provided 50 mg (65%) of the title compound (as off-white prismatic crystals), mp 197-199 °C (lit.[6] mp 204-205 °C).

Binding Studies. The radioligand binding assay was conducted in essentially the same manner as reported earlier.[11,18] Following decapitation, the brains of male Sprague-Dawley rats (ca. 220 g) were removed and placed in 0.9% ice-cold saline and dissected over ice until the tissue was prepared. Tissues were stored in ice-cold saline for no longer than 1 h and, following blot drying and weighing, were prepared and frozen at -30 °C until used. Freshly dissected tissue was homogenized (Polytron setting 6 for 20 s) in 30 volumes of ice-cold buffer containing 50 mM Tris·HCl (pH 7.4 at 37 °C; pH 8.0 at 4 °C), 0.5 mM Na_2EDTA, and 10 mM $MgSO_4$, and centrifugated at 30000*g* for 15 min. The supernatant was discarded, and the pellet was resuspended and preincubated for 15 min at 37 °C. The pellet was washed twice by centrifugation and resuspension. The final assay buffer contained 50 mM Tris·HCl (pH 7.7), 10 μM pargyline, 0.1% ascorbate, 10 mM $MgSO_4$, and 0.5 mM Na_2EDTA. The agonist high-affinity state of the 5-HT_2 receptor was labeled with 0.4 nM [^{3}H]DOB (40 Ci/mmol; New England Nuclear) with 20 mg wet weight of tissue prepared from rat frontal cortex. Cinanserin (1 μM) was used to define nonspecific binding. 5-HT_2 sites were also labeled with [^{3}H]ketanserin (76 Ci/mmol; New England Nuclear) and 3 mg wet weight of rat frontal cortex tissue. The 5-HT_{1A} receptor was labeled with 0.1 nM [^{3}H]8-OH-DPAT (120 Ci/mmol; New England Nuclear) and 6 mg wet weight of rat hippocampal tissue. 8-OH-DPAT (1 μM) was used to determine nonspecific binding. The 5-HT_{1B} receptor was labeled with 2 nM [^{3}H]serotonin (23 Ci/mmol; New England Nuclear) and 8 mg wet weight of rat striatum. Serotonin (10 μM) was used to define nonspecific binding; 8-OH-DPAT (100 nM) and mesulergine (100 nM) were used to block 5-HT_{1A} and 5-HT_{1C} sites, respectively. Eleven concentrations of nonradioactive competing drugs were made fresh daily in assay buffer. Following incubation with membranes and radioligand at 37 °C for 15 min (5-HT_2 assays) or for 30 min (5-HT_1 assays), samples were rapidly filtered with glass-fiber filters and were washed with 10 mL of ice-cold 50 mM Tris·HCl buffer. Individual filters were inserted into vials and equilibrated with 5 mL of scintillation fluid for 6 h before counting at 45% efficiency in a Beckman 3801 counter. Results were analyzed with RS1 (BBN Software).

Discrimination Studies. The present study used male Sprague-Dawley rats that had been previously trained[17,19] to discriminate either 1.0 mg/kg of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) or 0.2 mg/kg of 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) from 0.9% saline. The studies were conducted with use of standard two-lever operant chambers (Coulbourn Instruments Model E10-10), a variable-interval 15-s schedule of reinforcement for food (sweetened milk) reward, and a 15-min presession injection interval. Details of the training and testing procedures have been previously reported.[17,19] Briefly, in the tests of stimulus generalization (during which maintenance of the original DOM/saline or 8-OH-DPAT/saline discrimination was insured by continuation of training sessions throughout the studies), the animals were allowed 2.5 min to respond under extinction conditions and were then returned to their individual home cages. An odd number of training sessions (usually five, but never less than three) separated any two test sessions. During the test sessions, doses of the challenge drugs were administered by intraperitoneal injection in a random order to, routinely, groups of five animals. Once disruption of behavior occurred, only lower doses of the compound would be investigated. A 15-min presession injection interval was used throughout. Stimulus generalization was said to have occurred when the animals made ≥80% of their total responses on the drug-appropriate (i.e., either DOM-appropriate or 8-OH-DPAT-appropriate) lever. Animals making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. Where stimulus generalization occurred, ED50 values (i.e., doses at which the animals would be expected to make approximately 50% of their responses on the drug-appropriate lever) were calculated from the dose-response data.

Acknowledgment. We express our appreciation to M. Seggel and N. Naiman for their assistance with certain synthetic aspects of this project.

