*). [c] Administered in 0.9% NaCl and water, for ip and po routes, respectively. [d] Administered in a mixture of intralipid and DMSO, for ip and po routes, respectively. [e] The ip/po ratio is generally accepted as a raw indicator of oral bioavailability.

derivative of albitiazolium as an intermediate was first required and followed by the ring-opening of the thiazolium to further generate the disulfide in situ (Scheme 3).

The two hydroxyl groups of starting material **1** were phosphorylated using the Yoshikawa method.[24,25] Thus, albitiazolium was reacted with an excess of $POCl_3$, leading to a bis-phosphorodichloridate intermediate which was hydrolyzed in situ with 1 M TEAB. The bis-phosphate intermediate **9** (Scheme 3) was isolated by reverse phase chromatography with moderate to good yields. Unfortunately, attempts to obtain the desired phosphorylated disulfide prodrugs by treatment of **9** (either one-pot reaction or after isolation) with sodium hydroxide and rapid addition of an alkyl thiosulfate salt were not successful. The sole product identified was the cyclic derivative, bisthietane **10** (Scheme 3). We hypothesized that once the thiolate is generated, the intramolecular nucleophilic attack onto the phosphorylated side chain occurs rapidly, and preferentially, compared to the disulfide bond formation. Indeed, a test reaction was performed (phosphorylation and subsequent addition of sodium hydroxide) in the absence of any electrophile to trap the thiolate intermediate. Completion of the reaction was observed after 30 min and led to the isolation of derivative **10** in 73% yield.

Consequently, we envisaged the two previous steps in the reverse order, i.e., the introduction of the phosphate group on the preformed disulfide prodrug (Scheme 4). This methodology has been reported for the di- and triphosphorylation of thiamine and one corresponding disulfide derivative.[26]

At first we chose to investigate the diethylphosphoryl protecting group, which is one of the most widely used for phosphate protection and is usually cleaved with bromotrimethylsilane (Me_3SiBr).[27,28] Thus, the disulfide prodrug **2b** was converted to its phosphorylated analogue **11**, by reaction of diethyl chlorophosphate in the presence of triethylamine (TEA) and a catalytic amount of DMAP (Scheme 4). Unfortunately, all attempts to convert the phosphate diester **11** to its free acid using Me_3SiBr failed. Therefore, we turned our attention to the "CycloSal" protecting group, derived from salicylic acid, which is described to be very labile when exposed to smooth basic conditions and thereby could be eliminated under soft conditions.[29−31] To introduce the CycloSal group on our disulfide derivatives, we used a chlorophosphate strategy derived from the method of Casida et al.[32,33] Our various assays showed that without DMAP (at least 1 equiv), only the starting material and the monophosphorylated product were recovered from the reaction mixture. A large excess of the chlorophosphate of CycloSal was necessary to observe the conversion of the disulfide prodrug to its phosphorylated analogue **12b** or **12d**. The first attempt to remove the CycloSal protecting group from compound **12b** was carried out following the conditions described by Meier's group and using a mixture of TEA/water in acetonitrile.[34] After 48 h at room temperature, the reaction did not go further. Partially deprotected intermediates were isolated and their structure assigned by ^1H and ^{31}P NMR (data not shown). The selectivity of this reaction (P−O or C−O bond cleavage) was rather poor, and increasing the reaction time or the amount of TEA did not allow shifting the reaction

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in favor of the final compound **13b**. Under microwave irradiation, **13b** was finally obtained in 35% yield. To optimize the deprotection step, we tested various bases such as lithium hydroxide, and compound **13b** was then isolated with 75% yield. When the same procedure was applied to protected *S*-benzyl prodrug **12d**, lower yields were observed (12%).

All the disulfide prodrugs and their aminoacyl and phosphate conjugates were fully characterized by ^{1}H NMR, eventually ^{31}P and ^{13}C NMR, and HR-MS and then evaluated for their in vitro and in vivo antimalarial activity. Note that the ring-opening reaction of thiazolium ions under basic conditions, followed by in situ trapping of the amidoenothiolates, leads to the formation of a pair of amide rotamers. These two rotational isomers are related by a slow rotation about the C−N bond of the amide group and can be readily observed by NMR spectroscopy. This phenomenom gave rise to broadening and/or duplication of the formamide signal as well as the signals of vicinal groups. Consequently, for all our prodrugs, two characteristic signals for the *N*-formyl function (in the 7.5−8.0 ppm range) and for the methyl group (∼2.0 ppm) appeared and were attributed to the major and the minor conformers.

We also calculated the logP value for final derivatives in order to estimate their lipophilicity and compared compounds to each other within the series (Table 1). Data corresponding to chloroquine were introduced as a reference.

Biological Activities. *A. In Vitro Studies of Disulfide Prodrugs.* The antimalarial activities of the compounds were first evaluated in vitro against the blood stage of *P. falciparum* (Table 1). The activities were determined using [^{3}H]-hypoxanthine incorporation to assess parasite growth after contact of the compounds with the parasite for one parasite cycle (48 h). Parasitic viability was expressed as IC_{50}, the drug concentration causing 50% parasite growth inhibition. IC_{50} values presented in Table 1 indicate that disulfide prodrugs (**2a−d**, **3**, and **8**) have potent antimalarial activity against *P. falciparum* with an IC_{50} in the very low nanomolar range. As the prodrug scaffold is devoid of antimalarial activity,[11] this attests that all compounds were converted into the parent drug and behave as prodrugs of albitiazolium. Remarkably, the linear disulfide prodrugs **2a−c** showed an IC_{50} lower than 10 nM, and compound **2c** (IC_{50} = 1.3 nM) was more potent than the parent drug **1** (IC_{50} = 2.2 nM). The presence of the *n*-propyl disulfide promoiety leads to a highly lipophilic derivative (clogP = 7.4) which can easily penetrate into infected erythrocytes and thereby give rise, after reduction of the disulfide bond, to a higher intracellular concentration of the drug. The prodrug **2c** (R_1 = *n*-propyl, IC_{50} =1.3 nM) exhibited a substantially increased activity compared to **2d** (R_1 = benzyl, IC_{50} = 14.4 nM) despite a similar clogP, which may be attributed to an increase in the enzymatic stability of the *S*-benzyl promoiety (owing to steric hindrance and/or electronic effect) compared to the *n*-propyl promoiety.

