Synthesis and Pharmacological Evaluation of Fluorescent and Photoactivatable Analogues of Antiplasmodial Naphthylisoquinolines

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The naphthylisoquinoline (NIQ) alkaloids from tropical Ancistrocladaceae and Dioncophyllaceae plants show high antiplasmodial activities in vitro and in vivo, even against chloroquine-resistant strains of the malaria pathogen. For the directed optimization of these activities, an investigation of the mode of action seems most rewarding. We have therefore embarked on the identification of the respective target protein in *Plasmodium falciparum*. For this purpose, we have developed a flexible pathway for the synthesis of a chemically divergent series of photoactive and fluorescent derivatives of such alkaloids and succeeded in preparing the first functionalized NIQ derivatives, **10**, **12**, and **35**, suited for fluorescence and photoaffinity labeling experiments. Pharmacological investigations ensured that the modified alkaloid derivatives retained their antiplasmodial activity. The work may pave the way for a further improvement of the activity of these natural products and will thus increase their pharmacological potential as a valuable lead structure against the widespread tropical disease malaria.

Introduction

Extracts of tropical lianas of the plant families Dioncophyllaceae and Ancistrocladaceae have been widely used in traditional medicine for the treatment of infectious diseases like malaria.[1,2] Indeed, the secondary metabolites isolated from these plants, the naphthylisoquinoline alkaloids (Figure 1),[1] have proven to exhibit remarkable pharmacological activities.[3] Thus, dioncophylline C (**1**) and dioncopeltine A (**2**) display excellent antiplasmodial activities, both in vitro (against *P. falciparum*, *P. berghei*)[4,5] and in vivo (*P. berghei*, mouse model).[6] Other representatives of this class of alkaloids show good antitrypanosomal or antileishmanial activities.[7] Also from a stereochemical and biochemical point of view, these alkaloids are unique: Thus, the rotationally hindered biaryl axis between the two molecular portions (i.e., the naphthalene and the isoquinoline part), which, in connection with the stereogenic centers at C-1 and C-3, leads to rotational isomers, has triggered the development of efficient new methods for the atropo-selective synthesis of axially chiral compounds.[8] Furthermore, the unusual molecular framework of these secondary metabolites originates from an unprecedented biosynthetic origin of isoquinoline alkaloids from acetate units, both for the naphthalene and the isoquinoline portion.[9]

Some first hints at a possible mode of action of the antiplasmodial naphthylisoquinoline alkaloids were already obtained from NMR, IR, and Raman investigations on drug−heme interactions: Similar to the antimalarial drug chloroquine,[10] these biaryls form, albeit weaker, complexes with ferriprotoporphyrin,[11] thus possibly affecting the hemozoin precipitation, the essential heme detoxification process in intraerythrocytic stages of *P. falciparum*.[12] The fact that the naphthylisoquinolines likewise show antiplasmodial activity against chloroquine-resistant strains of *P. falciparum*[4] suggested that either the molecular mechanism of chloroquine-resistance in *P. falciparum* is circumvented by these compounds or that NIQs[a] have a different mode of action, possibly by selective inhibition of one of the essential proteins of the pathogen. Therefore, these secondary metabolites are promising candidates for the development of new antimalarial drugs.

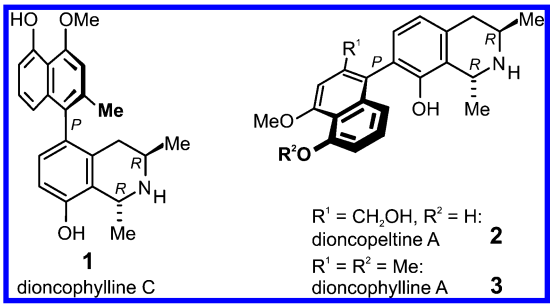

Figure 1. Structures of antiplasmodial naphthylisoquinoline alkaloids.

In this paper, we describe the synthesis of fluorescence-labeled antiplasmodial naphthylisoquinoline derivatives and the first investigations aiming at their localization in parasite-infected cells. For this purpose, a synthetic route to photoactivatable NIQ analogues for photoaffinity studies is described to identify possible molecular targets of NIQs within the parasite.

Results and Discussion

Basic Considerations. For the localization of NIQs in target cells, dioncophylline A (**3**) was chosen as a first representative of this class of compounds to elaborate the best conditions for

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[a] Abbreviations: NIQ, naphthylisoquinoline; IC_{50}, half-maximal inhibitory concentration; QSAR, quantitative structure−activity relationship; DMF, dimethylformamide; THF, tetrahydrofuran; LC-MS, liquid chromatography coupled to mass spectrometry; NBS, *N*-bromosuccinimide; AIBN, azobisisobutyronitrile; TMSCl, trimethylsilyl chloride; ESI-MS, electrospray ionization mass spectrometry; HPLC, high-performance liquid chromatography; NBO, natural bond order; HF, Hartree-Fock; HRMS, high-resolution mass spectrometry; TFA, trifluoroacetic acid; CD, circular dichroism.

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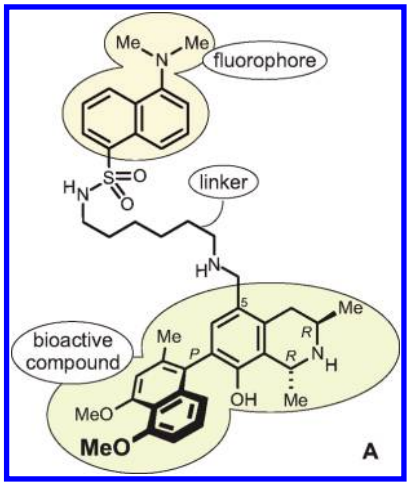


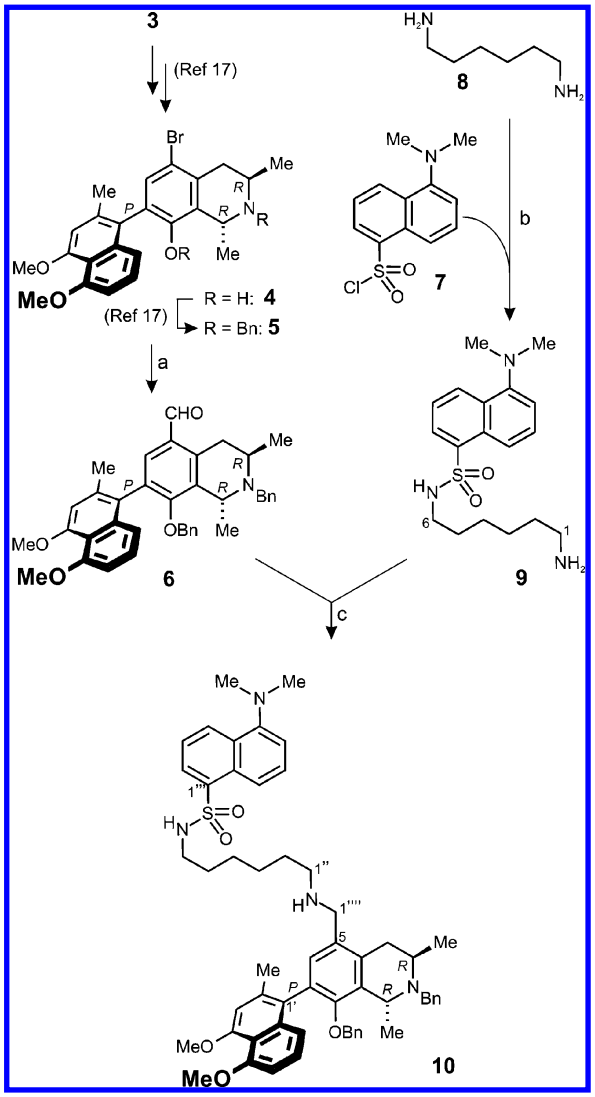
Figure 2. Fluorescent derivative **A** of dioncophylline A (**3**).

the attachment of a fluorescent probe. Although not reaching the excellent antiplasmodial activities of dioncophylline C (**1**) and dioncopeltine A (**2**), dioncophylline A (**3**) still shows an acceptable activity against *P. falciparum* ($IC_{50} = 0.14\ \mu g/mL$)[4] and is, in particular, easily accessible by isolation from *Triphyophyllum peltatum*[13] or by total synthesis.[14] Furthermore, the structural requirements of this compound for bioactivity are particularly well-known from extensive QSAR studies.[15,16] Thus, while the OH function and in particular the NH group in the isoquinoline part of **3** are crucial for the antiplasmodial activity,[16] the 5-position does not belong to the pharmacophore of the molecule, so that a functionalization at this C-atom should be possible without substantial loss of activity. For this reason, dioncophylline A (**3**) was connected in this particular position, via a flexible alkyl linker to prevent steric interference, at first to a fluorophore and then, in addition, to a photolabile group.

For the in vitro and in vivo identification of the compound (e.g., after photoaffinity experiments), the dansyl residue was chosen as an easily available and robust fluorophore. In contrast to other chromophores that emit blue light, it exhibits a green luminescence, which is detectable in a cellular environment.

Synthesis of a Fluorescent Derivative of Dioncophylline A (3). In a first, simplified approach, only the dansyl fluorophore was planned to be attached to **3** via C-5, using a hexamethylenediamine linker as in **A** (Figure 2). For this purpose, dioncophylline A (**3**) was brominated and O- and N-protected according to a known procedure.[17] The resulting *N*,8-*O*-dibenzyl-5-bromodioncophylline A (**5**) was lithiated and then formylated with DMF to give the aldehyde **6** in good yields (Scheme 1). This slight detour led to much better results than a direct Vilsmeyer formylation of dioncophylline A (**3**). The precursor **9** to the dansyl linker was prepared from 1,6-hexanediamine (**8**), by treatment with dansyl chloride (**7**) in the presence of Et_3N.[18] Besides the desired monoamine **9**, the respective bisdansylated compound was obtained as a byproduct (ratio 1:5), which was separable by column chromatography. The dansyl derivative **9** was attached to the protected 5-formyldioncophylline A **6** by reductive amination,[19] yielding the still O- and N-benzylated fluorescent analogue **10** of dioncophylline A as the major product (Scheme 1) and traces of a byproduct, presumably an overreacted tertiary amine, as deduced from LC-MS data. The last step of the synthesis toward a fluorescent analogue **12** of dioncophylline A (**3**) was the cleavage of the two benzyl groups of **10** by hydrogenation. However, under all conditions applied, no debenzylation products were isolated in significant yields. To overcome this difficulty, the reaction

Scheme 1. Synthesis of the Fluorescent Dioncophylline A Derivative **10**[a]



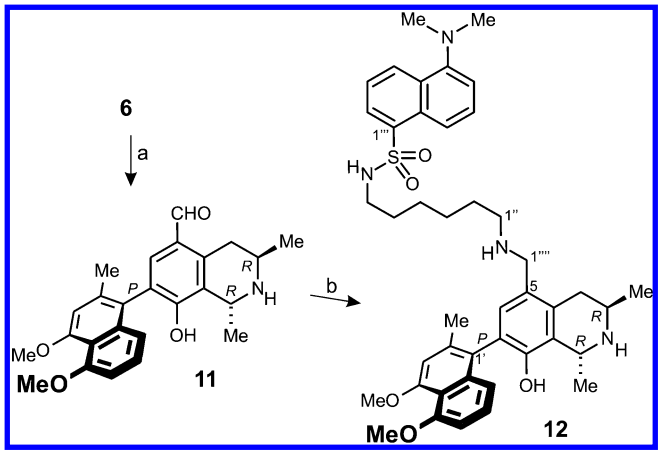
[a] Reagents and conditions: (a) 1: *n*-BuLi, THF, −78 °C, 110 min; 2: DMF, 45 min, 83%; (b) Et_3N, CH_2Cl_2, rt, 3 h, 55%; (c) $NaBH(OAc)_3$, CH_2Cl_2, rt, 24 h, 68%.

sequence was changed (Scheme 2). Accordingly, *N*,8-*O*-dibenzyl-5-formyldioncophylline A (**6**) was first debenzylated to give compound **11**, which was successfully coupled with the amine **9** to yield the fluorescent derivative **12** of dioncophylline A (**3**).

Before submitting **12** to biological testings, its fluorescence emission spectrum, along with that of free dioncophylline A (**3**), was recorded, to exclude quenching effects. From the measured spectra (Figure 3), it becomes obvious that the respective emission maxima, 524 nm in the case of **12** and 355 nm for **3**, are clearly separated, thus avoiding mutual interferences.