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Peptide Derivatives of Primaquine as Potential Antimalarial Agents

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Three peptide derivatives of primaquine were synthesized. The compounds were tested for radical curative antimalarial activity against *Plasmodium cynomolgi* in rhesus monkeys and blood schizonticidal antimalarial activity against *Plasmodium berghei* in mice. All three peptide derivatives showed activity against *P. cynomolgi* greater than that expected for the primaquine content of each prodrug. The toxicity of one of the peptide derivatives was less than that of primaquine in mice.

Malaria is caused by more than 50 different species of the protozoa *Plasmodium*.[1] However, only four species attack humans: *P. faciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. If not treated properly, *vivax* malaria may subside spontaneously, only to recur at a later date. Primaquine (*1*) is the drug of choice for the radical cure of *vivax* malaria, or in combination with other antimalarial drugs such as chloroquine for prophylaxis. The major drawback of primaquine is its low therapeutic index. The drug is known to cause hemolytic lesions, particularly in patients deficient in glucose-6-phosphate dehydrogenase. Many analogues of **1** have been prepared, some less toxic than **1**.[2,3] Recently, reduced toxicity was achieved by encapsulating primaquine in liposomes[4] or by linking **1** to a macromolecular protein.[5,6] Interestingly, Trouet and co-

(1) Sweeney, T. R.; Strube, R. E. In *Burger's Medicinal Chemistry*, 4th Ed.; Wolff, M. E., Ed.; Wiley-Interscience: New York, 1979; p 333.

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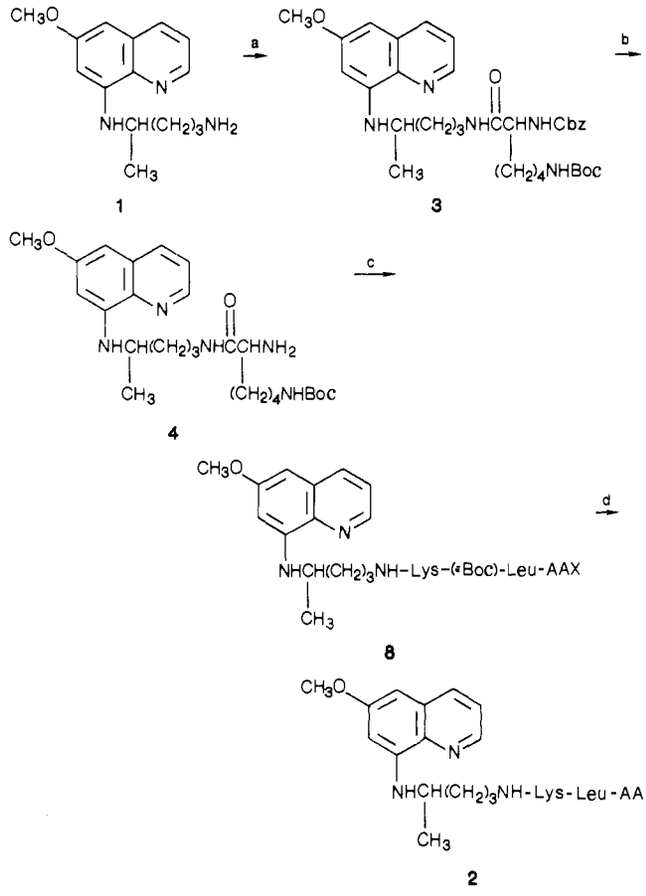
Scheme I[a]



a: AA = D-valine, X = Boc; **b**: AA = D-alanine, X = Cbz; **c**: AA = L-valine, X = Cbz

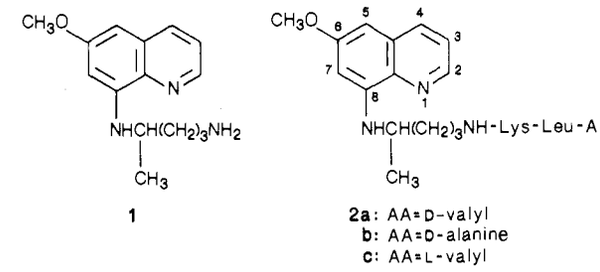
[a] (a) Et_3N, HOBT, N^{ϵ}-Boc-*N*-Cbz-Lys, DCC. (b) Pd/C, H_2. (c) HOBT, X-AA-Leu-OH (**7a–c**), DCC. (d) CF_3CO_2H, CH_2Cl_2.