The cyclic disulfide prodrugs **3** and **8** showed an IC_{50} on the same order of magnitude. However, the significantly lower IC_{50} value of the cyclic acetylated prodrug **8** (24 nM) in comparison to **3** (IC_{50} = 65 nM) was unexpected, taking into account the double conversion step (disulfide reduction and ester hydrolysis) to give albitiazolium. This result suggests that, in this case, the enzymatic conversion of the pro-prodrugs is rapid and is not a limiting step, and that the pro-prodrugs might have a few advantages to reach the targets (or easier access to the intraparasitic target).

In the second set of compounds, we introduced amino acid residues on the side chain of the thiazolium to improve aqueous solubility of the resulting pro-prodrugs. Thus, the IC_{50} values of corresponding aminoacyl disulfide prodrugs **6a,b** and **7a,b** ranged from 120 to 590 nM. In that case, the polarity of the second pro-moiety (amino acid) might delay the bioconversion of the pro-prodrugs to the corresponding parent prodrug **2a** and then to albitiazolium. This postponed bioconversion might be advantageous for oral absorption.

We also designed and synthesized O-phosphorylated derivatives **9**, **13b**, and **13d**, which led to much contrasted results. Only, the bis-O-phosphorylated drug **9** (IC_{50} = 6.6 nM) exhibited activity similar to that of the parent drug **1** (IC_{50} = 2.25 nM), proving that the phosphate group is efficiently hydrolyzed in biological media to release the active parent drug. However, phosphorylated disulfide prodrugs **13b** and **13d** (which involved two enzymatic conversion steps presumably mediated by alkaline phosphatase and reductase) showed very low activity in vitro with an IC_{50} of 5.7 and 4.4 μM, respectively. We hypothesized that in the presence of the highly polar phosphate groups, the conversion of the pro-prodrugs into the parent prodrugs **2b** or **2d** occurred very slowly. Indeed, related prodrugs **2b** and **2d** exhibited an IC_{50} of 5.4 and 14.4 nM, respectively, indicating that in the absence of the phosphate group the reduction of the disulfide bond proceeded rapidly. Phosphorylated prodrug intermediates **11**, **12b**, and **12d** were also screened and showed a modest to low ability to inhibit *P. falciparum* growth (60 nM < IC_{50} < 900 nM), thus indicating that the disulfide promoieties were hydrolyzed (more rapidly than for compounds **13b** and **13d**) to generate the corresponding bis-thiazolium heads. However, we have no evidence that the diethyl or the CycloSal phosphoester groups were removed (or not) from the molecule either through a chemical and/or an enzymatic process.

B. In Vivo Antimalarial Activity of Disulfide Prodrugs. All derivatives were also evaluated in vivo against *P. vinckei* in mice by both intraperitoneal (i.p.) and oral (p.o.) routes (Table 1). When administered intraperitoneally once daily for 4 days, most of the compounds exhibited very potent curative activity against the pathogenic parasite. Efficient doses ED_{50} ranged from 0.41 to 5 mg/kg, which are close to that of albitiazolium (ED_{50} of 0.2 mg/kg) and comparable to that of chloroquine (ED_{50} of 1.1 mg/kg). Consequently, both the disulfide prodrugs (**2a−d**, **3**) and related pro-prodrugs (**6a** and **6b**, **7a** and **7b**, **8**, **12b**, **13b**, and **13d**) are readily bioconverted. The ED_{50} values for compounds **2a−d**, **3**, **8**, and **9** are in agreement with their potent in vitro antimalarial activity against *P. falciparum*.

Surprisingly, the aminoacyl and phosphorylated disulfide pro-prodrugs **6a** and **6b**, **7a and 7b**, **12b**, **13b**, and **13d**, which were significantly less active in vitro with an IC_{50} ranging from 120 to 5700 nM, appeared as potent as the parent prodrugs with an ED_{50} of 0.9 to 5 mg/kg. This observation may be attributed to the higher and wide enzymatic activity present in animal models than in the culture medium and corroborated the hypothesis made above that the kinetic of the decomposition of these pro-prodrugs was very slow in the culture model. Overall, these results point out that the pro-prodrug approaches envisaged are valuable ones. In addition, it is important to note that, as expected, an improvement in the aqueous solubility of the related compounds was observed. As an example, aminoacyl derivatives **6b** and **7b** could be administered as aqueous solutions.

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To further study the ability of our pro-prodrug approach to improve the oral activity of albitiazolium, we orally administered all the compounds studied to *P. vinckei*-infected mice. Linear disulfide prodrugs (**2a**–**d**) bearing various lipophilic moieties showed a good correlation between oral antimalarial activity and their clogP, which was used for comparison of lipophilicity within our prodrug series. Thus, compound **2a**, with a relatively low clogP value (5.3), was the more active compound after oral administration with an efficient dose of 15 mg/kg close to that of the parent drug (**1**, ED_{50}(po) = 13 mg/kg), meaning that the prodrug is quickly converted in the gastrointestinal tract. The cyclic prodrug **6** was the less lipophilic prodrug in this series (clogP = 4.4) but still showed interesting activity, with an ED_{50}(po) = 22 mg/kg. Increasing the lipophilicity of this cyclic derivative by acetylation of the hydroxyl groups (compound **8**, clogP = 6.2) led to a remarkable enhancement of the oral activity of the prodrug (ED_{50}(po) = 7.3 mg/kg). This result illustrates, for the first time in our series of bis-thiazolium prodrugs, that the global shape of the molecule also plays a crucial role in the absorption phenomenon. One could easily imagine that the intramolecular cyclization of the drug is "freezing" the 3D-structure of the molecule and decreasing the number of the rotatable bounds in comparison to the linear prodrugs.

As the clogP and molecular weight of all disulfide prodrugs were higher than the usually suggested cutoff for orally druglike compounds, we also explored the effect of decreasing their lipophilicity by appending amino acids and phosphate groups as polar moieties. Among the disulfide pro-prodrugs incorporating L-valine (**6a** and **7a**), L-lysine (**6b** and **7b**), and phosphate groups (**13b** and **13d**), only the L-lysine disulfide prodrugs were soluble in water at the test concentration condition (27 mg/mL). Unfortunately, their pharmacological activities were lower than that of albitiazolium, meaning that improvement of the aqueous solubility for the studied disulfide prodrugs has no significant benefits.

CONCLUSION

The difficult challenge in optimizing the orally druglike feature of our promising antimalarial drug albitiazolium by means of a prodrug approach is somehow limited by intrinsic nonfavorable parameters of the parent drug, such as its high molecular weight and lipophilicity associated with the flexible C12-alkyl chain. Thus, the range of alternative structural modulation of the prodrug moiety for optimizing oral bioavailability is very narrow. Nevertheless, within the series of disulfide pro-prodrugs designed and studied herein, the cyclic compound **8** was identified as the best derivative, exhibiting a substantial increase in oral activity (ED_{50} of 7.3 mg/kg and ratio ED_{50}(ip)/ED_{50}(po) of 13.7%), that is nearly twice more active than the parent drug.