Biological Investigations with Fluorescent Derivatives of Dioncophylline A. Given the good physical properties of the fluorescent derivates **10** and **12** of dioncophylline A (**3**), their bioactivities against several parasites were tested (Table 1) before using these compounds as valuable alternatives to **3** for investigation of the cellular localization of dioncophyllines, e.g., in parasite-infected red blood cells, in order to minimize the risk of having a loss of activity or a change of the mode of action due to the synthetic modifications.

Scheme 2. Synthesis of the Unprotected Fluorescent Dioncophylline A Derivative **12**[a]



[a] Reagents and conditions: (a) Pd/C, H_2, THF, 9 h, 73%; (b) **9**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 24 h, 48%.

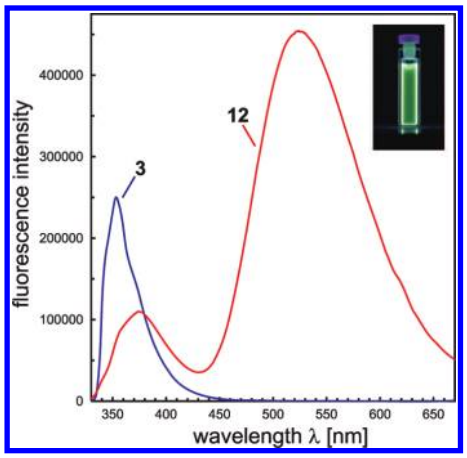


Figure 3. Fluorescence emission spectra of **3** (blue; excitation at 305 nm) and of **12** (red; excitation at 310 nm), together with a fluorescing sample of **12** (right upper corner).

Table 1. Bioactivity Results for **10** and **12**[a]

	P. falciparum	cytotoxicity[b]
standard	0.055[c]	0.010[d]
3	0.381	29.1
10	0.045	2.50
12	0.602	16.5

[a] All values in μM. [b] Against L-6 cells (rat skeletal myoblast cells). [c] Chloroquine. [d] Podophyllotoxin.

As seen from Table 1, the benzylated precursor **10** possesses almost the same antiplasmodial activity as the standard chloroquine, thus being even more active than dioncophylline A (**3**) itself, simultaneously exhibiting a lower toxicity, while the deprotected target compound, **12**, was less active against *P. falciparum*. Therefore and because of its initially better synthetic availability, **10** was chosen as a first fluorescent probe to perform in vitro labeling of *P. falciparum* blood stages.

For fluorescence-microscopic examinations, *P. falciparum* trophozoites were incubated with **10** (0.5 μM) for 4 h under normal culture conditions. As a result, substance **10** obviously affected early blood stages of *Plasmodium*. Moreover, a fluorescent labeling and accumulation of **10** was detected in infected erythrocytes, whereas noninfected erythrocytes were not labeled[20] (Figure 4). The fluorescence was found to be clearly restricted to the parasite in the host cell. Like noninfected erythrocytes, the host cell cytoplasm was totally unlabeled. In

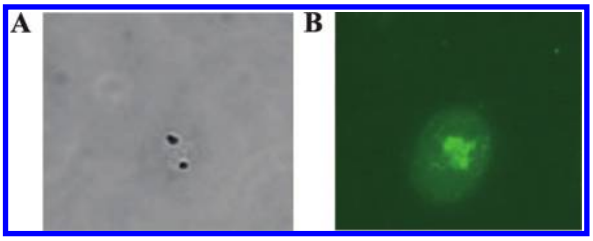


Figure 4. Microscopic visualization of **10** in *Plasmodium*-infected red blood cells. Only the infected cell is labeled (A: transmission mode; B: fluorescence mode).

conclusion, **10** significantly differentiates between the parasite and the erythrocyte (Figure 4).

A possible explanation of this phenomenon is either that the plasma membrane of infected erythrocytes is not permeable for **10** or that the substance can freely diffuse but its concentration in the host cell may be below the detection limit. By contrast, the accumulation of **10** by the intraerythrocytic parasite leads to a visible fluorescence. It remains to be solved whether accumulation of **10** in the parasite cell takes place by an active transport or by diffusion. Possibly **10** is trapped in the parasite by an affinity-driven complex formation with an as yet unknown target molecule.

As seen in Figure 4B, the parasite is labeled distinctly within its confines to the host cell cytosol. Although the fluorescence appears not to be distributed uniformly inside the parasite, the localization of **10** cannot be assigned to a single subcellular compartment, like, e.g., to the digestive vacuole. The hemozoin appears as dark dots, which may indicate that it does not agglomerate **10** (Figure 4B) or that the fluorescence is quenched by hemozoin. Our findings hint at a different behavior of **10** in comparison to chloroquine, which accumulates in the food vacuole, suggesting that **10** and chloroquine have different modes of action. Consequently, the exact cellular localization and molecular target of **10** is restricted to the parasite cell but remains to be further investigated. Therefore, additional experiments are required and are presently in progress, e.g., time-dependent effects of **10** against different intraerythrocytic stages of *P. falciparum*.

The deprotected labeled compound **12** was also used for incubation experiments in *P. falciparum* blood stages, leading to the same specific fluorescent labeling of infected erythrocytes as detected for the precursor **10** (for the microscopic visualization of **12**, see Supporting Information).

Synthesis of Photoactive and Fluorescent Derivatives of Dioncophylline A for Photoaffinity Labeling. In a second, more comprehensive approach, dioncophylline A (**3**) was planned to be equipped with a fluorescent and, in addition, with a photoaffinity probe. For the assembly of such multifunctional molecules, which can be used in protein purification, several different strategies are known.[21] In our case a three-functional core unit was planned to serve as the central module to connect the required building blocks, for which the hydroxylated amino acids L-serine (**13**) and L-tyrosine (**14**) were chosen (see Figure 5). These were planned to be linked to the dansyl residue as the fluorescence label via the amino group. To the free hydroxy function of the amino acid, by contrast, we intended to fix three different types of photoactive groups. With a variety of photoactive substances at hand, we wanted to check for an incorporation of the respective compounds into the cell and cross-linking to the target protein. The use of different photoactive chromophores, in this case a benzophenone, a trifluoromethyldiazirine, and a tetrafluorophenylazide (to generate radicals, carbenes, and nitrenes, respectively), is a common

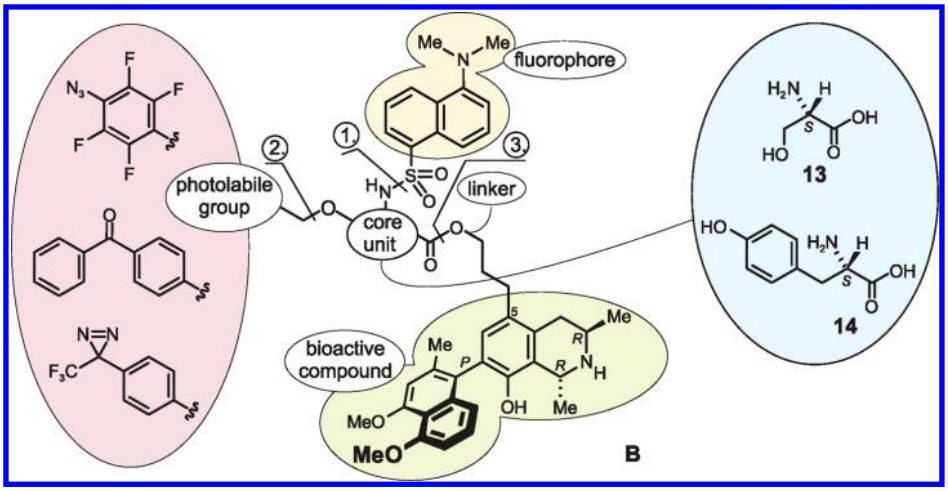
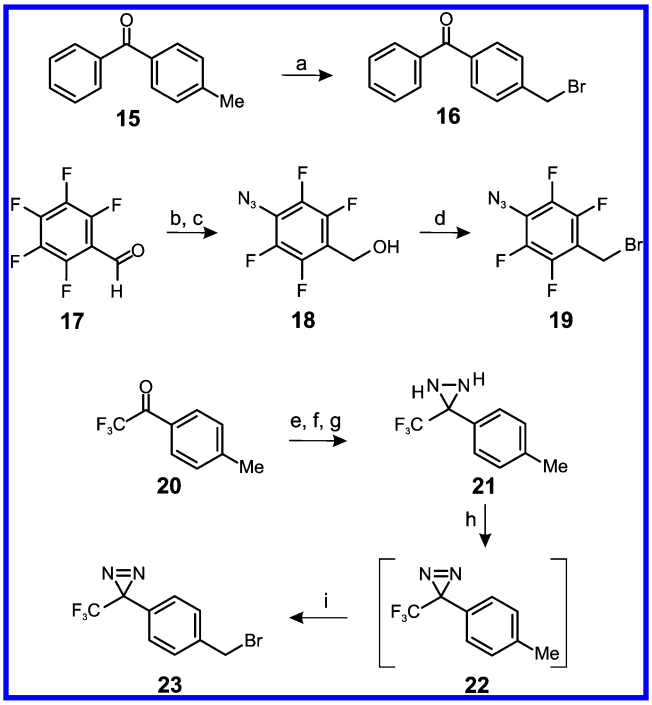


Figure 5. Schematic overview of possible photoactive and fluorescent derivatives of dioncophylline A (**3**); the numbers indicate the order of synthetically connecting the different modules.

Scheme 3. Synthesis of the Photosensitive Benzophenone **16**, the Arylazide **19**, and the Diazirine **23**[a]

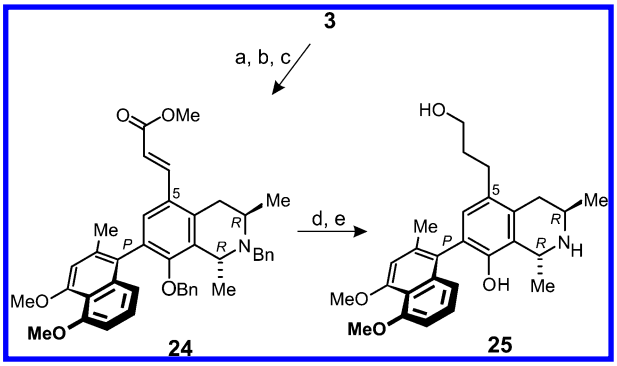


[a] Reagents and conditions: (a) NBS, CCl_4, AIBN, reflux, 3 h, 89%; (b) NaN_3, acetone/H_2O, reflux, 6 h, 64%; (c) $HNMe_2{\cdot}BH_3$, AcOH, rt, 1.5 h; (d) $(CCl_2Br)_2$, PPh_3, CH_2Cl_2, 0 °C, 1 h, 68%; (e) $H_2NOH{\cdot}HCl$, MS 3 Å, pyridine, 80 °C, 2 h, 89%; (f) TosCl, pyridine, reflux, 3 h, 98%; (g) NH_3(liq.), Et_2O, −35 °C, 8 h, 60%; (h) MnO_2, CCl_4, rt, 30 min, quant.; (i) NBS, CCl_4, AIBN, reflux, 1 h, 56%.

procedure to meet the a priori unpredictable situation in the particular biological system.[21−23] And, finally, the amino acid module as the central building block should connect the fluorophore and the photoactive moiety to the bioactive naphthylisoquinoline portion.

The first of the photoactive precursors, 4-(bromomethyl)-benzophenone (**16**),[24] was readily available by radical bromination of the cheap 4-methylbenzophenone (**15**) (Scheme 3). For the synthesis of the aryl azide **19** and the diazirine **23**, several routes had been reported,[23,25,26] starting with the aldehyde **17** and the ketone **20**, respectively (Scheme 3). By optimization of the reaction conditions, better yields were achieved in both cases (vide infra). A nucleophilic substitution reaction of **17** with NaN_3 in acetone/water provided the desired para-substituted

Scheme 4. Synthesis of a Dioncophylline A Derivative **25** Equipped with a C_3 Linker for Attachment of Analytical Probes[a]



[a] Reagents and conditions: (a) Ag_2SO_4, I_2, CH_2Cl_2, rt, 82%; (b) BnBr, Cs_2CO_3, acetone, reflux, 10 h, 64%; (c) $Pd(OAc)_2$, Cs_2CO_3, *n*-Bu_4NBr, PPh_3, H_2O, MeCN, reflux, 1.5 h, 82%; (d) $LiAlH_4$, THF, −78 °C, 63%; (e) Pd/C, H_2, MeOH, 5 h, 65%.