workers showed that linking **1** to small peptides both reduced toxicity and enhanced its therapeutic effect.[7]

It is known that tumors are associated with increased levels of plasmin. On this basis, Carl and co-workers[8] reported that peptide prodrugs of several anticancer agents designed to be specific plasmin substrates showed selective toxicity in vitro. If malarial parasites were to possess increased levels of plasmin, similarly designed peptide derivatives of primaquine should show selective toxicity for the parasite. In order to test this hypothesis, we report the synthesis and antimalarial evaluation of the three peptide primaquine analogues **2a–c** which were designed to be substrates for plasmin. Compound **2a** contains the same peptide that Carl and co-workers used in their design of plasmin activated anticancer prodrugs.[8]

Chemistry

Scheme I outlines the procedure used to prepare **2a–c**. Primaquine free base (**1**) was coupled with N^{ϵ}-Boc-*N*-Cbz-lysine with use of HOBT and DCC to give the protected lysylprimaquine derivative **3**. Hydrogenolysis of the *N*-Cbz group of **3** using palladium on carbon catalyst



yielded the free amino compound **4** with the ϵ-nitrogen still protected. The free amino compound **4** was coupled with Boc-D-Val-Leu-OH (**7a**) (this dipeptide was prepared from leucine methyl ester (**5**) and Boc-D-valine via **6a** by a known procedure[8]), again with use of HOBT and DCC as the coupling reagent, to give the protected tripeptide derivative **8a**. Treatment of **8a** with trifluoroacetic acid in methylene chloride gave the target compound **2a**.

If the free amino compound **4** was coupled with Cbz-D-Ala-Leu-OH (**7b**),[9] **8b** was obtained. Since the amino groups in tripeptide **8b** were protected by the Cbz group as well as the BOC group, a two-step deprotection was needed. Removal of the BOC group proceeded smoothly with trifluoroacetic acid in methylene chloride. The completion of the reaction was confirmed by ^1H NMR spectroscopy as well as by TLC analysis. The resulting product was subjected to hydrogenolysis with 10% palladium-on-carbon catalyst to give **2b**.

The dipeptide **7c** needed for the synthesis of **2c** was prepared by saponification of commercially available Cbz-Val-Leu-OCH_3 and was coupled to **4** by the DCC method to afford the diprotected peptide **8c**. The protective groups were removed by hydrogenolysis, followed by treatment with trifluoroacetic acid to give **2c**.

The ^1H NMR spectral data for **2a–c** are given in the Experimental Section. However, we would like to point out the following variations noted in the ^1H NMR spectrum of these target compounds. The ^1H NMR spectra of **2a–c** in D_2O showed a time-dependent variation of intensity for the 5-proton. This behavior was observed also with primaquine phosphate.[10] It appears that the 5-proton of primaquine as well as the primaquine peptide derivatives exchange with deuterium in the solvent. In the case of primaquine phosphate in D_2O, we found that the exchange was complete within 30 min, while in 40% D_2O in DMSO the exchange required almost 2.5 h to reach the minimum detectable limit by ^1H NMR analysis. In addition, it was also observed that the 7-proton on primaquine phosphate was exchangeable but at a considerably slower rate.

Biological Testing

Compounds **2a–c** were tested for radical curative activity against *Plasmodium cynomolgi* in rhesus monkeys at the SEATO Medical Research Laboratory, Bangkok.[11,12] The data, along with a comparisoin of the data for primaquine, are shown in Table I. For comparison the dose is given as primaquine base/kilogram. At a dose of 1.0 mg of

(5) Hofsteenge, J.; Capuano, A.; Altszuler, R.; Moore, S. *J. Med. Chem.* **1986**, *29*, 1765.
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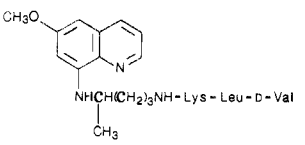
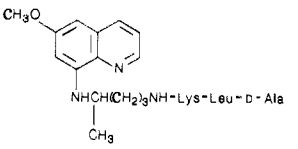
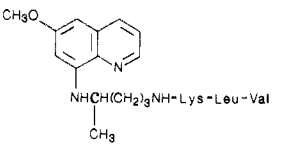
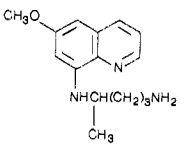
(9) Geiger, R.; Jaeger, G.; Koenig, W.; Volk, A. *Z. Naturforsch B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1969**, *24*, 999.
(10) McChesney and Sarangan have reported a rapid exchange of the 5-proton in D_2O (McChesney, J. D.; Saranyan, S. *Pharm. Res.* **1984**, 184.
(11) Schmidt, L. N.; Rossan, R. N.; Fradkin, R.; Woods, *J. Bull. WHO* **1966**, *34*, 783.
(12) The test procedure is described in World Health Organization (1972b); WHO/MAL/72.763 (cyclostyled report), World Health Organization, Geneva.