EXPERIMENTAL SECTION

Biology. *In Vitro Antimalarial Activity.* Drug effects on in vitro *P. falciparum* growth (Nigerian strain) were measured in microtiter plates according to a modified Desjardins test.[35,36] Parasite growth was assessed by measuring the incorporation of [^{3}H]hypoxanthine into nucleic acids after 48 h of contact with the drug, as previously described.[37] Results were expressed as the concentration resulting in 50% inhibition (IC_{50}).

In Vivo Antimalarial Activity. Antimalarial activities were determined against the *Plasmodium vinckei petteri* (279BY) strain in female Swiss mice. Compounds were injected ip and orally in DMSO, or 0.9% NaCl, or a mixture of intralipid/DMSO (90/10). Parasitemia levels were monitored at day 5 after four days of treatment.[11] Results were expressed as the dose inhibiting 50% of parasitemia (ED_{50}, efficient dose 50).

Chemistry. *General Information.* All moisture-sensitive reactions were carried out under rigorous anhydrous conditions and argon atmosphere and using oven-dried glassware. Solvents were dried and distilled prior to use, and solids were dried over P_2O_5 under reduced pressure. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at ambient temperature on a Bruker 250 or 300 Avance. Chemical shifts (δ) are quoted in parts per million (ppm) referenced to the residual solvent peak ($CDCl_3$ fixed at 7.26 ppm and 77 ppm, DMSO-d_6 fixed at 2.49 ppm and 39.5 ppm) relative to tetramethylsilane. Deuterium exchange and 2D-COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in hertz. ESI mass and high resolution mass spectra were recorded in the positive or negative-ion mode on a Micromass Q-TOF. Chromatography was performed on Merck silica gel 60 (230–400 mesh ASTM). Analytical HPLC traces were obtained using a Waters HPLC system (Separation module 2695, 996 Photodiode Array Detector 2996) and a Waters Symmetry Shield (50 × 4.6 mm, 3.5 μm) RP-18-column, with a 1 mL/min flow rate The elution solvents were water containing 0.1% (v/v) of TFA (solvent A) and acetonitrile containing 0.1% (v/v) TFA (solvent B). A linear gradient was performed from 100% of solvent A to 100% of solvent B over 15 min. Purity was determined by HPLC, and most of the tested compounds were confirmed to have ≥95% purity.

Preparation of Alkyl Thiosulfate (Bünte salts). The appropriate alkyl halide (1 equiv) was dissolved in ethanol (0.5 mL/mmol of alkyl halide), and sodium thiosulfate (1 equiv) in the minimum amount of water was added. The reaction mixture was vigorously stirred until completion of the reaction (ca. overnight) and concentrated in vacuum. The Bünte salt was isolated as a white solid after freeze-drying. Following this procedure, methyl, ethyl, propyl, and benzyl thiosulfate were isolated in quantitative yields and used in the next step without further purification.

General Procedure A. Preparation of linear disulfide prodrugs: Compound **1** was dissolved in NaOH (2 N) aqueous solution (5 equiv), and the mixture was stirred in an ice bath for 30 min. Chloroform and then the appropriate alkyl thiosulfate (4–8 equiv) were added, and stirring was continued for 1–2 h. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (gradient: CH_2Cl_2 to CH_2Cl_2/MeOH, 95:5) to afford the expected compound.

N,N′-(Dodecan-1,12-diyl)bis[(1Z)-4-hydroxy-1-methyl-2-(methyldisulfanyl)buten-1-yl)]diformamide ***2a***. According to general procedure A, the title compound (941 mg, 84%) was obtained, as a yellow oil, from **1** (1.18 g, 1.92 mmol), NaOH (4.8 mL), methyl thiosulfate (2.3 g, 7.68 mmol), and $CHCl_3$ (10 mL). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.01 and 7.93 (2s, 2H, major and minor rotamers), 3.84 (t, *J* = 6.7, 4H), 3.45–3.36 (m, 4H), 2.96–2.88 (m, 4H), 2.38 and 2.37 (2s, 6H), 2.05–1.70 (m, 8H), 1.69–1.15 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.4, 161.7, 135.3, 133.0, 132.1, 131.1, 60.6, 60.1, 53.3, 48.1, 42.8, 32.9, 32.8, 29.3, 29.3, 28.9, 27.6, 27.1, 26.7, 23.4, 23.2, 18.9, 18.3. MS (ESI+): 581.0 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{26}H_{49}N_2O_4S_4$ $[M + H]^+$: 581.2575, found: 581.2566.

N,N′-(Dodecan-1,12-diyl)bis[(1Z)-2-(ethyldisulfanyl)-4-hydroxy-1-methylbuten-1-yl)]diformamide ***2b***. According to general procedure A, the title compound (1.14 g, 94%) was obtained, as a yellow oil, from **1** (1.22 g, 2 mmol), NaOH (5 mL), ethyl thiosulfate (1.57 g, 9.6 mmol), and $CHCl_3$ (10 mL). Purity was determined by analytical HPLC and was 92%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 and 7.85 (2s, 2H, major and minor rotamers), 3.76–3.70 (m, 4H), 3.33 (t, *J* = 7.2, 4H), 2.86–2.78 (m, 4H), 2.62–2.55 (m, 4H), 1.94 and 1.92 (2s, 6H), 1.53–1.41 (m, 4H), 1.23–1.14 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.7, 161.9, 134.6, 132.8, 132.5, 131.8, 60.6, 60.1, 48.8, 42.9, 33.4, 33.2, 32.9, 29.4, 29.2, 29.1, 27.8, 27.2, 26.8, 19.1, 18.6, 14.2. MS (ESI+): 609.4 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{28}H_{53}N_2O_4S_4$ $[M + H]^+$: 609.2888, found: 609.2880.