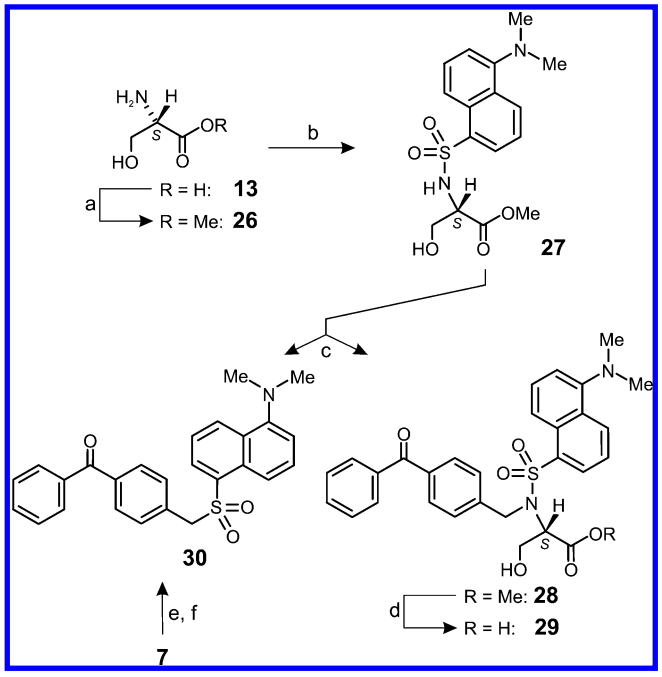
aldehyde, which was reduced under mild conditions with $Me_2NH{\cdot}BH_3$ in acetic acid to give the alcohol **18**. In contrast to the known procedures,[23] the final hydroxyl−bromine exchange was carried out by the use of the improved reagent combination[27] $PPh_3/(CCl_2Br)_2$ in CH_2Cl_2, which increased the over-all-yield of the synthesis.[28]

The synthesis of **23** started with the commercially available ketone **20**, whose oxime (as an *E/Z*-mixture) was tosylated in pyridine and then treated with liquid NH_3 for 8 h to provide the diaziridine **21**. For the subsequent last two steps in the synthesis of **23**, the oxidation of **21** to the diazirine **22** and its side-chain halogenation, the introduction of a one-pot procedure resulted in much higher yields compared to the literature protocols:[23] Oxidation of **21** was carried out with MnO_2 in CCl_4 followed by the filtration of the heterogeneous oxidation reagent and immediate radical bromination with NBS/AIBN right from the crude filtrate. With the oxidation yielding the desired diazirine **22** as the only product, no purification was necessary at this level. This is a significant improvement since it avoids the material-consuming and thus yield-diminishing workup of the sensitive, and highly volatile, intermediate **22**.

Both the aryl azide **19** and the diaziridine **23** proved to be highly photoactive by UV spectroscopy. Irradiation at 254 and 356 nm led to rapid decomposition of the starting material.[29]

For the functionalization of **3** according to the concept presented in Figure 5, the alkaloid was halogenated at C-5 (Scheme 4). This was readily achieved by the use of Ag_2SO_4

Scheme 5. Preparation of the Two-Fold Functionalized Serine Building Block **29** and Independent Synthesis of the Unexpected Side Product **30**[a]

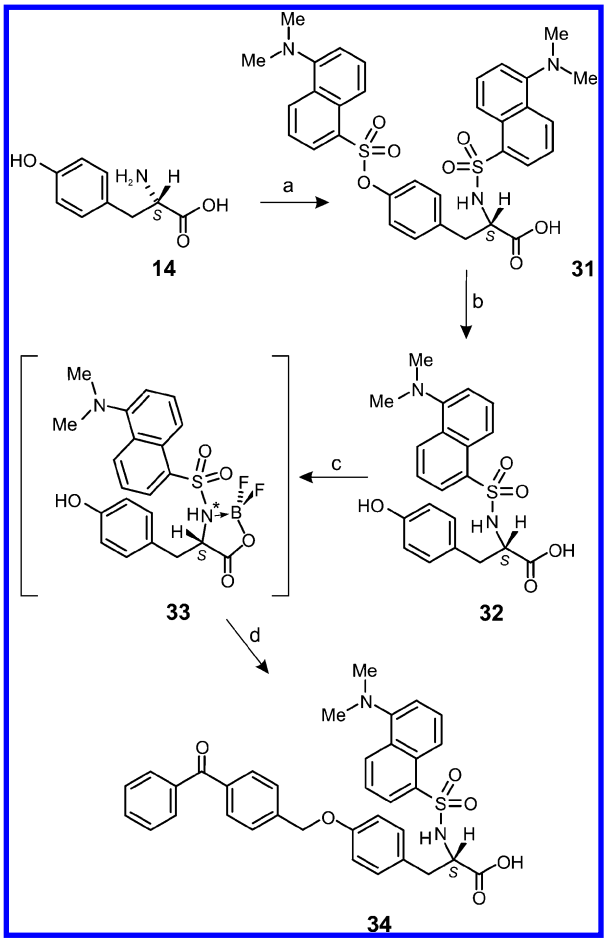


[a] Reagents and conditions: (a) TMSCl, MeOH, rt, 14 h, 70%; (b) dansyl chloride, pyridine, rt, 24 h, 85%; (c) **16**, Cs_2CO_3, NaI, acetone, reflux, 9 h, 45% of **28**; (d) NaOH, THF/H_2O, reflux, 76%; (e) Na_2SO_3, H_2O, rt, 3 h, 67%; (f) **16**, NaOH, EtOH, 80 °C, 12 h, 50%.

and I_2, which produced exclusively 5-iododioncophylline A. After standard *O*- and *N*-benzyl protection in acetone/Cs_2CO_3, an optimized Heck reaction with methyl acrylate gave the desired cinnamate ester **24**. Its reduction was achieved with $LiAlH_4$. While no incomplete reduction to the corresponding aldehyde occurred, some over-reduction of the enone system took place, leading to an inseparable mixture. This, however, did not need to be resolved, since the final hydrogenation of the exocyclic double bond with simultaneous cleavage of the N- and O-protecting groups gave the intermediate **25** with the fully saturated side chain, anyhow.

While the alkaloid and the photoactive groups were now available, the preparation of the desired *N*-dansyl-L-serine and -L-tyrosine-based amino acid linkers turned out to be challenging. L-Serine (**13**) itself could not be dansylated in any solvent/base system, because of its high hydrophilicity and the resulting low solubility in organic solvents. The corresponding methyl ester **26**, by contrast, prepared in MeOH/TMSCl, was readily N-functionalized (Scheme 5), the best results being obtained by using dansyl chloride in pyridine.[30] The following reactions involving photoactive groups were performed, exemplarily, with the benzophenone derivative **16** as the most stable of these groups, because their now-required attachment to the hydroxy function imposed some unforeseen difficulties. Thus, the scheduled substitution reaction of the benzophenone derivative **16** did not take place in THF with KH as the base, while alkali metal carbonates, in particular Cs_2CO_3 in acetone, produced the N-alkylated serine derivative **28**, because of the higher acidity of the sulfonamide proton as compared to that of the aliphatic hydroxy group. Besides the unexpected, but in principle usable, N,N-disubstituted serine derivative **28**, the formation of the 4-(dansylmethyl)benzophenone (**30**) as a side product was observed in varying quantities, depending on the equivalents of base used and on the reaction time.[31] For a further confirmation of the postulated structure of this undesired product, the compound was prepared in a directed two-step synthesis. Thus, reduction of dansyl chloride (**7**) to give the corresponding sulfinic acid and substitution reaction with 4-bromomethylbenzophenone (**16**) provided the same compound **30**, identical in all chromatographic and spectral (NMR and ESI-MS) data to those of the undesired side product.

Scheme 6. Synthesis of the Building Block **34** with a Central Tyrosine Unit[a]



[a] Reagents and conditions: (a) dansyl chloride, Et_2O, NaOH, rt, 20 h, 69%; (b) NaOH, MeOH, reflux, 2 h, 93%; (c) NaH, BF_3·Et_2O, THF, reflux, 2 h; (d) **16**, Cs_2CO_3, acetone, reflux, 9 h; then: H_2O, reflux, 30 min, 63%.

To avoid the decreased yields resulting from the formation of this side product and from the following ester hydrolysis of **28**, the phenolic, and thus more OH-acidic, amino acid L-tyrosine (**14**) instead of L-serine (**13**) was chosen as the central module. Although the more lipophilic amino acid **14** even underwent double dansylation in the two-phase system Et_2O/H_2O/NaOH (Scheme 6), the resulting *N*,*O*-didansyl-L-tyrosine (**31**) was easily cleaved back to the desired mono-*N*-dansyl derivative **32** in refluxing NaOH/MeOH, by selective hydrolysis of the sulfonate ester.

Again the following steps were, exemplarily, optimized for the benzophenone derivative **16** as the most stable of the available photoactive groups, so that it should later be possible to adopt these optimum reaction conditions to the subsequent synthesis of the more sensitive diazirine and arylazide derivatives. According to LC-MS and NMR, the 'classic' conditions for the O-alkylation of phenolic hydroxy functions with alkali carbonates in acetone resulted in a complex reaction mixture of N-,O-, and N,O-alkylated tyrosine derivatives, which was hard to resolve by column chromatography. For the selective O-alkylation, an in situ protection−deprotection strategy was

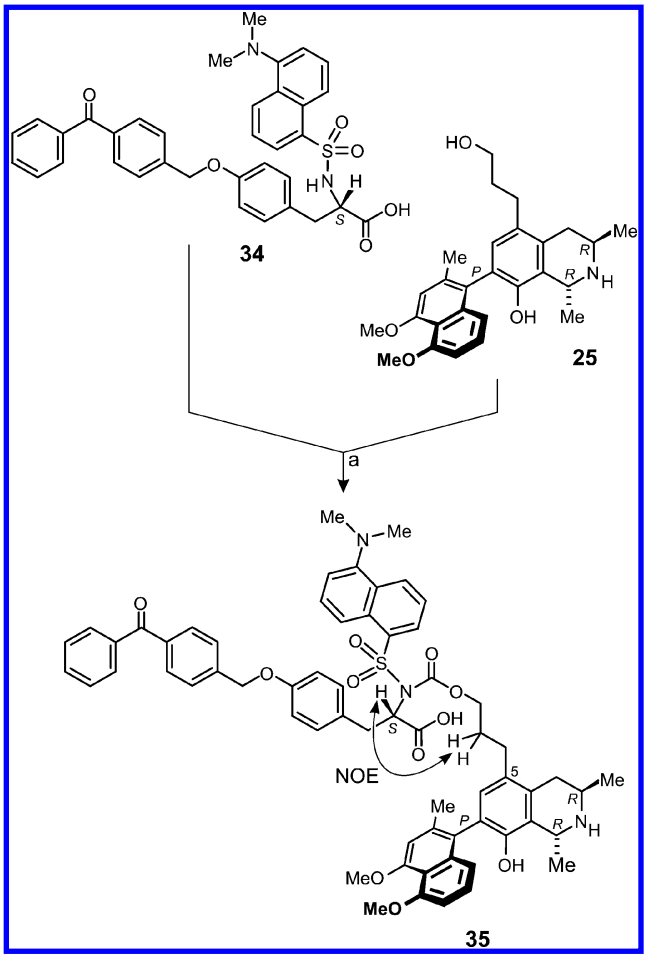
applied, as previously developed for serine and threonine.[32] Thus, reaction of the deprotonated amino acid with BF_3 led to the formation of the oxazaborolidinone **33**, which was characterized by isolation and NMR spectroscopy, but could, after removal of the $NaBF_4$ by filtration, also be immediately subjected to the selective O-alkylation, right in the crude filtrate. Eventual addition of an excess of water after completion of the reaction according to TLC effected the in situ hydrolysis of the intermediate oxazaborolidinone heterocycle and thus set free the desired O-alkylated tyrosine **34** as the only regioisomer, hence completing the efficient one-pot synthesis.