Table I. Antimalarial Activities of Primaquine Prodrugs against *P. cynomolgi* in Rhesus Monkeys[a]

compound	structure	dose,[b,c] mg/kg	cures[d]	relapse[e]
2a		0.032 0.10 0.319 1.0	0/2 0/2 0/2 2/2	6, 7 6, 9 65, 79
2b		0.319 1.0	0/1 2/2	13
2c		0.319 1.0	0/1 1/1	16
primaquine[f]		0.316 1.0	0/2 1/2	

[a] Data were supplied by Walter Reed Army Institute of Research. [b] Dose administered via stomach tube once daily for 7 days with 10 mg of base/kg of chloroquine. [c] Doses in milligrams of primaquine base/kilogram. [d] Monkeys that did not relapse are considered cured (see ref 12). [e] The number given is the days between the end of treatment and relapse. [f] Taken from LaMontagne, M. P.; Blumbergs, P.; Strube, R. E. *J. Med. Chem.* **1982**, *25*, 1094.

primaquine base/kg, compound **2a** and **2b** gave 2/2 cures, and **2c** showed 1/1 cure. Primaquine gave only 1/2 cures at a dose of 1.0 mg/kg. Monkeys dosed at 0.032 and 0.1 mg of primaquine base/kg of **2a** relapsed on days 6–9. When two monkeys were dosed at 0.319 mg of primaquine base/kg with **2a**, the monkeys relapsed on day 79 and day 65. In the case of **2b** and **2c**, the monkeys relapsed on days 13 and 16, respectively, when dosed at 0.319 mg of primaquine base/kg.

Compounds **2a–c** were tested for blood schizonticidal activity against *P. berghei* in mice[13] at the Rane Malaria Screening Laboratory, University of Miami, Miami, FL. The data along with a comparison of the data for primaquine are shown in Table II. For comparison the dose is given as primaquine diphosphate/kilogram. The only compound that showed activity in this screen was the primaquine prodrug analogue **2a**, which has a D-Val-Leu-Lys peptide connected to the terminal side-chain amino function. This compound was active at 90, 180, and 400 mg/kg but was also toxic at 180 and 400 mg/kg. Surprisingly, the primaquine prodrug analogue **2c**, which has a Val-Leu-Lys peptide connected to the terminal side-chain amino function, was not active but toxic at 90 and 362 mg/kg.

Table II. Antimalarial Activity against *P. berghei* in Rodents[a]

compd	dose, mg/kg[b]	ΔMST[c]	toxic deaths[d]
1	80	9.0	
	160		2
	320		5
	640		5
2a	90	6.9	
	180	7.6	2
	400	9.9	3
2c	20	3.2	
	91		5
	362		5

[a] Tests were carried out by the Rane Laboratory, University of Miami, Miami, FL, using blood-induced *P. berghei* infected mice (five animals per group) by the method described by Osdene et al.[13] Test data were supplied by Walter Reed Army Institute of Research. [b] Doses in milligrams of primaquine diphosphate/kilogram. [c] ΔMST, mean survival time over controls (6.2 ± 0.5 days). A compound is considered active if MST of the treated group is more than twice that of the control group. [d] Number of toxic deaths occurring on days 2–5 after infection.

Discussion

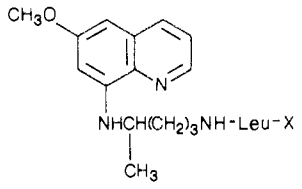
Trouet and co-workers[7] reported the preparation, antimalarial activity, and toxicity of several amino acid and peptide derivatives of primaquine. The activity was assessed against *P. berghei* in mice by a procedure that was designed to show causal prophylactic antimalarial activity. The toxicity was established by determining the LD_{50}.