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N,N′-(Dodecan-1,12-diyl)bis[(1Z)-4-hydroxy-1-methyl-2-(propyldisulfanyl)-buten-1-yl)]diformamide **2c**. According to general procedure A, the title compound (1.8 g, 87%) was obtained, as a yellow oil, from **1** (2 g, 3.25 mmol), NaOH (8.5 mL), *n*-butyl thiosulfate (5.5 g, 19.5 mmol), and $CHCl_3$ (15 mL). The compound was purified by column chromatography (CH_2Cl_2/MeOH, 98:2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.93 and 7.85 (2s, 2H, major and minor rotamers), 3.78−3.62 (m, 4H), 3.49−3.27 (m, 4H), 2.85 (t, J = 6.6, 4H), 2.57 (t, J = 7.2, 4H), 1.96 (s, 6H), 1.70−0.80 (m, 30H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1, 162.3, 135.0, 133.3, 132.8, 132.2, 61.0, 60.5, 53.9, 48.8, 43.3, 41.9, 41.7, 33.3, 29.9, 29.7, 29.6, 29.5, 28.2, 27.6, 27.3, 22.7, 19.5, 19.0, 13.5. MS (ESI+): 637.4 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{30}H_{57}N_2O_4S_4$ $[M + H]^+$: 637.3201, found: 637.3205.

N,N′-(Dodecan-1,12-diyl)bis[(1Z)-2-(benzyldisulfanyl)-4-hydroxy-1-methyl-buten-1-yl)] diformamide **2d**. According to general procedure A, the title compound (1.11 g, 94%) was obtained, as a yellow oil, from **1** (1 g, 1.62 mmol), NaOH (4.1 mL), benzyl thiosulfate (1.84 g, 8.13 mmol), and $CHCl_3$ (10 mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 and 7.80 (2s, 2H, major and minor rotamers), 7.27−7.15 (m, 10H), 3.81 (s, 4H), 3.65−3.3.50 (m, 4H), 3.35−3.22 (m, 4H), 2.72−2.65 (m, 4H), 1.90 and 1.88 (2s, 6H), 1.47−1.38 (m, 4H), 1.25−1.10 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8, 161.9, 136.5, 136.3, 134.7, 132.9, 132.5, 131.6, 129.3, 129.2, 128.7, 128.6, 127.7, 127.6, 60.5, 60.2, 48.4, 44.6, 44.5, 43.1, 33.1, 29.5, 29.3, 29.2, 27.9, 27.2, 26.9, 19.3, 18.7. MS (ESI+): 733.4 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{38}H_{57}N_2O_4S_4$ $[M + H]^+$: 733.3201, found: 733.3203.

(3Z,19Z)-3,20-Bis(2-hydroxyethyl)-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-5,18-dicarbaldehyde **3**. Compound **1** (2 g, 3.27 mmol) was dissolved in a 2 N NaOH aqueous solution (8 mL, 16.33 mmol, 5 equiv), and the mixture was stirred in an ice bath for 30 min. Then dichloromethane (330 mL) was added to the reaction mixture and, under vigorous stirring, *tert*-butyl nitrite (1.73 mL, 13.06 mmol, 4 equiv) was added dropwise. After 10 h stirring, the organic layer was separated and washed with aqueous sodium bicarbonate solution followed by brine. The organic layer was dried over magnesium sulfate, filtered, and evaporated in vacuum. The residue was purified by silica gel chromatography (gradient: CH_2Cl_2 to CH_2Cl_2/MeOH, 95:5) to give compound **3** (400 mg, 25%) as an oily yellow liquid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 and 7.81 (2s, 2H, major and minor rotamers), 4.70 (m, 2H), 3.60−3.30 (m, 4H, overlap with water), 2.75−2.55 (m, 4H), 2.10−1.80 (m, 8H), 1.45−1.15 (m, 20H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.1, 162.0, 161.9, 161.8, 161.5, 137.8, 137.7, 136.1, 129.7, 129.4, 129.3, 129.2, 66.3, 60.0, 59.9, 41.9, 41.6, 34.4, 29.0, 28.9, 28.8, 27.3, 27.2, 26.7, 26.3, 18.3, 18.1, 17.9. MS (ESI+): 487.5 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{24}H_{43}N_2O_4S_2^+$ $[M + H]^+$: 487.2648, found: 487.2664.

General Procedure B. Preparation of *t*Boc-aminoacyl disulfide prodrugs: To an ice-cold solution of the protected amino acid (3 to 4.5 equiv) in dichloromethane was added *N,N′*-dicyclohexylcarbodiimide (3 or 4 equiv) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (4 or 4.5 equiv) and DMAP (0.5 or 0.6 equiv). The mixture was stirred for 15 min, and a solution of the required disulfide prodrug (either **2a** or **3**) in dichloromethane was added. After 2−3 h stirring at room temperature, the solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane, and the resulting organic layer was washed successively with a 1 M aqueous $KHSO_4$ solution, a saturated aqueous $NaHCO_3$ solution, and brine and then dried over Na_2SO_4. The solvent was removed in vacuum, and the residue was purified by column chromatography to afford the expected compound.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-4-(formylamino)3-(methyldisulfanyl)penten-3-yl] Bis[(2-(tert-butoxycarbonyl)amino)-3-methylbutanoate] **4a**. According to general procedure B, the title compound (617 mg, 85%) was obtained, as a white amorphous solid, from **2a** (430 mg, 0.742 mmol), Boc-Val (483 mg, 2.22 mmol), *N,N′*-dicyclohexylcarbodiimide (459 mg, 2.22 mmol), DMAP (45 mg, 0.371 mmol), and CH_2Cl_2 (8 mL). The compound was purified by column chromatography (cyclohexane/AcOEt, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 and 7.79 (2s, 2H, major and minor rotamers), 4.98−4.90 (m, 2H), 4.36−4.10 (m, 6H), 3.40−3.23 (m, 4H), 2.92 (t, J = 6.7, 4H), 2.29 (s, 6H), 2.14−1.95 (m, 8H), 1.60−1.10 (m, 38H), 0.91, 0.88, 0.84, 0.81 (2d, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 172.3, 162.2, 161.3, 155.6, 136.2, 135.1, 130.4, 130.0, 79.9, 62.8, 60.4, 58.5, 53.4, 48.8, 42.9, 31.3, 29.5, 29.3, 29.3, 29.2, 28.3, 27.8, 27.2, 26.8, 23.6, 23.3, 19.0, 18.9, 18.7, 17.6, 17.5. MS (ESI+): 979.5 $[M + H]^+$.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-4-(formylamino)3-(methyldisulfanyl)penten-3-yl] Bis[(2,6-bis(tert-butoxycarbonyl)-diamino)hexanoate] **4b**. According to general procedure B, the title compound (665 mg, 68%) was obtained, as a white amorphous solid, from **2a** (453 mg, 0.78 mmol), $(Boc)_2$-Lys (1.08 g, 3.11 mmol), *N,N′*-dicyclohexylcarbodiimide (643 mg, 3.11 mmol), DMAP (57 mg, 0.467 mmol), and CH_2Cl_2 (10 mL). The compound was purified by column chromatography (cyclohexane/AcOEt, 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 and 7.79 (s, 2H, major and minor rotamers), 5.02 (bs, 2H), 4.52 (bs, 2H), 4.35−4.10 (m, 6H), 3.45−3.23 (m, 4H), 3.10−2.85 (2 m, 8H), 2.29 (s, 6H), 1.98 and 1.95 (2s, 6H), 1.80−1.10 (m, 66H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 162.3, 156.1, 136.2, 129.9, 80.0, 79.2, 62.9, 53.3, 48.7, 42.9, 40.0, 32.3, 29.7, 29.5, 29.3, 28.5, 28.3, 27.8, 27.2, 26.9, 23.3, 22.5, 18.9, 18.7. MS (ESI+): 1237.4 $[M + H]^+$.