With the photoactive and fluorescent amino acid derivatives **29** and **34** in hand, we focused on their subsequent attachment to the alkaloid derivative **25** via an ester bridge. Despite the expectation of regioisomers due to the presence of three possible reaction sites, viz. the aliphatic and the phenolic OH groups and the secondary amine, and the hence possible formation of different products, we tried the esterification reaction directly on **34**. None of the classical reagents used, carbonyldiimidazole, dicyclohexylcarbodiimide, $SOCl_2$, or $(COCl)_2$, however, led to any conversion of the starting material, leaving open whether the activated carboxylic group was too inactive to be attacked by the nucleophile or whether even the amino acid was not activated at all, maybe for sterical reasons. Indeed, no reaction of the acid to any kind of activated species was observed, neither by TLC nor by HPLC. Under Mitsunobu conditions,[33] i.e., with specific activation of the aliphatic hydroxy group to react, again no formation of the desired ester occurred. In view of the assumption that the reaction might have failed because the amino acid had not been activated, we finally used the strong, but toxic and more difficult to handle, activation reagent phosgene, which now, for the first time, did succeed in converting the amino acid into a reactive species as observed by TLC (more rapidly eluting spot), eventually leading to the isolation of a product in which both, the amino acid and the alkaloid, were present according to MS and NMR, albeit only in a poor yield (Scheme 7).

A more in-depth NMR and MS analysis, however, revealed that the compound isolated was not the desired ester, but the carbamate **35**. The formation of this unexpected product, which, expectedly, had retained the phosgene-derived CO_2, may be explained by the structure and reactivity of the activated carboxylic acid. It is most probable that the reaction of the acid **34** with phosgene yields the respective oxazolidindione **36** (Figure 6), which apparently does not react in the anticipated way. Thus, instead of the initially expected attack on the lactone carbonyl function (C-5), to provide a carbaminic acid, which would then decarboxylate spontaneously to give the desired ester, the 'open-chain' carbamate ester **35** is formed with the observed retention of the CO_2 portion. The actually occurring attack of the O-nucleophile on the carbamate-CO group (C-2) rather than on the lactone-CO group (C-5) might be due to the electron-withdrawing properties of the dansyl moiety and the sterical properties of the heterocycle, making quantum chemical calculations on the structure and reactivity of the presumed intermediate **36** rewarding.

An NBO population analysis of an HF/6-31G(d)[34] optimized structure of **36** revealed a small −I effect of the dansyl moiety with respect to the carbamate carbonyl. This effect is, however, not significant and results in a slightly more polarized carbamate CO double bond in comparison to the unsubstituted analogue. The calculations also show that the lone pair of the ring nitrogen interacts with the dansyl moiety; thus the +M effect to the ring observed in an unsubstituted anhydride is significantly lowered

Scheme 7. Eventual Linkage of the Two Portions **34** and **25** by the Use of Phosgene[a]



[a] Reagents and conditions: (a) 1: phosgene (20% solution in toluene), pyridine, reflux, 1.5 h; 2: **25**, THF, rt, 12 h, 14%.

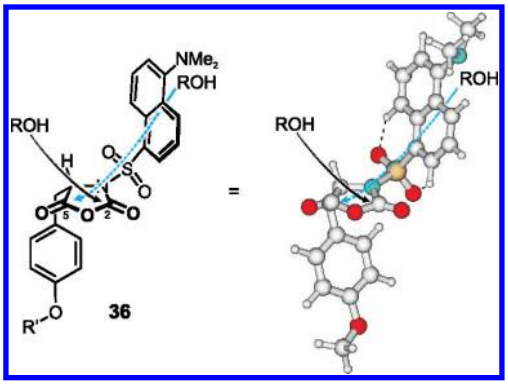


Figure 6. Structure of the assumed intermediate activated amino acid derivative **36**. The substituent below the ring plane rotates easily, whereas the dansyl moiety has a fixed position due to hydrogen bondings (dashed lines). The arrows show the possible (full black arrow) and impossible (disrupted blue arrow) approach vectors of the nucleophile from above the ring plane; the benzophenone-4-methylene portion is abbreviated as R′ (left) or CH_3 (right).

and the carbamate carbonyl should be more reactive. The interaction of the ring nitrogen lone pair with the dansyl moiety also affects the structure: one of the two diastereotopic *S*-oxygen atoms, viz. the upper one, is coplanar with the heterocyclic ring. Therefore and because of hydrogen bondings between the proximal aromatic protons and the SO_2 oxygens, the dansyl moiety has a fixed position above the plane of the cyclic carbamate (Figure 6). HF/6-31G(d) calculations of **36** showed

Table 2. IC_{50} Bioactivity Results for Dioncophylline A (**3**) and Its Derivatives **24**, **25**, and **35**[a]

	P. falciparum	cytotoxicity[b]
standard	0.12[c]	0.007[d]
3	0.38	29.1
24	0.19	>140
25	2.42	99.0
35	0.96[e]	69.5

[a] All values in μM. [b] Against L-6 cells (rat skeletal myoblast cells). [c] Chloroquine. [d] Podophyllotoxin. [e] In the case of **35** the IC_{50} value of the standard was 0.309.

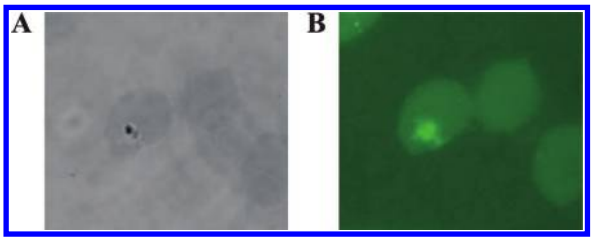


Figure 7. Microscopic visualization of **35** in *Plasmodium*-infected red blood cells. Only the infected cell is labeled (A: transmission mode; B: fluorescence mode).

that all local minima found are within 4−20 kJ/mol above the energetically lowest structure found, and in all these conformers the dansyl moiety has this fixed position, whereas the large substituent of the amino acid rotates easily (rotational barrier: 29 kJ/mol), thus shielding the bottom face of the heterocycle toward an approach of the electrophile from below. On the upper face, the dansyl moiety blocks the approach vector of the nucleophilic attack at the lactone carbonyl, leaving only the trajectory to the carbamate CO double bond unhindered. Considering these electronic and steric effects the observed reaction to the product **35** becomes understandable.

Still, despite the poor yield in the last step and the unexpected structure, compound **35** does fulfill the desired properties of a molecule that joins together the active alkaloid to a fluorescence and a photoaffinity probe via an amino acid core. Thus, we decided to first exemplarily test **35** for its applicability to photoaffinity labeling before preparing the corresponding diazirine and azide substances.

Antiplasmodial Activities and Cellular Biodistribution of the Fluorescence and Photoaffinity-Labeled Derivative 35 of Dioncophylline A. As for the fluorescent derivatives **10** and **12**, the synthetic key precursors **24** and **25** and the final photoactivatable complex **35** were tested for their antiplasmodial and cytotoxic activities before investigation of the cellular distribution of **35** in parasite-infected erythrocytes. The methyl acrylate derivative **24** of dioncophylline A (**3**) did show relatively low IC_{50} values (Table 2), while attachment of an *n*-propanol moiety in the 5-position of **3**, as in **25**, decreased the activity against *P. falciparum* blood stages, thus also providing valuable data for our ongoing QSAR investigations.[15,16] Most importantly, the designated photoaffinitiy tool **35** itself did not only reveal antiplasmodial activity ($IC_{50} = 0.96$ μM), which is only slightly weaker than that of genuine dioncophylline A (**3**), but also entered the parasite cell in the microscopic studies (Figure 7), showing that it is indeed a promising first model compound for performing photoaffinity labeling assays.

Significantly, its distribution in a culture of *P. falciparum* blood stages was the same as observed for the exclusively dansyl-labeled dioncophylline A derivatives, **10** and **12** (see above). For this reason, photoaffinity labeling experiments with this first photoactivatable and fluorescent naphthylisoquinoline derivative should be rewarding and are presently being performed in our laboratories.

From the fluorescence distribution one might assume the cysteine protease falcipain-2 to be a possible target. This protein is one of the principal cysteine proteases in the digestive vacuole but has also been found in other cell compartments.[35] Moreover falcipain-2 is known to be inhibited by some isoquinoline derivatives, making it a possible target of the naphthylisoquinolines.[36] However, the genuine alkaloid **3** did not inhibit recombinantly expressed plasmodial falcipain-2 expressed plasmodial falcipain-2 (data now shown). Consequently, dioncophylline A (**3**) and presumably its derivatives may act on other plasmodial target molecules of the parasite.

Conclusions. In the search for the target protein(s) of the highly antiplasmodial naphthylisoquinolines,[3] the two first approaches are described in this paper. The dansyl-functionalized dioncophylline A derivatives **10** and **12** were shown to be good candidates for being capable of visualizing the in vitro distribution of these alkaloids. The remarkable ability of these naphthylisoquinoline derivatives to accumulate exclusively in the pathogen inside infected erythrocytes raised the question if a transport mechanism exists for these biaryl alkaloids or whether they exhibit a high affinity to the pathogens proteins. The new dioncophylline A analogues like **12** constitute a promising basic structure to develop malaria-specific diagnostics, e.g., for the sensitive, fluorescence-based detection of parasites in blood samples of a clinical patient, even with a low degree of parasitemia. Toward the identification of putative target molecules of naphthylisoquinolines, a first route to photoactive fluorescent derivatives of dioncophylline A was developed. Tyrosine turned out to be a possible central building block to connect the dansyl and benzophenone moieties to a dioncophylline A derivative. Pharmacological investigations and microscopic studies of the distribution of the photoaffinity complex **35** indicated that this alkaloid derivative should be suitable for first exploratory photoaffinity studies. These experiments are presently in progress. After the first access to photoactivatable antiplasmodial naphthylisoquinolines reported here, related, further optimized compounds will soon follow, thus establishing a variety of instruments required for the identification of the target protein for antiplasmodial naphthylisoquinolines.

Experimental Section

General Information. All used solvents were distilled before use. THF was predried over CaH_2 and freshly distilled from potassium. Commercially available material was used without further purification. Reactions with water- or oxygen-sensitive compounds were carried out in predried glassware under argon. The reaction vessels in which phosgene solution was handled were equipped with a washing bottle filled with aqueous NaOH solution to neutralize gaseous phosgene. Reactions involving aryl azides and diazirines were carried out in brown flasks preventing the solutions from direct exposure to light.

Thin-layer chromatography was carried out using silica gel 60 F_{254} or C-18 F_{254s} aluminum foil. Detection of the compounds was achieved by fluorescence quenching at 254 nm, fluorescence at 356 nm, or staining with ninhydrin or $KMnO_4$ solutions. Flash chromatography was performed using silica gel (20−63 mesh), which was deactivated with 7.5% NH_3 solution (25%) if needed. NMR spectra were obtained on a Bruker DMX 600, Avance 400, Avance 250, or Avance 200 apparatus and are reported in ppm relative to internal solvent signal, with coupling constants (*J*) in Hertz (Hz). For ^{19}F spectroscopy $CFCl_3$ was used as the internal standard. EI mass spectrometry was carried out on a Finnigan MAT 8200; ESI-HRMS was measured on a Bruker Daltonik micrOTOF-focus.

Analytical HPLC was performed on a Jasco System (DG-1580, LG-1580, PU-1580, CO-1560, AS-1555, MD-1510) using a Chromolith Performance RP-18e (100 × 4.6 mm) with a MeCN (A)/H_2O(B) solvent mixture stabilized with 0.05% TFA: 0.5 min 10% A, 3 mL/min; 5 min 50% A, 5 mL/min; 9 min 100% A, 5 mL/min; 13 min 100% A, 6 mL/min; 15 min 10% A, 6 mL/min. Preparative HPLC was carried out on a Jasco System (PU-2087 Plus, MD-2010 Plus) with a Waters 600 Controller an a Waters 996 Photodiode Array Detector. As a column a Chromolith RP-18e (100 × 10 mm) was used with a MeCN (A)/H_2O(B) solvent mixture stabilized with 0.05% TFA: 0.5 min 10% A, 12 mL/min; 5 min 50% A, 12 mL/min; 9 min 100% A, 12 mL/min; 13 min 100% A, 20 mL/min; 15 min 10% A, 20 mL/min.