Trouet and co-workers[7] prepared leucylprimaquine (**9a**), alanylleucylprimaquine (**9b**), and alanylleucylalanylleucylprimaquine (**9c**) and found that **9a** was not hydrolyzed in the presence of serum whereas **9b** and probably **9c** were slowly hydrolyzed to release **9a**. Compound **9b** and **9c** were found to be less toxic and more active than primaquine while **9a** showed activity and toxicity similar to that of primaquine. The authors suggested that **9b** and **9c** are products that have to be hydrolyzed to **9a** in the serum in order to be active. Since **9a** is not hydrolyzed in serum, it is either active itself or hydrolyzed to primaquine inside the cells. The reasons why the peptide derivatives of primaquine are less toxic and more active than primaquine are not known. Trouet and co-workers[7] suggested that the uptake and distribution of the peptide prodrugs in the tissue are different from those of **1** and thus might explain their different chemotherapeutic properties.

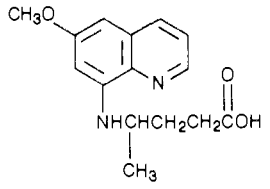
Baker and co-workers[14] found that 35–83% of the primaquine dose was converted to 8-[(3-carboxy-1-methyl-

(13) Osdene, T. S.; Russell, P. B.; Rane, L. *J. Med. Chem.* **1967**, *10*, 431.

(14) Baker, J. K.; Bedford, J. A.; Clark, A. M.; McChesney, J. D. *Pharm. Res.* **1984**, *98*.



9a: X = OH
b: X = alanyl
c: X = alanylleucylalanyl



10

propyl)amino]-6-methoxyquinoline (**10**) in rhesus monkeys. Interestingly, the plasma concentration of **10** exceeded that of **1** within 15 min. Thus, the peptide residue may serve to protect the primary amino function of **1** against this rapid metabolism.

Regardless of the mechanism of their operation, the extremely encouraging results of Trouet and co-workers[7] suggested the synthesis of other amino acid and peptide derivatives of **1** as a means of improving the chemotherapeutic properties of **1**.

Carl and co-workers[8,15] reported that several anticancer agents with different modes of action can be converted to inactive peptidyl prodrugs that can then be reactivated by the plasmin generated by tumors, thus leading to selective cytotoxicity. The peptidyl drugs were specifically designed to serve as plasmin substrates since many types of malignant cells and human tumors display increased concentration of the protease, plasminogen activator, which converts plasminogen to the highly active protease, plasmin. Thus, peptidyl prodrugs that contain the appropriate peptide specifier are locally activated by the higher concentration of tumor-associated plasmin to a degree greater than that by normal cells, resulting in selective toxicity. The particular peptidyl drugs, D-Val-Leu-Lys-drug, were chosen based on the following: (a) plasmin is a protease with specificity for lysine, (b) in 14 of 19 known plasmin cleavage sites on the fibrinogen molecule, the amino acid next to lysine is hydrophobic, and (c) D-amino acids on the terminal position help prevent degradation by aminopeptidases.

Since a variety of invading organisms such as bacteria[16] and herpes viruses[17] contain proteases that can activate the serum plasminogen to the active protease plasmin, one could speculate that the malarial parasites might also show an increased production of plasminogen activator. If this hypothesis is correct, then the peptidyl prodrugs **2a–c** would be expected to show selective toxicity. Each of the proposed peptidyl prodrugs of **1** contain a lysine connected directly to **1** with a hydrophobic leucine next to the lysine. Compound **2a** is analogous to the anticancer prodrugs used by Carl and co-workers.[8,15] Compound **2b** differs from **2a** in that a D-alanine has replaced the terminal D-valine in **2a**. Compound **2c**, which differs from **2a** by possessing a terminal natural L-valine residue, was prepared for comparison.

A comparison of the data of the three primaquine peptide prodrugs **2a–c** to that of primaquine shows that each of the prodrugs displays activity in the *P. cynomolgi* screen greater than that expected from the primaquine content of the prodrugs. Compound **2a**, which cured 2/2 monkeys at a dose of 1.0 mg of primaquine base/kg, appears to be the most active analogue since two monkeys at a lower dose of 0.319 mg of primaquine base/kg did not relapse for 65 and 79 days, respectively.

Toxicity studies were not determined in monkeys. However, prodrug **2a** is less toxic than **2c** and primaquine in mice (see Table II).

Conclusion

The antimalarial activity of D-valylleucyllysylprimaquine (**2a**) and D-alanylleucyllysylprimaquine (**2b**) and toxicity of **2a** were compared to the activity and toxicity of valylleucyllysylprimaquine (**2c**) and primaquine (**1**). The data imply that **2a** is less toxic and more active than **2c** and **1**. This limited study suggests that additional peptide derivatives of primaquine should be prepared. For example, **2a–c** were all prepared from racemic primaquine, and since it is known that (+)- and (−)-primaquine possess similar activities but different levels of toxicity,[18] it would be particularly interesting to prepare peptide derivatives of (+)- and (−)-primaquine.