2,2′-((3Z,19Z)-5,18-Diformyl-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-3,20-diyl)bis(ethane-2,1-diyl) Bis[(2-(tert-butoxycarbonyl)amino)-3-methylbutanoate] **5a**. According to general procedure B, the title compound (381 mg, 70%) was obtained, as a white amorphous solid, from **3** (300 mg, 0.616 mmol), Boc-Val (401 mg, 1.85 mmol), *N,N′*-dicyclohexylcarbodiimide (382 mg, 1.85 mmol), 4-(dimethylamino)pyridine (38 mg, 0.31 mmol), and CH_2Cl_2 (8 mL). The compound was purified by column chromatography (cyclohexane/AcOEt, 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 and 7.81 (s, 2H, major and minor rotamers), 5.0−4.83 (m, 2H), 4.35−4.02 (m, 6H), 3.55−3.12 (m, 4H), 3.02−2.75 (2 m, 4H), 2.15−1.80 (m, 8H), 1.50−1.10 (m, 22H), 0.90, 0.88, 0.83, 0.81 (2d, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 162.0, 161.3, 155.6, 129.4, 79.9, 62.4, 58.5, 47.7, 42.5, 31.3, 30.1, 28.3, 28.0, 27.7, 27.5, 27.1, 27.0, 28.9, 26.8, 26.4, 19.018.5, 17.6. HRMS (TOF-ESI+) calcd for $C_{34}H_{61}N_4O_6S_2$ $[M - H - 2Cl]^+$: 685.4033, found: 685.4003.

2,2′-((3Z,19Z)-5,18-Diformyl-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-3,20-diyl)bis(ethane-2,1-diyl) Bis[(2,6-bis-(tert-butoxycarbonyl)diamino)hexanoate] **5b**. According to general procedure B, the title compound (479 mg, 70%) was obtained, as a white amorphous solid, from **3** (290 mg, 0.517 mmol), $(Boc)_2$-Lys (829 mg, 2.39 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (423 μL, 2.39 mmol), DMAP (37 mg, 0.30 mmol), and CH_2Cl_2 (8 mL). The compound was purified by column chromatography (cyclohexane/AcOEt, 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.0−7.86 (m, 4H), 5.20−5.05 (m, 2H), 4.85−4.60 (m, 2H), 4.42−4.10 (m, 8H), 3.80−3.20 (m, 8H), 3.15−2.70 (m, 4H), 2.0−1.90 (m, 6H), 1.85−1.60 (m, 4H), 1.50−1.20 (m, 36H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 162.5, 162.4, 161.8, 156.3, 156.2, 155.5, 136.8, 136.2, 131.9, 130.6, 129.6, 80.1, 79.1, 62.6, 60.8, 60.4, 60.0, 53.3, 48.1, 47.5, 42.3, 42.2, 40.0, 34.0, 33.4, 32.3, 28.5, 28.4, 28.3, 28.2, 27.6, 27.3, 27.1, 27.0, 26.5, 22.5, 18.7, 18.6, 18.4, 17.8, 17.4. MS (ESI+): 1165.8 $[M + Na]^+$.

General Procedure C. Deprotection of *t*Boc-aminoacyl disulfide prodrugs: Protected pro-prodrug was dissolved in a cold hydrogen chloride solution (4 M in 1,4-dioxane). The mixture was stirred for 45 min at 0 °C and then concentrated in vacuum. The residue was triturated in diethyl ether. After removal of the diethyl ether, the residue was dissolved in water and freeze-dried to afford the desired compound as its hydrochloride salt.

(3Z,3′Z)-4,4′-(Dodecan-1,12-diyl)bis[4-(formylamino)-3-(methyldisulfanyl)penten-3-yl] Bis[(2-amino-3-methylbutanoate) hydrochloride] **6a**. According to general procedure C, the title compound (406 mg, 78%) was obtained as a white powder from **4a** (597 mg, 0.610 mmol) and 4 N hydrogen chloride solution in 1,4-dioxane (22 mL). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.72 (bs, 6H), 8.01 and 7.79 (2s, 2H, major and minor rotamers), 4.40−4.25 (m, 4H), 3.90−3.8 (m, 2H), 3.40−3.30 (m, 4H), 3.05−2.90 (m, 4H), 2.39 and 2.35 (2s, 6H), 2.30−2.15 (m, 2H), 2.02 and 1.94 (2s, 6H), 1.50−

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1.22 (m, 20H), 1.0, 0.98, 0.96, 0.94 (2d, 12H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.9, 168.8, 161.9, 161.5, 137.6, 135.2, 127.6, 63.2, 63.0, 57.3, 57.2, 47.3, 41.7, 29.2, 28.9, 28.7, 27.3, 26.6, 26.2, 22.9, 22.8, 18.5, 18.4. HRMS (TOF-ESI+) calcd for $C_{36}H_{67}N_4O_6S_4^+$ [M − H − 2Cl]$^+$: 779.3943, found: 779.3913.