***N*,8-*O*-Dibenzyl-5-formyldioncophylline A (6).** To a solution of **5**[17] (100 mg, 0.157 mmol) in absolute THF (10 mL) at −78 °C was added a solution of *n*-butyllithium (0.25 mL of 1.6 M in hexane, 0.393 mmol) dropwise. The reaction mixture was stirred at −78 °C under argon for 110 min. In parallel, a DMF solution (90 μL, 1.17 mmol) in THF (0.5 mL) was cooled down to −78 °C and was then quickly added into the reaction mixture. The resulting mixture was stirred at −78 °C for 45 min. Ethanol (0.55 mL) was added followed by saturated aqueous NH_4Cl solution (1 mL). The suspension was warmed to room temperature and extracted with diethyl ether. The ether phases were combined and dried over $MgSO_4$. After column chromatography on deactivated (7.5% NH_3) silica gel (petroleum ether−EtOAc, 8:2 elution), **6** was obtained as a yellow solid (76 mg, 0.13 mmol, 83%): mp 83−85 °C (petroleum ether−EtOAc); $[\alpha]_D^{23}$ +13.5 (c = 0.12, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, J = 6.7 Hz, 3H, 3-CH_3), 1.40 (d, J = 6.6 Hz, 3H, 1-CH_3), 2.13 (s, 3H, 2′-CH_3), 3.00 (dd, J = 11.4, 18.5 Hz, 1H, 4-H_{ax}), 3.39 (dd, J = 4.6, 18.6, 1H, 4-H_{eq}), 3.52 (d, J = 13.8 Hz, 1H, N-CH_2Ph), 3.57 (m_c, 1H, 3-H), 3.91 (d, J = 13.8 Hz, 1H, N-CH_2Ph), 3.99 (s, 3H, 5′-OCH_3), 4.00 (s, 2H, O-CH_2Ph), 4.02 (s, 3H, 4′-OCH_3), 4.12 (q, J = 6.7 Hz, 1H, 1-H), 6.39 (d, J = 6.9 Hz, 2H, Ph), 6.78 (s, 1H, 3′-H), 6.82 (d, J = 7.7 Hz, 1H, 6′-H), 6.97−7.08 (m, 3H, Ph), 7.17−7.40 (m, 7H, Ar−H), 7.54 (s, 1H, 6-H), 10.13 (s, 1H, CHO); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 16.9 (1-Me), 22.8 (3-Me), 23.4 (2′-Me), 25.0 (4-C), 29.5 (1-C), 29.8 (3-C), 32.1 (NCH_2Ph), 56.6 (4′-OMe), 59.8 (5′-OMe), 70.4 (OCH_2Ph), 91.9, 103.4, 107.8, 107.9, 108.9, 128.1, 128.2, 136.1, 138.9, 153.6, 154.2, 156.6, 165.7 (4′-C), 173.4 (5-C), 193.3 (*C*HO); IR (KBr) 3060, 3040, 2960, 2940, 2860, 2848, 1693, 1593, 1455, 1393, 1360, 1263, 1210, 1130, 1070, 738 cm^{-1}; CD (MeOH): $\Delta\epsilon_{221}$ −17.6, $\Delta\epsilon_{240}$ +11.3, $\Delta\epsilon_{261}$ −4.8, $\Delta\epsilon_{301}$ +1.8, $\Delta\epsilon_{321}$ −1.5, $\Delta\epsilon_{353}$ +2.5; MS (EI): *m*/*z* (%): 585 $[M]^+$ (4), 370 $[M\text{-}CH3]^+$ (100), 91 $[C_7H_7]^+$ (32); MS (ESI positive): *m*/*z* (%): 586 $[M + H]^+$; HREIMS Calcd for $C_{38}H_{36}O_4N^+$ $(M - CH_3)^+$: 570.26389, found: 570.26360.

***N*,8-*O*-Dibenzyl-5-[*N*′-(1″,6″-hexanediamino-*N*″-dansyl)]-methyldioncophylline A (10).** *N*-benzyl-8-*O*-benzyl-5-formyldioncophylline A (**6**) (13 mg, 0.022 mmol) and *N*-dansyl-1,6-hexanediamine (**9**) (11.65 mg, 0.033 mmol) were mixed in CH_2Cl_2 (2 mL), cooled to 0 °C for 15 min, and then treated with sodium triacetoxyborohydride (7 mg, 0.033 mmol). The mixture was stirred at room temperature under Ar for 24 h. The reaction was stopped by adding aqueous saturated $NaHCO_3$, and the product was extracted with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by preparative TLC (CH_2Cl_2−MeOH, 95:5 elution) to give **10** (13.8 mg, 0.015 mmol, 68%) as a pale yellow solid: mp 64 °C (CH_2Cl_2); $[\alpha]_D^{23}$ +18.7 (c = 0.08, MeOH); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.08−1.10 (m, 4H, 3″-CH_2, 4″-CH_2), 1.28−1.32 (m, 4H, 5″-CH_2, 2″-CH_2), 1.30 (d, J = 6.7 Hz, 3H, 3-CH_3), 1.42 (d, J = 6.6 Hz, 3H, 1-CH_3), 2.09 (s, 3H, 2′-CH_3), 2.58 (t, J = 7.5 Hz, 2H, 1″-CH_2), 2.71 (dd, J = 11.1, 17.1 Hz, 1H, 4-H_{ax}), 2.80 (s, 6H, $(H_3C)_2$-N), 2.78−2.83 (m, 3H, 6″-CH_2 and 4-H_{eq}), 3.51 (d, J = 13.4 Hz, 1H, N-CH_2Ph), 3.62 (m_c, 1H, 3-H), 3.80 (s, 2H, 1‴-CH_2), 3.86 (s, 2H, O-CH_2Ph), 3.91 (s, 3H, 5′-OCH_3), 3.93 (d, J = 13.8 Hz, 1H, N-CH_2Ph), 3.94 (s, 3H, 4′-OCH_3), 4.15 (q, J = 6.7 Hz, 1H, 1-H), 6.28 (d, J = 6.9 Hz, 2H, Ph), 6.84−7.04 (m, 6H, aromatic H and Ph), 7.15−7.38 (m, 8H, aromatic H and Ph), 7.44−7.54 (m, 2H, 7‴ and 3‴ aromatic H), 8.13 (d, J = 7.3 Hz, 1H, 4‴ aromatic H), 8.33 (d, J = 8.7 Hz, 1H, 8‴ aromatic H), 8.49 (d, J = 8.6 Hz, 1H, 2‴ aromatic H); ^{13}C NMR (62.5 MHz, methanol-d_4) δ 19.6 (1-Me), 20.2 (3-Me), 20.9 (2′-Me), 23.7 (4-C), 26.9 (1-C), 27.2 (3-C), 28.1, 30.3, 30.4, 30.5, 30.6, 30.8 (2×), 33.1, 36.5, 43.6 (5-CH_2), 45.8 (NMe_2), 47.1, 51.3, 52.4 (4′-OMe), 56.9 (NCH_2Ph), 57.0 (5′-OMe), 75.7 (OCH_2Ph), 106.8, 110.3, 116.4, 117.7, 119.7, 120.6, 124.3, 127.7, 127.8, 128.2, 128.7, 128.8, 129.0 (2×), 129.5, 130.1 (2×), 130.9, 131.0, 131.2, 132.9, 134.6, 135.5, 137.2, 137.9, 138.0, 140.8, 153.2, 158.7, 159.3 (4′-C), 170.8 (5-C); IR (KBr) 3453, 3293, 3060, 3040, 2930, 2853, 1593, 1455, 1393, 1323, 1263, 1155, 1145, 1130, 1070, 793 cm^{-1}; CD (MeOH): $\Delta\epsilon_{218}$ −10.6, $\Delta\epsilon_{238}$ +6.4, $\Delta\epsilon_{262}$ −2.5, $\Delta\epsilon_{342}$ +1.4, $\Delta\epsilon_{362}$ −1.7; MS (EI): *m*/*z* (%): 919 $[M]^+$ (6), 904 $[M - CH_3]^+$ (83), 169 $[C_{12}H_{11}N]^+$ (83), 91 $[C_7H_7]^+$ (100); MS (ESI positive): *m*/*z* (%): 920 $[M + H]^+$; HRMS (ESI): $C_{57}H_{66}N_4O_5S$ requires for M + H at *m*/*z* 919.48267; found: 919.48271.

5-Formyldioncophylline A (11). *N*,8-*O*-Dibenzyl-5-formyldioncophylline A (**6**) (3 mg, 0.005 mmol) was hydrogenated in dry THF (1.6 mL) in the presence of Pd/C (10%, 0.8 mg) at room temperature and 3 bar H_2 for 9 h. After filtration and evaporation of the solvent, the residue was purified by preparative TLC (CH_2Cl_2−MeOH, 95:5 elution) to give **11** (1.5 mg, 0.0037 mmol, 73%) as a white solid: mp 110−112 °C (CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.57 (d, J = 6.2 Hz, 3H, 3-CH_3), 1.72 (d, J = 6.8 Hz, 3H, 1-CH_3), 2.17 (s, 3H, 2′-CH_3), 3.09 (dd, J = 10.9, 18.1 Hz, 1H, 4-H_{ax}), 3.32−3.36 (m, 1H, 4-H_{eq}), 3.89−3.95 (m, 1H, 3-H), 3.92 (s, 3H, 5′-OCH_3), 3.98 (s, 3H, 4′-OCH_3), 4.12 (q, J = 6.7 Hz, 1H, 1-H), 6.77 (d, J = 8.5 Hz, 1H, 6′-H), 6.89 (d, J = 8.0 Hz, 1H, 8′-H), 6.94 (s, 1H, 3′-H), 7.21 (t, J = 8.2 Hz, 1H, 7′-H), 7.57 (s, 1H, 6-H), 9.95 (s, 1H, CHO); ^{13}C NMR (100 MHz, methanol-d_4) δ 18.8 (1-Me), 25.5 (3-Me), 28.8 (2′-Me), 34.6 (3-C), 39.4 (4-C), 41.1 (4′-OMe), 64.5 (5′-OMe), 105.4, 107.7, 129.2, 129.8, 135.4, 136.0, 167.7, 169.5, 170.7, 176.1 (4′-C), 180.1 (5-C), 191.2 (*C*HO); IR (KBr): ν 2962, 2925, 2852, 1686, 1592, 1457, 1394, 1261, 1087; 1025, 802; 698 cm^{-1}; MS (ESI positive): *m*/*z* (%): 406 $[M + H]^+$; HRMS (ESI): $C_{25}H_{27}NO_4$ requires for M + H at *m*/*z* 406.20128; found: 406.20135.

5-[*N*′-(1″,6″-Hexanediamino-*N*″-dansyl)]methyldioncophylline A (12). A solution of 5-formyldioncophylline A (**11**) (2 mg, 4.9 μmol) and *N*-dansyl-1,6-hexanediamine (**9**) (2.6 mg, 7.4 μmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C for 15 min and then treated with sodium triacetoxyborohydride (1.6 mg, 7.5 μmol). The mixture was stirred at room temperature under an Ar atmosphere for 24 h. After being quenched by addition of aqueous saturated $NaHCO_3$, the product was exacted with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by preparative TLC (CH_2Cl_2−MeOH, 95:5 elution) to give **12** (1.7 mg, 2.3 μmol, 48%) as a pale-yellow solid: $[\alpha]_D^{23}$ +6.5 (c = 0.18, MeOH); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.26−1.31 (m, 2H, 4″-CH_2), 1.30−1.42 (m, 2H, 3″-CH_2), 1.55−1.65 (m, 4H, 5″-CH_2, 2″-CH_2), 1.59 (d, J = 6.3 Hz, 3H, 3-CH_3), 1.70 (d, J = 6.7 Hz, 3H, 1-CH_3), 2.16 (s, 3H, 2′-CH_3), 2.80 (t, J = 6.6 Hz, 2H, 1″-CH_2), 2.84−2.94 (m, 2H, 4-H_{ax}, 4-H_{eq}), 2.86 (s, 6H, $(H_3C)_2$-N), 3.00−3.05 (m, 2H, 6″-CH_2), 3.88−3.98 (m, 1H, 3-H), 3.90 (s, 3H, 5′-OCH_3), 3.93−3.96 (m, 2H, 1‴-CH_2), 3.96 (s, 3H, 4′-OCH_3), 4.20 (q, J = 6.9 Hz, 1H, 1-H), 6.79 (d, J = 8.5 Hz, 1H, 6′-H), 6.87 (d, J = 7.7 Hz, 1H, 8′-H), 6.91 (s, 1H, 3′-H), 7.14 (s, 1H, 6-H), 7.18 (t, J = 8.2 Hz, 1H, 7′-H), 7.26 (d, J = 7.6 Hz, 1H, 6‴-H), 7.50−7.58 (m, 2H, 3‴ and 7‴-H), 8.12 (dd, J = 1.3, 7.3 Hz, 1H, 4‴-H), 8.32 (d, J = 8.6 Hz, 1H, 8‴-H), 8.53 (d, J = 8.5 Hz, 1H, 2‴-H); ^{13}C NMR (100 MHz, methanol-d_4) δ 17.5 (1-Me), 24.6 (3-Me), 27.0 (2′-Me), 28.4 (4-C), 28.5 (1-C), 30.3 (2×), 30.6 (1-C), 30.8 (3-C), 33.5, 40.9 (5-CH_2), 42.5 (NMe_2), 50.4, 55.8 (4′-OMe), 58.5 (5′-OMe), 116.1, 120.3, 121.2, 122.6, 124.4, 126.1, 129.3, 130.9, 133.8, 140.4, 141.6, 141.8, 142.3, 145.8, 147.0, 152.2, 153.7, 154.3, 155.6, 157.2, 164.0 (4′-C), 169.0 (5-C); IR (KBr) 2925, 2853, 1656, 1458, 1385, 1261, 1028, 1075, 798, 722, 626 cm^{-1}; MS (ESI positive): *m*/*z* (%): 740 $[M + H]^+$; HRMS (ESI): $C_{43}H_{54}N_4O_5S$ requires for M + H at *m*/*z* 739.38877; found: 739.38881.