(3Z,3′Z)-4,4′-(Dodecan-1,12-diyl)bis[4-(formylamino)-3-(methyldisulfanyl)penten-3-yl] Bis[(2,6-diaminohexanoate) dihydrochloride] ***6b***. According to general procedure C, the title compound (464 mg, 89%) was obtained as a white powder from **4b** (660 mg, 0.53 mmol) and 4 N hydrogen chloride solution in 1,4-dioxane (10 mL). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (bs, 6H), 8.13 (bs, 6H), 8.04 and 7.80 (s, 2H, major and minor rotamers), 4.40−4.25 (m, 4H), 4.10−3.92 (m, 2H), 3.11−2.90 (m, 4H), 2.88−2.63 (m, 4H), 2.41 and 2.38 (2s, 6H), 2.04 and 1.96 (2s, 6H), 1.90−1.78 (m, 4H), 1.70−1.20 (m, 30H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.4, 162.0, 137.6, 134.8, 128.4, 127.4, 63.4, 51.5, 47.1, 41.6, 29.2, 28.9, 28.7, 28.3, 27.3, 26.6, 26.1, 22.9, 22.7, 21.2, 18.3, 17.9. MS (ESI+): 837.2 [M + H]$^+$. HRMS (TOF-ESI+) calcd for $C_{38}H_{72}N_6O_6S_4$·2HCl [M − H − 2Cl]$^+$: 837.4479, found: 837.4486.

2,2′-((3Z,19Z)-5,18-Diformyl-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-3,20-diyl)bis(ethane-2,1-diyl) Bis[(2-amino-3-methylbutanoate) hydrochloride] ***7a***. According to general procedure C, the title compound (218 mg, 95%) was obtained as a white powder from **5a** (267 mg, 0.301 mmol) and 4 N hydrogen chloride solution in 1,4-dioxane (6 mL). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (bs, 6H), 7.84 and 7.79 (s, 2H, major and minor rotamers), 4.31−4.20 (m, 4H), 3.85−3.70 (m, 2H), 3.55−3.20 (m, 4H, overlap with water), 3.0−2.80 (m, 4H), 2.20−2.10 (m, 2H), 2.05−1.90 (m, 6H), 1.40−1.15 (m, 20H), 1.05−0.90 (m, 12H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.1, 169.0, 161.9, 127.3, 66.3, 63.0, 57.3, 29.3, 29.0, 28.8, 27.3, 27.1, 26.8, 26.7, 26.6, 26.4, 26.3, 26.1, 25.6, 18.4, 18.3, 18.1, 17.9, 17.6. MS (ESI+): 685.6 [M + H]$^+$. HRMS (TOF-ESI+) calcd for $C_{34}H_{61}N_4O_6S_2$.2HCl [M − H − 2Cl]$^+$: 685.4033, found: 685.4003.

2,2′-((3Z,19Z)-5,18-Diformyl-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-3,20-diyl)bis(ethane-2,1-diyl) Bis[(2,6-diaminohexanoate) dihydrochloride] ***7b***. According to general procedure C, the title compound (138 mg, 85%) was obtained as a white powder from **5b** (207 mg, 0.181 mmol) and 4 N hydrogen chloride solution in 1,4-dioxane (6 mL). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10−7.80 (m, 8H), 4.25 (m, 4H), 4.02 (m, 2H), 3.05−2.55 (m, 8H), 2.10−1.72 (m, 12H), 1.68−1.10 (m, 42H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.8, 162.1, 162.0, 138.0, 136.8, 129.4, 127.6, 63.2, 59.0, 58.9, 54.9, 51.8, 51.7, 41.1, 40.8, 38.1, 34.0, 33.5, 33.1, 29.4, 29.0, 28.4, 27.2, 27.0, 26.8, 26.5, 26.4, 26.3, 26.2, 25.7, 25.6, 22.4, 21.7, 21.2, 19.8, 19.2, 18.0, 17.9, 17.2, 14.1, 11.2. MS (ESI+): 743.5 [M + H]$^+$. HRMS (TOF-ESI+) calcd for $C_{36}H_{67}N_6O_6S_2$ 2HCl [M − H − 2Cl$^-$]$^+$: 743.4564, found: 743.4581.

(3Z,19Z)-3,20-Bis[2-(acetoxy)ethyl)]-4,19-dimethyl-1,2-dithia-5,18-diazacycloicosa-3,19-diene-5,18-dicarbaldehyde ***8***. To a cold solution (0 °C) of **3** (72.8 mg) and TEA (85 μL, 0.59 mmol, 4 equiv) in dichloromethane (1.5 mL) was added dropwise acetyl chloride (50 μL, 0.59 mmol, 4 equiv). The mixture was stirred for 15 min at this temperature and then allowed to warm to room temperature (ca. 1 h). The mixture was diluted with dichloromethane, and the organic layer was washed with water and finally dried over Na_2SO_4. The solvent was removed in vacuum, and the residue was purified by column chromatography (gradient: CH_2Cl_2 to CH_2Cl_2/MeOH, 98:2) to afford **15** (80 mg, 94%) as an oily yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 and 7.90 (s, 2H, major and minor rotamers), 4.22−4.15 (m, 4H), 3.60−3.20 (m, 4H), 3.05−2.75 (m, 4H), 2.10−1.90 (m, 12H), 1.50−1.00 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 162.4, 162.1, 161.3, 136.9, 131.1, 129.9, 61.9, 61.8, 61.7, 47.7, 42.4, 42.3, 30.0, 29.7, 29.3, 28.3, 28.1, 27.8, 27.7, 27.6, 27.3, 27.1, 26.9, 26.6, 26.5, 20.9, 18.6, 18.4, 17.5. MS (ESI+): 571.4 [M + H]$^+$. HRMS (TOF-ESI+) calcd for $C_{28}H_{47}N_2O_6S_2$ [M + H]$^+$: 571.2870, found: 571.2876.

N,N′-(Dodecane-1,12-diyl)bis[2-(4-methyl-1,3-thiazol-3-ium-5-yl)ethyl dihydrogen phosphate] ***9***. Compound **1** (0.63 g, 1.02 mmol) was suspended in dry triethyl phosphate (4 mL), freshly distilled $POCl_3$ (0.38 mL, 4.07 mmol, 4 equiv) was added, and stirring was maintained overnight at room temperature. The homogeneous clear yellow solution was hydrolyzed by adding TEAB (1M, 10 mL) until pH ≈ 8. Triethyl phosphate was removed by extracting the aqueous layer with Et_2O. Water was evaporated, and free triethylammonium phosphate salts resulting from the hydrolysis of excess $POCl_3$ with TEAB were precipitated from acetone. The residue was purified by reverse phase RP18 chromatography, eluting with H_2O/CH_3CN system (H_2O to H_2O/CH_3CN, 85:15). A colorless oil (0.43 g, 49%) was obtained after evaporation and freeze-drying. ^{1}H NMR (300 MHz, D_2O): δ 9.85 (s, 2H, major and minor rotamers), 4.43 (t, J = 7.2, 4H), 3.90−3.75 (m, 4H), 3.38 (t, J = 6.0, 4H), 2.49 and 2.47 (2s, 6H), 1.90−1.75 (m, 4H), 1.30−1.0 (m, 16H). ^{13}C NMR (75 MHz, D_2O): δ 156.8, 156.2, 145.1, 143.5, 137.7, 136.3, 63.8 (d, J_{CP} = 5.3), 55.4, 46.1, 30.9, 30.5, 30.4, 30.3, 30.0, 27.1, 27.0, 17.3 (d, J_{CP} = 6.8), 13.1, 12.9. ^{31}P NMR (121 MHz, D_2O): δ 0.7. MS (ESI+): 730.9 [M + 2TFA]$^+$, 307.0 [(M + 2H)/2]$^{2+}$.

N,N′-(Dodecane-1,12-diyl)bis[(1Z)-1-(2-thietanylidene)ethyl]-diformamide ***10***. To an ice-cooled solution of compound **9** (0.18 g, 0.29 mmol) in water (0.5 mL) was added a 2 N NaOH aqueous solution (0.58 mL, 1.16 mmol, 4 equiv), the mixture was stirred for 10 min, and then 2 mL of dichloromethane was added. After 30 min stirring at room temperature, the reaction mixture was taken up in dichloromethane, and the resulting aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 97:3), affording compound **10** (96 mg, 73%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 and 7.88 (2s, 2H, major and minor rotamers), 3.50−3.40 (m, 4H), 3.35−3.20 (m, 4H), 3.10−3.05 (m, 4H), 1.66 and 1.63 (2s, 6H), 1.45−1.39 (m, 4H), 1.23−1.10 (m, 16H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.5, 160.1, 130.1, 129.2, 122.3, 120.5, 45.9, 40.7, 33.2, 30.9, 29.3, 28.7, 28.5, 28.4, 28.2, 27.4, 26.0, 25.6, 20.2, 19.5, 14.3, 14.1. MS (ESI+): 453.2 [(M + 2H)/2]$^{2+}$.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-3-(ethyldisulfanyl)-4-(formylamino)penten-3-yl] Bis[diethyl phosphate] ***11***. In a dried round-bottom flask, the prodrug **2b** (0.26 g, 0.43 mmol) was dissolved in CH_2Cl_2 (10 mL). Diethyl chlorophosphate (0.15 mL, 1.07 mmol, 2.5 equiv), previously dried under vacuum, was added slowly at 0 °C under neutral atmosphere, followed by TEA (0.17 mL, 1.20 mmol, 2.8 equiv) and DMAP (0.016 g, 0.13, 0.3 equiv). The ice bath was removed, and the reaction mixture was stirred for 1 h. The solvent was evaporated, and the crude residue was directly purified on silica gel chromatography (CH_2Cl_2/MeOH, 97:3), affording a clear yellow oil (0.28 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 and 7.83 (s, 2H, major and minor rotamers), 4.23−4.00 (m, 12H), 3.34−3.29 (m, 4H), 2.94 (t, J = 5.7, 4H), 2.58 (q, J = 7.2, 4H), 1.94 (s, 6H), 1.48−1.43 (m, 4H), 1.34−1.12 (m, 34H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.3, 161.2, 1135.9, 135.0, 130.2, 130.0, 65.0 (d, J_{CP} = 6.7), 64.8 (d, J_{CP} = 6.0), 63.8 (d, J_{CP} = 7.2), 63.7 (d, J_{CP} = 6.7), 48.9, 42.9, 33.2, 33.1, 30.6 (d, J_{CP} = 7.5), 29.5, 29.2, 27.8, 27.2, 26.8, 19.0, 18.8, 16.1 (d, J_{CP} = 6.8), 16.0 (d, J_{CP} = 6.8), 14.1, 14.0. ^{31}P NMR (121 MHz, $CDCl_3$): δ −0.9 and −0.8. MS (ESI+): 903.4 [M + Na]$^+$, 452.2 [(M + Na)/2]$^{2+}$. HRMS (TOF-ESI+) calcd for $C_{36}H_{71}N_2O_{10}P_2S_4$ [M + H]$^+$: 881.3467, found: 881.3484.

General Procedure D. Preparation of CycloSal phosphorylated prodrug **12b** and **12d**: In a flame-dried round-bottom glass filled with argon, anhydrous dichloromethane was cooled to −50 °C. Freshly distilled $POCl_3$ (15 equiv) was added, and a combination of dried 2-hydroxybenzoic acid (15 equiv) and TEA (30 equiv) in dichloromethane (2 mL/mmol of $POCl_3$) was slowly added in 2 h. After addition, the reaction mixture was warmed to 0 °C for 1 h and cooled down to −50 °C for the slow addition of a combination of the disulfide prodrug (1 equiv), TEA (15 equiv) and DMAP (1−2 equiv) in dichloromethane (6.5 mL/mmol of the prodrug). The mixture was allowed to reach room temperature. The reaction was quenched after overnight stirring by adding water. The organic layer was washed with water, dried with $MgSO_4$, filtered and concentrated to afford brown oil which was further purified on silica gel chromatography, eluting with CH_2Cl_2/MeOH system.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-3-(ethyldisulfanyl)-4-(formylamino)penten-3-yl] Bis[(2-oxo-4H-2l^5-benzo[1,3,2]dioxa)-