***N*,8-*O*-Dibenzyl-5-iododioncophylline A.** A mixture of dioncophylline A (200 mg, 0.53 mmol) and $AgNO_3$ (182 mg, 0.58 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C, and iodine (148 mg, 0.58 mmol) dissolved in EtOH (2 mL) was added. The mixture was stirred at 0 °C until completion of the reaction according to TLC and then treated with an aqueous $Na_2S_2O_3$ solution (5%, 5 mL) and extracted with CH_2Cl_2. After evaporation of the solvent, the purification of the residue by column chromatography on silica gel (CH_2Cl_2−MeOH, 99:1 to 95:5) led to *N*,8-*O*-dibenzyl-5-iododioncophylline A (292 mg, 580 μmol, 82%) as a colorless solid, which was recrystallized from MeOH: mp 154 °C; $[\alpha]_D^{25} = -107.4$ (c = 0.15, EtOH); IR (KBr) 3423, 2923, 2360, 1587, 1452, 1379, 1263, 1189, 1099, 963, 806, 760, 578 cm^{-1}; ^1H NMR (600 MHz, methanol-d_4) δ 1.35 (d, $^3J_{H-H}$ = 6.30 Hz, 3H, 3-Me), 1.48 (d, $^3J_{H-H}$ = 6.60 Hz, 3H, 1-Me), 2.16 (s, 3H, 2′-Me), 2.76 (dd, $^2J_{H-H}$ = 17.20 Hz, $^3J_{H-H}$ = 11.00 Hz, 1H, 4-H), 2.84 (dd, $^2J_{H-H}$ = 17.30 Hz, $^3J_{H-H}$ = 4.50 Hz, 1H, 4-H), 3.39 (m, 1H, 3-H), 3.94 (s, 3H, 5′-OMe), 3.98 (s, 3H, 4′-OMe), 4.45 (q, $^3J_{H-H}$ = 6.70 Hz, 1H, 1-H), 6.76 (s, 1H, 3′-H), 6.79 (d, $^3J_{H-H}$ = 7.50 Hz, 1H, 6′-H), 6.90 (d, $^3J_{H-H}$ = 8.4 Hz; 1H, 8′-H), 7.24 (dd, $^3J_{H-H}$ = 8.2 Hz, $^3J_{H-H}$ = 8.2 Hz, 1H, 7′-H), 7.44 (s, 1H, 6-H); MS (EI, 70 eV): *m*/*z* (%): 503 (17) $[M]^+$, 488 (100) $[M - CH_3]$, 376 (2) [M − I], 361 (7) $[M - I - CH_3]$; Anal. ($C_{24}H_{26}INO_3$) C, H, N.

Methyl 3-(*N*,8-*O*-Dibenzyldioncophylline-A-5-yl)-acrylate (24). A mixture of nBu_4NBr (189 mg, 586 μmol), Cs_2CO_3 (478 mg, 1.47 mmol), and PPh_3 (15.7 mg, 59.9 μmol) in acetonitrile (20 mL) was degassed for 10 min in an ultrasonic bath. The *N*,8-*O*-dibenzyl-5-iododioncophylline A (400 mg, 586 μmol) and methyl acrylate (126 mg, 1.46 mmol) were added, and the solution was again degassed for 10 min. After addition of $Pd(OAc)_2$ (13.5 mg, 58.6 μmol), the mixture was stirred for 1.5 h at 70 °C before it was filtered over celite. The filtrate was portioned between Et_2O and H_2O, the organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo at 20 °C. Flash chromatography eluting with petroleum ether−EtOAc (8:2 to 1:1) gave a pale yellow solid (310 mg, 82%): mp 103 °C; $[\alpha]_D^{25} = -88.4$ (c = 0.05, MeOH; IR (KBr) 2932, 2838, 1716, 1585, 1454, 1389, 1263, 1070, 1073 cm^{-1}; ^1H NMR (400 MHz, methanol-d_4) δ 1.30 (d, $^3J_{H-H}$ = 6.80 Hz, 3H, 3-Me), 1.43 (d, $^3J_{H-H}$ = 6.60 Hz, 3H, 1-Me), 2.10 (s, 3H, 2′-Me), 2.76 (dd, $^2J_{H-H}$ = 17.32 Hz, $^3J_{H-H}$ = 11.36 Hz, 1H, 4-H), 2.93 (dd, $^2J_{H-H}$ = 17.32 Hz, $^3J_{H-H}$ = 4.92 Hz, 1H, 4-H), 3.47 (d, $^2J_{H-H}$ = 13.52 Hz, 1H, NC*H*HPh), 3.52−3.58 (m, 1H, 3-H), 3.74 (s, 3H, COOMe), 3.88−3.95 (m, 3H, NCH*H*Ph, OCH_2Ph), 3.92 (s, 3H, 5′-OMe), 3.95 (s, 3H, 4′-OMe), 4.13 (q, $^3J_{H-H}$ = 6.68 Hz, 1H, 1-H), 6.27 (d, $^3J_{H-H}$ = 15.8 Hz, 1H, C*H*COOMe), 6.29 (d, $^3J_{H-H}$ = 7.00 Hz, 2H), 6.85−6.89 (m, 2H, 6′-H, 3′-H), 6.94−6.98 (m, 2H), 7.03−7.06 (m, 1H), 7.14−7.21 (m, 2H), 7.24−7.31 (m, 3H), 7.33−7.38 (m, 3H, 6-H), 8.07 (d, 1 H, $^3J_{H-H}$ = 15.76 Hz, C*H*CHCOOMe); ^{13}C NMR (100 MHz, methanol-d_4) δ 19.6 (1-Me), 20.1 (3-Me), 20.9 (2′-Me), 30.9 (4-C), 47.0 (1-C), 51.2 (3-C), 52.2 (COO*C*H_3), 52.3 (NCH_2Ph), 56.9 (4′-OMe), 56.0 (5′-OMe), 75.7 (O*C*H_2Ph), 107.0, 110.4, 117.7, 119.1, 119.6, 127.6, 127.9, 128.1, 128.7, 128.8, 129.0, 129.5, 130.0, 130.1, 130.1, 132.3, 134.5, 136.3, 137.2, 137.9, 138.0, 141.0, 142.7, 158.1 (8-C), 158.3 (4′-C), 158.8 (5-C), 169.2 (*C*OOCH$_3$); MS (EI, 70 eV): *m*/*z* (%): 641 (3) $[M]^+$, 626 (100) $[M - CH_3]^+$, 542 (22) [M − methylacrylate, $C_4H_6O_2$ − Me]$^+$, 535 (6) [M − Me − Bn]$^+$, 520 (17), 518 (14), 434 (6) [M − 2Bn − 2Me]$^+$, 91 (51) [CH_2-Ph]$^+$; Anal. ($C_{42}H_{43}NO_5$) C, H, N.

3-(*N*,8-*O*-Dibenzyldioncophylline-A-5-yl)-2-propen-1-ol. A solution of ester **24** (20.0 mg, 31.2 μmol) in dry THF (2 mL) was cooled to −78 °C, and $LiAlH_4$ (2.48 mg, 65.4 μmol) was added. After 2 h stirring at that temperature, the solution was allowed to warm to room temperature, neutralized with 0.05 n HCl, and extracted with EtOAc (3 × 2 mL). The organic phase was washed with water and dried over $MgSO_4$. After removal of the solvent in vacuo at 20 °C, the crude product was purified by flash chromatography using deactivated silica gel and eluting with petroleum ether−EtOAc (8:2) to obtain a yellow solid (12.0 mg, 63%). ^1H NMR showed a 3 to 1 ratio of 3-(*N*,8-*O*-dibenzyldioncophylline-A-5-yl)-2-propen-1-ol to 3-(*N*,8-*O*-dibenzyldioncophylline-A-5-yl)-propan-1-ol. The main portion of the material was used as such in the next step. For analytical purposes the desired compound was further purified by preparative HPLC: mp 100 °C; $[\alpha]_D^{25} = -39.6$ (c = 0.07, MeOH); IR (KBr) 3416, 2964, 2931, 2870, 2837, 1736, 1592, 1454, 1389, 1261, 1128, 1073, 1013 cm^{-1}; ^1H NMR (400 MHz, methanol-d_4) δ 1.55 (d, $^3J_{H-H}$ = 6.32 Hz, 3H, 3-Me), 1,72 (d, $^3J_{H-H}$ = 6.96 Hz, 3H, 1-Me), 2.14 (s, 3H, 2′-Me), 3.05−3.14 (m, 2H, 4-CH_2), 3.94−4.00 (m, 10H, 5′/6′-OMe, NCH_2Ph, OCH_2-Ph), 4.21−4.23 (m, 1H, 3-H), 4.25 (dd, $^2J_{H-H}$ = 5.32 Hz, $^3J_{H-H}$ = 1.64 Hz, 2H, CH_2OH), 4.56−4.58 (m, 1H, 1-H), 6.22 (dt, $^3J_{H-H}$ = 5.28 Hz, $^3J_{H-H}$ = 15.56 Hz, 1H, HC=C*H*CH_2OH), 6.42 (d, $^3J_{H-H}$ = 6.44 Hz, 2H), 6.88 (s, 2H, 6′-H, 3′-H), 6.92−6.94 (m, 1H, *H*C=CHCH_2OH), 7.03−7.07 (m, 2H), 7.10−7.19 (m, 3H), 7.33−7.41 (m, 5 H); ^{13}C NMR (100 MHz, methanol-d_4) δ 19.6 (1-Me), 20.1 (3-Me), 20.9 (2′-Me), 29.0 (4-C), 37.0 (1-C), 40.1 (3-C), 51.3 (N*C*H_2Ph), 56.9 (4′-OMe), 57.1 (5′-OMe), 63.5 (CH_2OH), 75.4 (O*C*H_2Ph), 106.9, 110.5, 119.9, 128.0, 128.3, 128.9, 129.2, 130.2, 131.4, 131.9, 133.1, 137.0, 137.1, 137.0, 137.2, 137.8, 150.3, 155.8 (4′-C), 158.7 (5-C); MS (EI, 70 eV): *m*/*z* (%): 613 (2) $[M]^+$, 601 (42), 600 (100), 582 (18) $[M - CH_2OH]$, 493 (12), 492 (33), 490 (17), 91 (79) $[CH_2Ph]^+$. HRMS (ESI): $C_{41}H_{44}NO_4$ requires M + H at *m*/*z* 614.32703; found: 614.32661.

3-(Dioncophylline-A-5-yl)-propan-1-ol (25). To a mixture of 3-(*N*,8-*O*-dibenzyldioncophylline-A-5-yl)-2-propen-1-ol and its 3,4-reduced derivative (164 mg, 267 μmol) in MeOH (20 mL), Pd/C (10%, 35.0 mg) was added, and the atmosphere was replaced with H_2 (1 bar). After stirring for 5 h, the solution was filtered over celite and the solvent was removed in vacuo at 20 °C. The crude product was purified by chromatography on Sephadex LH-20 with EtOAc−MeOH (1:1) as the eluent to give a brownish solid (92.0 mg, 79%): mp 138 °C; $[\alpha]_D^{25} = -40.0$ (c = 0.12, MeOH); IR (KBr) 3421, 2925, 2853, 1594, 1461, 1394, 1261, 1070, 1027 cm^{-1}; ^1H NMR (400 MHz, methanol-d_4) δ 1.33 (d, $^3J_{H-H}$ = 6.20 Hz, 3H, 3-Me), 1.51 (d, $^3J_{H-H}$ = 6.68 Hz, 3H, 1-Me), 1.76−1.79 (m, 2H, CH_2), 2.01 (s, 3H, 2′-Me), 2.47 (dd, $^2J_{H-H}$ = 16.92 Hz, $^3J_{H-H}$ = 11.08 Hz, 1H, 4-H), 2.61−2.65 (m, 2H, CH_2), 2.96 (dd, $^2J_{H-H}$ = 16.91 Hz, $^3J_{H-H}$ = 4.28 Hz, 1H, 4-H), 3.43−3.49 (m, 1H, 3-H), 3.57−3.61 (m, 2H, CH_2OH), 3.91 (s, 3H, 5′-OMe), 3.96 (s, 3H, 4′-OMe), 4.50 (q, $^3J_{H-H}$ = 6.56 Hz, 1H, 1-H), 6.69 (s, 1H, 3′-H), 6.84−6.88 (m, 2H, 6′-H, 8′-H), 6.91 (s, 1H, 6-H), 7.11−7.16 (m, 1H, 7′-H); ^{13}C NMR (100 MHz, methanol-d_4) δ 20.3 (1-Me), 20.9 (3-Me), 22.2 (2′-Me), 29.2 (CH_2), 34.4 (CH_2), 34.9 (3-C), 43.3 (4-C), 57.0 (4′-OMe), 57.1 (5′-OMe), 62.7 (OCH_2), 107.2, 110.6, 117.7, 120.1, 124.9, 127.1, 127.5, 131.2, 132.5, 133.1, 137.6, 138.6, 150.3, 158.0 (4′-C), 158.6 (5-C); MS (EI, 70 eV): *m*/*z* (%): 435 (24) $[M]^+$, 434 (42) $[M - H]^+$, 420 (100) $[M - CH_3 - H]^+$, 390 (7) $[M - CH_2CH_2OH]^+$, 210 (25); HRMS (ESI): $C_{27}H_{34}NO_4$ requires M + H at *m*/*z* 436.24878; found: 436.24802.

***N*-(Benzophenone-4-methylene)-*N*-dansyl-l-serine Methyl Ester (28).** *p*-Bromomethylbenzphenone (**16**) (39.0 mg, 142 μmol) and NaI (42.6 mg, 284 μmol) were suspended in acetone (1.50 mL) and refluxed for 30 min, *N*-dansyl-L-serine methyl ester (**27**) (10.0 mg, 28.4 μmol) and Cs_2CO_3 (185 mg, 56.8 μmol) were added, and the solution was refluxed for 9 h. The mixture was acidified (pH 4−5) with 0.05 N HCl and extracted with EtOAc (3 × 2 mL). The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography eluting with petroleum ether−EtOAc (1:1) gave a pale yellow solid (7.00 mg, 45%), which was recrystallized from petroleum ether−EtOAc: mp 53 °C; $[\alpha]_D^{25} = -12.7$ (c = 0.10, MeOH); IR (KBr) 3337, 2950, 2856, 1742, 1656, 1608, 1447, 1318, 1280, 1145, 1060, 941, 792, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.88 (s, 6H, NMe_2), 3.38 (s, 3H), 3.83−3.92 (m, 2H, β-CH_2), 4.54 (d, $^2J_{H-H}$ = 16.28 Hz, 1H), 4.82 (d, $^3J_{H-H}$ = 12.24 Hz, 1H), 4.85 (d, $^2J_{H-H}$ = 16.28 Hz, 1H, 7.19 (d, $^3J_{H-H}$ = 7.44 Hz, 1H), 7.31 (d, $^3J_{H-H}$ = 8.08 Hz, 1H), 7.42−7.49 (m, 3H), 7.52−7.60 (m, 4H), 7.72 (d, $^3J_{H-H}$ = 7.08 Hz, 1H), 8.21 (d, $^3J_{H-H}$ = 7.32 Hz, 1H), 8.34 (d, $^3J_{H-H}$ = 8.72 Hz, 1H), 8.52 (d, $^3J_{H-H}$ = 8.48 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.6 (NMe_2), 50.4 (CH_2), 52.3 (OMe), 61.0 (α-C), 61.8 (β-CH_2), 115.4, 119.4, 123.3, 126.5, 128.4, 128.7, 130.0, 130.1, 130.3, 130.4, 130.5, 131.0, 131.0, 132.6, 134.7, 137.0, 137.6, 141.6, 170.0, 196.2; MS (EI, 70 eV): *m*/*z* (%): 546 (6) $[M]^+$, 171 (100) [1-*N*,*N*-

dimethylnaphthalene]$^{+,38}$ 170 (51), 105 (44) [PhCO]$^+$, 77 (31) [Ph]$^+$; HRMS (ESI): $C_{30}H_{30}N_2O_6S$ requires M + Na at *m*/*z* 569.17223; found: 569.17091.

***N*-(Benzophenone-4-methylene)-*N*-dansyl-l-serine (29).** A mixture of *N*-(*p*-benzophenoylmethyl)-*N*-dansyl-L-serine methyl ester (**28**) (40.0 mg, 73.2 μmol) and 2 N NaOH (146 μL, 293 μmol) in THF−H_2O (1:1, 5 mL) was refluxed for 4 h. Acidification (pH 4−5) of the solution followed by extraction with EtOAc (3 × 3 mL) and evaporation of volatile compounds yielded the crude product. Purification by flash chromatography eluting with petroleum ether−EtOAc (1:1) gave a yellow oil (28.2 mg, 72%). $[\alpha]_D^{25}$ = +8.8 (*c* = 0.28, $CHCl_3$); IR (KBr) 3443, 2924, 1711, 1658, 1317, 1280, 1204, 1143, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (s, 6H, NMe_2), 3.88 (m, 2H), 4.59 (d, $^2J_{H-H}$ = 16.16 Hz, 1H, ArNC*H*H), 4.80 (m, 1H, α-H), 4.85 (d, $^2J_{H-H}$ = 16.17 Hz, 1H, ArNCH*H*), 7.24 (d, 1H), 7.31 (d, $^3J_{H-H}$ = 8.01 Hz, 1H), 7.47 (m, 3H), 7.57 (m, 4H), 7.80 (d, $^3J_{H-H}$ = 6.99 Hz, 1H), 8.21 (d, $^3J_{H-H}$ = 7.16 Hz, 1H), 8.41 (d, $^3J_{H-H}$ = 8.70 Hz, 1H), 8.55 (d, $^3J_{H-H}$ = 8.08 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.8 (NMe_2), 50.5 (CH_2), 60.6 (α-C), 62.0 (β-CH_2), 126.7, 128.3, 128.5, 128.8, 128.9, 130.2, 130.3, 130.5, 130.6, 131.0, 132.3, 132.4, 132.7, 137.1, 137.6, 141.5, 172.2, 196.4; HRMS (ESI): $C_{29}H_{28}N_2O_6S$ requires for M + Na at *m*/*z* 555.15657; found: 555.15745.

4-(5-Dimethylaminonaphthalene-1-sulfonylmethyl)-benzophenone (30). A solution of dansyl sulfinic acid[39] (30.0 mg, 0.13 mmol), 4-bromomethyl-benzophenone (**16**) (124 mg, 0.45 mmol), and 2 N NaOH (65.0 μL, 0.13 mmol) in EtOH (1 mL) was stirred over night at 80 °C. The mixture was extracted with CH_2Cl_2 (3 × 2 mL), and the organic phase was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent in vacuo yielded the crude product, which was purified by flash chromatography eluting with petroleum ether−EtOAc (7:3) to obtain a yellow solid (32 mg, 50%). The following analytical data completely matched that of the observed byproduct (**30**): mp 61−63 °C; IR (KBr) 2940, 1658, 1608, 1574, 1448, 1412, 1314, 1278, 1141, 1124, 794, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (s, 6H, NMe_2), 4.59 (s, 2H, CH_2), 7.10 (d, $^3J_{H-H}$ = 8.20 Hz, 2H), 7.22 (d, $^3J_{H-H}$ = 7.36 Hz, 1H), 7.45−7.50 (m, 3H), 7.56−7.63 (m, 4H), 7.72 (d, $^3J_{H-H}$ = 7.95 Hz, 2H), 7.99 (d, $^3J_{H-H}$ = 7.33 Hz, 1H), 8.46 (d, $^3J_{H-H}$ = 8.59 Hz, 1H), 8.58 (d, $^3J_{H-H}$ = 8.58 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.6 (NMe_2), 62.1 (CH_2), 115.5, 118.5, 123.4, 129.1, 129.8, 130.2, 130.8, 130.9, 131.6, 131.9, 132.8, 133.3, 137.4, 137.8, 152.4, 196.2; MS (EI, 70 eV): *m*/*z* (%): 429 (87) [M]$^+$, 364 (19), 234 (35) [dansyl]$^+$, 195 (32) [*p*-methyl-benzophenone − H]$^+$, 170 (100) [1-*N*,*N*-dimethylnaphthalene − H]$^{+,38}$ 169 (89), 168 (68), 167 (83), 105 (55) [benzaldehyde − H]$^+$, 77 (46) [Ph]$^+$; HRMS (ESI): $C_{26}H_{23}NO_3S$ requires for M + Na at *m*/*z* 452.12963; found: 452.12998; Anal. ($C_{26}H_{23}NO_3S$): C, H, N, S.

***N*,*O*-Didansyl-l-tyrosine (31).** Dansyl chloride (**7**) (50.0 mg, 0.19 mmol) was suspendend in Et_2O (2 mL) and added to a solution of L-tyrosine (26.0 mg, 0.14 mmol) in 2 N NaOH, and the mixture was stirred for 20 min at room temperature. A pale yellow gel was formed during the reaction. The aqueous phase was washed with Et_2O, acidified (pH 4−5) with 0.05 N HCl, and extracted with EtOAc (3 × 3 mL). The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo, yielding a yellow solid (32.0 mg, 69%), which was recrystallized from EtOAc: mp 122 °C; $[\alpha]_D^{25}$ = −38.8 (*c* = 0.10, MeOH); IR (KBr) 2945, 1720, 1573, 1504, 1456, 1367, 1145, 866, 789, 623, 572 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.74 (dd, $^2J_{H-H}$ = 14.04 Hz, $^3J_{H-H}$ = 6.96 Hz, 1H, β-C*H*H), 2.81−2.85 (m, 1H, β-CH*H*), 2.85 (s, 6H, NMe_2), 2.90 (s, 6H, NMe_2), 4.01−4.03 (m, 1H, α-H), 5.48 (d, $^3J_{H-H}$ = 8.32 Hz, 1H, N*H*), 6.50 (d, $^3J_{H-H}$ = 8.60 Hz, 2H), 6.69 (d, $^3J_{H-H}$ = 8.60 Hz, 2H), 7.14 (d, $^3J_{H-H}$ = 7.48 Hz, 1H), 7.27 (d, $^3J_{H-H}$ = 6.92 Hz, 1H), 7.37−7.64 (m, 3H), 7.66 (t, $^3J_{H-H}$ = 7.68 Hz, 1H), 8.00 (d, $^3J_{H-H}$ = 7.32 Hz, 1H), 8.09−8.12 (m, 2H), 8.43−8.48 (m, 2H), 8.56 (d, $^3J_{H-H}$ = 8.75 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.9 (β-C), 45.7 (NMe_2), 57.1 (α-C), 115.8, 116.0, 119.9, 120.0, 121.8, 123.4, 123.5, 128.4, 129.0, 129.4, 129.5, 129.6, 129.6, 130.2, 130.5, 130.5, 131.1, 131.4, 131.9, 134.5, 134.5; MS (EI, 70 eV): *m*/*z* (%): 647 (2) [M]$^+$, 253 (18), 171 (71) [1-*N*,*N*-dimethylnaphthalene]$^{+,38}$ 170 (100), 169 (26), 168 (36), 155 (21), 154 (27), 128 (23), 127 (29); HRMS (ESI): $C_{35}H_{32}N_2O_6S$ requires for M + Na at *m*/*z* 670.16576; found: 670.16475.