phosphate] **12b**. According to procedure D, the title compound (0.92 g, 53%) was obtained as a clear yellow oil from 2-hydroxybenzoic acid (3.43 g, 27.6 mmol), $POCl_3$ (2.53 mL, 27.6 mmol), TEA (7.7 mL, 55.3 mmol), the disulfide prodrug **2b** (1.12 g, 1.84 mmol), TEA (3.84 mL, 27.66 mmol), and DMAP (0.25 g, 1.84 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 and 7.72 (s, 2H, major and minor rotamers), 7.30−7.20 (m, 2H), 7.15−6.95 (m, 6H), 5.37−5.20 (m, 4H), 4.31−4.23 (m, 4H), 3.32−3.20 (m, 4H), 3.02−2.90 (m, 4H), 2.55 (q, J = 7.2, 4H), 1.89 (s, 6H), 1.45−1.32 (m, 4H), 1.25−1.15 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.2, 161.2, 150.1(d, J_{CP} = 7.0), 136.2, 129.9, 129.7, 129.6, 125.3 (d, J_{CP} = 4.1), 124.4, 124.2, 120.6, 120.5, 118.6 (d, J_{CP} = 8.8), 68.6 (d, J_{CP} = 6.9), 66.1 (d, J_{CP} = 6.0), 49.0, 42.9, 33.3, 33.1, 30.7, 30.6, 29.5, 29.2 (d, J_{CP} = 8.2), 27.9, 27.2, 26.8, 19.1, 14.2, 14.1. ^{31}P NMR (121 MHz, $CDCl_3$): δ −9.4 and −9.5. MS (ESI+): 945.5 $[M + H]^+$. HRMS (TOF-ESI+) calcd for $C_{42}H_{63}N_2O_{10}P_2S_4$ $[M + H]^+$: 945.2841, found: 945.2835.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-3-(benzyldisulfanyl)-4-(formylamino)penten-3-yl] Bis[(2-oxo-4H-2l^5-benzo[1,3,2]dioxa)-phosphate] **12d**. According to procedure D, the title compound (1.26 g, 77%) was obtained as a clear yellow oil from 2-hydroxybenzoic acid (3 g, 24.1 mmol), $POCl_3$ (2.21 mL, 24.1 mmol), TEA (6.72 mL, 48.33 mmol), the disulfide prodrug **2d** (1.24 g, 1.53 mmol), TEA (3.36 mL, 24.16 mmol) and DMAP (0.39 g, 3.22 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 and 7.69 (s, 2H, major and minor rotamers), 7.30−6.90 (m, 18H), 5.32−5.20 (m, 4H), 4.25−4.11 (m, 4H), 3.79 (s, 4H), 3.30−3.22 (m, 4H), 2.92−2.75 (m, 4H), 1.88, 1.85 (2s, 6H), 1.45−1.30 (m, 4H), 1.20−1.05 (m, 16H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.3, 161.3, 150.1 (d, J_{CP} = 7.5), 150.0, 136.6, 136.2, 136.1, 129.9 (d, J_{CP} = 1.5), 129.7, 129.6, 129.4, 129.2, 128.7, 128.6, 128.4, 127.8, 127.6, 125.4, 125.3, 124.4, 124.2, 120.7, 120.4, 118.5 (d, J_{CP} = 8.3), 68.6 (d, J_{CP} = 6.0), 66.0 (d, J_{CP} = 6.0), 53.5, 48.9, 44.5, 44.3, 43.6, 43.0, 30.7, 30.6, 29.5, 29.3, 29.2 (d, J_{CP} = 8.3), 27.9, 27.2, 26.8, 19.2, 18.8. ^{31}P NMR (121 MHz, $CDCl_3$): δ −9.6 and −9.5. MS (ESI+): 1069.5 $[M + H]^+$. HRMS (TOF-ESI+): calcd for $C_{52}H_{67}N_2O_{10}P_2S_4$ $[M + H]^+$: 1069.3154, found: 1069.3179.

General Procedure E. Removal of the CycloSal protecting groups: the protected phosphorylated prodrug (1 equiv) was dissolved in a mixture of acetonitrile and water (2:1, v/v, 12.5 mL/mmol of prodrug). Lithium hydroxide monohydrate (5 equiv) was added, and the reaction was stirred at 20 °C for 24 h. After concentration, the yellow residue was purified on reverse phase RP18 chromatography, eluting with H_2O/CH_3OH systems.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-3-(ethyldisulfanyl)-4-(formylamino)penten-3-yl] Bis(dilithium phosphate) **13b**. According to procedure E, the title compound (72 mg, 75%) was obtained as a white powder from derivative **12b** (115 mg, 0.12 mmol), $LiOH·H_2O$ (25.5 mg, 0.6 mmol) and acetonitrile/water (1.5 mL). ^{1}H NMR (300 MHz, D_2O): δ 7.87 and 7.83 (2s, 2H, major and minor rotamers), 3.90−3.75 (m, 4H), 3.50−3.30 (m, 4H), 3.0−2.80 (m, 4H), 2.66 (q, J = 6.9, 4H), 1.97 and 1.92 (2s, 6H), 1.50−1.40 (m, 4H), 1.25−1.10 (m, 22H). ^{13}C NMR (75 MHz, D_2O): δ 165.2, 163.4, 135.8, 132.2, 131.7, 131.0, 62.1 (d, J_{CP} = 4.0), 48.3, 42.3, 33.4, 33.2, 31.3 (d, J_{CP} = 3.5), 29.4, 29.3, 29.2, 28.4, 27.4, 27.2, 26.8, 17.8, 17.3, 14.1. ^{31}P NMR (121 MHz, D_2O): δ 3.5 and 3.4. MS (ESI+): 769.4 $[M + H]^+$. HRMS (TOF-ESI−): calcd for $C_{28}H_{53}N_2O_{10}P_2S_4$ $[M - H]^-$: 767.2058, found: 767.2050.

N,N′-(Dodecan-1,12-diyl)bis[(3Z)-3-(benzyldisulfanyl)-4-(formylamino)penten-3-yl] Bis(dilithium phosphate) **13d**. According to procedure E, the title compound (83 mg, 12%) was obtained as a white powder from derivative **12d** (783 mg, 0.73 mmol) and $LiOH·H_2O$ (153 mg, 3.65 mmol) and acetonitrile/water (9 mL). ^{1}H NMR (300 MHz, D_2O): δ 7.80 and 7.66 (2s, 2H, major and minor rotamers), 7.14−7.10 (m, 10H), 3.92 (bs, 4H), 3.76 (bs, 4H), 3.10 (bs, 2H), 2.80 (bs, 4H), 1.90 and 1.85 (2s, 6H), 1.30−1.20 (m, 4H), 1.10−0.90 (m, 22H). ^{13}C NMR (75 MHz, D_2O): δ 164.9, 163.3, 136.7, 136.6, 136.3, 132.4, 131.7, 130.5, 129.2, 128.7, 127.6, 62.9, 48.8, 48.0, 44.1, 42.3, 31.0, 29.5, 29.3, 28.5, 27.7, 27.3, 26.9, 18.0, 17.4. ^{31}P NMR (121 MHz, D_2O): δ 4.0 and 3.9. MS (ESI+): 893.4 $[M + H]^+$. HRMS (TOF-ESI+): calcd for $C_{38}H_{59}N_2O_{10}P_2S_4^+$ $[M + H]^+$: 893.2528, found: 893.2527.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral data (^{1}H and ^{31}P NMR, HRMS, HPLC traces) for final compounds **2a−d**, **3** and **6a,b**, **7a,b**, **8**, **9**, **11**, **12b,d**, **13b,d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACTs, artemisinin-based combination therapy; FPIX, ferriprotoporphyrin IX; DCC, dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TEA, triethylamine; TEAB, triethylammonium bicarbonate; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; 2D-COSY, two-dimensional correlation spectrocopy experiment

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