***N*-Dansyl-l-tyrosine (32).** A suspension of *N*,*O*-didansyl-L-tyrosine (**31**) (365 mg, 0.56 mmol) in a 1:1 mixture (40 mL) of 2 N NaOH and MeOH was refluxed for 2 h. The solution was acidified (pH 4−5) with 0.05 N HCl and extracted with EtOAc (3 × 20 mL). The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. The resulting pale yellow solid was recrystallized from EtOAc (218 mg, 93%): mp 119 °C; $[\alpha]_D^{25}$ = −60.1 (*c* = 0.07, MeOH); IR (KBr) 3550, 2925, 1612, 1578, 1396, 1146, 1091, 788, 591 cm^{-1}; ^{1}H NMR (400 MHz, methanol-d_4) δ 2.64 (dd, $^2J_{H-H}$ = 13.88 Hz, $^3J_{H-H}$ = 8.84 Hz, 1H, β-C*H*H), 2.83−2.91 (m, 1H, β-CH*H*), 2.87 (s, 6H, NMe_2), 3.90 (dd, $^2J_{H-H}$ = 8.72 Hz, $^3J_{H-H}$ = 5.18 Hz, 1H, α-H), 6.34 (d, $^3J_{H-H}$ = 8.56 Hz, 2H), 6.69 (d, $^3J_{H-H}$ = 8.56 Hz, 2H), 7.22 (d, $^3J_{H-H}$ = 6.84 Hz, 1H), 7.41−7.50 (m, 2H), 7.99 (d, $^3J_{H-H}$ = 6.08 Hz, 1H), 8.21 (d, $^3J_{H-H}$ = 8.72 Hz, 1H), 8.48 (d, $^3J_{H-H}$ = 8.44 Hz, 1H); ^{13}C NMR (62.5 MHz, methanol-d_4) δ 38.8 (β-C), 46.0 (NMe_2), 59.5 (α-C), 61.7 (OCH_2), 115.9, 116.3, 116.4, 121.1, 124.1, 124.2, 128.3, 129.9, 130.8, 130.9, 131.1, 131.2, 137.1, 137.1, 152.8, 157.0, 175.1; MS (EI, 70 eV): *m*/*z* (%): 428 (17), 414 (15) [M]$^+$, 236 (28), 172 (25), 171 (100) [1-*N*,*N*-dimethylnaphthalene]$^{+,38}$ 170 (87), 168 (29), 155 (18), 154 (19), 121 (24), 107 (57); HRMS (ESI): $C_{21}H_{22}N_2O_5S$ requires for M + Na at *m*/*z* 437.11471; found: 437.11491.

***N*-Dansyl-2,2-difluoro-4-(4-hydroxybenzyl)-[1,3,2]oxazaborolidin-5-one (33).** To a solution of *N*-dansyl-L-tyrosine (**36**) (13.0 mg, 31.4 μmol) in dry THF (0.5 mL) was added NaH (0.83 mg, 34.5 μmol), and the mixture was stirred at room temperature for 30 min. After addition of BF_3•OEt_2 (8.36 μL, 66.0 μmol), the solution was refluxed for 3 h. The precipitated $NaBF_4$ was filtered off under nitrogen, and the resulting solution was freed from volatile compounds in vacuo. The resulting yellow solid (14.4 mg, 99%) could be stored under nitrogen at 4 °C for several weeks. Because of its instability to hydrolysis it was only characterized by NMR. ^{1}H NMR (400 MHz, acetone-d_6) δ 2.62 (dd, $^2J_{H-H}$ = 13.88 Hz, $^3J_{H-H}$ = 10.72 Hz, 1H, β-C*H*H), 2.95 (dd, $^2J_{H-H}$ = 13.88 Hz, $^3J_{H-H}$ = 9.84 Hz, 1H, β-CH*H*), 3.75 (s, 6 H, NMe_2), 4.00−4.05 (m, 1H, α-H), 6.14 (d, $^3J_{H-H}$ = 8.44 Hz, 2H), 6.67 (d, $^3J_{H-H}$ = 8.32 Hz, 2H), 7.38 (d, $^3J_{H-H}$ = 9.44 Hz, 1H, NH), 7.78−7.82 (m, 2H), 8.18 (d, $^3J_{H-H}$ = 6.60 Hz, 1H), 8.29 (d, $^3J_{H-H}$ = 7.72 Hz, 1H), 8.50 (d, $^3J_{H-H}$ = 8.72 Hz, 1H), 8.76 (d, $^3J_{H-H}$ = 8.72 Hz 1H); ^{19}F NMR (400 MHz, acetone-d_6) δ −152.34, −150.10.

***N*-Dansyl-*O*-(4-benzoylbenzyloxy)-l-tyrosine (34).** A mixture of oxazaborolidinone **33**, *p*-bromomethyl-benzophenone (**16**) (13.5 mg, 49 μmol), and Cs_2CO_3 (22.5 mg, 69 μmol) in acetone (3 mL) was stirred at 50 °C until formation of a byproduct [R_f (EtOAc−PE = 1:1): 0.72] was observed (usually 6−8 h). Addition of water (1 mL) and further stirring for 30 min at 50 °C effected hydrolysis of the oxazaborolidinone. The solution was acidified (pH 4−5) with 0.5 N HCl and extracted with EtOAc (3 × 2 mL). The organic phase was washed with 0.05 N HCl and dried over $MgSO_4$. Evaporation of the solvent in vacuo resulted in a pale yellow solid (12.0 mg, 63%), which was recrystallized from EtOAc: mp 83 °C; $[\alpha]_D^{25}$ = −10.6 (*c* = 0.07, MeOH); IR (KBr) 3453, 3286, 2942, 1743, 1655, 1612, 1597, 1576, 1516, 1447, 1317, 1145, 1024, 790, 702, 627 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (dd, $^2J_{H-H}$ = 13.78 Hz, $^3J_{H-H}$ = 6.56 Hz, 2H, β-CH_2), 2.88 (s, 6H, NMe_2), 4.18−4.21 (m, 1H, α-H), 4.71 (d, $^2J_{H-H}$ = 12.52 Hz, 1H, OC*H*$_2$), 4.79 (d, $^2J_{H-H}$ = 12.50 Hz, 1H, OC*H*$_2$), 5.34 (d, $^3J_{H-H}$ = 9.20 Hz, 1H, NH), 6.38 (d, $^3J_{H-H}$ = 8.44 Hz, 2H), 6.63 (d, $^3J_{H-H}$ = 8.56 Hz, 2H), 7.10 (d, $^3J_{H-H}$ = 8.48 Hz, 2H), 7.18 (d, $^3J_{H-H}$ = 7.72 Hz, 1H), 7.45−7.52 (m, 5H), 7.59−7.67 (m, 1H), 7.66 (d, $^3J_{H-H}$ = 8.48 Hz, 2H), 7.79−7.81 (m, 2H), 8.21−8.23 (m, 2H), 8.51 (d, $^3J_{H-H}$ = 8.59 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.3 (β-C), 45.3 (NMe_2), 57.0 (α-C), 66.0 (OCH_2), 123.0, 126.0, 128.0, 128.3, 129.4, 129.5, 129.8, 130.0, 130.1, 130.5, 132.8, 134.4, 136.8, 137.6, 138.9, 154.7, 170.3, 196.1; MS (EI, 70 eV): *m*/*z* (%): 608 (21) [M]$^+$, 373 (16) [M − dansyl-H]$^+$, 235 (17) [dansyl-H]$^+$, 196 (41) [*p*-methyl-benzophenone]$^+$, 195 (31) [*p*-methyl-benzophenone − H]$^+$, 171 (100) [1-*N*,*N*-dimethylnaphthalene]$^{+,38}$ 170 (93) [1-*N*,*N*-

dimethylnaphthalene − H]$^+$, 107 (36), 77 (21) [Ph]$^+$; Anal. ($C_{35}H_{32}N_2O_6S$): C, N, H calcd: 5.30; found: 5.89; S calcd: 5.27; found: 4.77.

3-(Dioncophylline-A-5-yl)-*n*-propyl *N*-Dansyl-*O*-(4-benzoylbenzyloxy)-l-tyrosinecarbaminate (35). The obtained *N*-dansyl-*O*-(4-benzoylbenzyloxy)-L-tyrosine (**34**) (23.0 mg, 38.0 μmol) was treated with a solution of phosgene (20%, 0.80 mL, 1.53 mmol) and pyridine (3.00 mg, 38.0 μmol) in toluene, and the mixture was stirred for 1.5 h at 60 °C. A white precipitate was formed. The solvent was removed in vacuo, conveying the gases through a wash bottle filled with 2 N NaOH. The residue was dissolved in THF (1 mL), 3-(dioncophylline-A-5-yl)-propan-1-ol (**25**) (14.6 mg, 33.4 μmol) was added, and the mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was purified by preparative HPLC to give a brown solid (5.00 mg, 14%): mp 65−67 °C; $[\alpha]_D^{25} = +33.7$ ($c = 0.08$, $CHCl_3$); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.56 (d, $^3J_{H-H} = 6.32$ Hz, 3H, 3-Me), 1.70 (d, $^3J_{H-H} = 6.68$ Hz, 3H, 1-Me), 1.97−1.99 (m, 2H, CH_2), 2.13 (s, 3H, 2′-Me), 2.71−2.75 (m, 2H, CH_2), 2.76−2.93 (m, 10H, β-CH_2, 4-CH_2, NMe_2), 3.90−3.93 (m, 4H, 3-H, 5′-OMe), 3.97 (s, 3H, 4′-OMe), 4.06 (dd, $^3J_{H-H} = 8.60$ Hz, $^3J_{H-H} = 6.44$ Hz, 1H, α-H), 4.08−4.16 (m, 2H, CH_2OR), 4.84−4.88 (m, 1H, 1-H), 6.46 (d, $^3J_{H-H} = 8.60$ Hz, 2H), 6.79−6.88 (m, 5H, 3′-H, 6′-H, 8′-H, H−Ar), 6.90 (s, 1H, 6-H), 7.15−7.21 (m, 4H, 7′-H, H−Ar), 7.42−7.56 (m, 5H), 7.61−7.77 (m, 4H), 8.07 (d, $^3J_{H-H} = 6.04$ Hz, 1H), 8.21 (d, $^3J_{H-H} = 8.68$ Hz, 1H), 8.42 (d, $^3J_{H-H} = 8.60$ Hz, 1H); ^{13}C NMR (150 MHz, acetone-d_6): δ = 17.9 (1-Me), 19.2 (3-Me), 20.8 (2′-Me), 29.3 (CH_2), 30.6 (CH_2), 32.6 (4-C), 38.4 (β-C), 44.5 (3-C), 45.6 (NMe_2), 48.9 (1-C), 56.3 (4′-OMe), 56.5 (5′-OMe), 58.5 (α-C), 66.4 (ArOCH_2), 68.4 (OCH_2), 106.3, 109.5, 115.9, 117.3, 120.4, 121.5, 123.9, 127.5, 128.3, 129.3, 130.5, 130.7, 132.0, 132.2, 133.3, 134.5, 13.7, 137.3, 138.0, 138.1, 141.0, 150.8, 152.5 (NMe_2), 152.6, 154.0, 158.0 (4′-C), 158.6 (5-C), 171.4 (COOH), 196.1; HRMS (ESI): $C_{63}H_{65}N_3O_{11}S$ requires for M + H at *m*/*z* 1070.42616; found: 1070.42581.

Biological Materials and Methods. Cultivation and Fluorescence Labeling of Intraerythrocytic *P. falciparum*. *P. falciparum* (strain FCBR) was cultured in vitro under standard conditions. Briefly, parasites were grown in human red blood cells (blood group A^{Rh+}) in RPMI 1640 medium (Sigma), supplemented with 25 mM Hepes, 20 mM sodium bicarbonate, 10 mM D-glucose, and 0.5% w/v AlbuMAX II (GIBCO) at 2.5% hematocrit. Cultivation was performed at 37 °C with a gaseous phase of 90% N_2, 5% O_2, and 5% CO_2. For fluorescence labeling a culture of *P. falciparum* was incubated with 0.5 μM of the respective compound for 4 h under the culture conditions. After the incubation period, thin blood smears were prepared and immediately examined under a confocal-laser-scanning microscope (Leica TCS SP) using the laser and filter settings for DAPI fluorescence or an Axiovert S100 fluorescence microscope (Zeiss) using an emission filter of 470 ± 40 nm. Images were also taken in transmission mode to visualize nonfluorescent cells. The tests for protease inhibition were performed as previously described.[37]

Computational Chemistry. For the quantum chemical calculations the program package Gaussian 03[40] was used together with the Hartree−Fock method and the polarized valence double-ζ basis set 6-31G(d).[34] To establish the found stationary points as local minima (no imaginary frequencies) or transition states (exactly one imaginary frequency), frequency calculations were performed. These calculations were also used to get zero point corrected energies (zero-point vibrational energies were scaled with the factor 0.9135[41]). The population analyses were accomplished with the NBO software[42] as implemented in Gaussian 03.

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Supporting Information Available: Further microscopic visualizations, UV-spectroscopic investigations, mechanistic considerations, and HPLC, combustion analysis, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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