Experimental Section

Melting points were determined on a Koffler hot stage. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Ultraviolet spectra were run on a Varian Model 2290 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker 250 spectrometer. Chemical shifts were reported in δ values relative to tetramethylsilane (Me_4Si).

4′-*N*-(*N*$^\epsilon$-Boc-*N*-Cbz-lysyl)primaquine (3). A solution of primaquine phosphate (1.79 g, 0.005 mol) in DMF (10 mL) was neutralized with triethylamine (0.7 mL, 55 mmol) at 0 °C. *N*$^\epsilon$-Boc-*N*-cbz-lysine (1.88 g, 0.005 mol) and HOBT (0.680 g, 0.005 mol) were added, followed by a solution of DCC (1.14 g, 0.0055 mol) in CH_2Cl_2 (15 mL). The mixture was stirred at 0 °C for 4 h and overnight at room temperature. The urea formed was separated by filtration and washed with CH_2Cl_2. The filtrate and washings were evaporated to a syrupy liquid, which was dissolved in ether (100 mL), treated with charcoal, and evaporated to dryness to give 3.36 g of a yellow waxy solid. The residue was chromatographed on silica gel (200 g). Elution with 5% MeOH in $CHCl_3$ gave 2.91 g (95%) of **3** as a mixture of diastereoisomers: R_f 0.53 ($CHCl_3$–EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3, $CHCH_3$), 3.82 (s, 3, OCH_3), 5.01 (d, 2, benzylic), 6.22 (d, 1, C_7-H), 6.28 (d, 1, C_5-H), 7.23 (dd, 1, C_3-H), 7.26 (s, 5 C_6H_5), 7.85 (d, 1, C_4-H), and 8.46 (d, 1, C_2-H).

Compound **3** as a mixture of diastereoisomers was used for the preparation of **4**.

4′-*N*-(*N*$^\epsilon$-Boc-lysyl)primaquine (4). A solution of **3** (790 mg, 1.29 mmol) in 15 mL of MeOH and 10% Pd/C (0.10 g) was hydrogenated at atmospheric pressure for 40 min. The catalyst was separated by filtration, and the filtrate was evaporated to dryness. The product was purified by chromatography (60 g silica gel) with 10% MeOH–$CHCl_3$ as eluant. The product (0.54 g, 87%) was obtained as a yellow waxy solid diastereoisomeric mixture of **4**: R_f 0.43 (MeOH–$CHCl_3$, 1:9); ^{1}H NMR ($CDCl_3$–CD_3OD) δ 1.32 (d, 3, $CHCH_3$), 1.43 [s, 9, $C(CH_3)_3$], 3.89 (s, 3, OCH_3), 6.32 (s, 1, C_7-H), 6.40 (s, 1, C_5-H), 7.34 (dd, 1, C_3-H), 7.96 (d, 1, C_4-H), 8.50 (d, 1, C_2-H).

Compound **4** as a mixture of diastereoisomers was used for the preparation of **8a–c**.

4′-*N*-[Boc-D-valylleucyl(*N*$^\epsilon$-Boc-lysyl)]primaquine (8a). To a solution of **4** (4.77 g, 0.01 mol), **7a**[8] (3.30 g, 0.010 mol), and HOBT (1.35 g, 0.010 mol) in 15 mL of dry DMF at 0 °C was added dropwise a solution of DCC (2.27 g, 0.011 mol) in 25 mL of methylene chloride over a period of 30 min. After being stirred for 4.5 h, the mixture was diluted with 200 mL of methylene chloride and filtered. The filtrate was evaporated under reduced pressure at 25–30 °C. The residue was dissolved in 300 mL of ethyl acetate, washed with 5% sodium bicarbonate and NaCl solution, and dried (Na_2SO_4). The residue obtained on evaporation was chromatographed on silica gel (400 g). Elution with 10% MeOH in chloroform gave 5.6 g (70%) of **8a** as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 0.90 [d, 6, $C(CH_3)_2$], 1.27 [d, 6, $C(CH_3)_2$], 1.45 [s, 18, $C(CH_3)_3$], 3.89 (s, 3, OCH_3), 5.32 (s, 2, OCH_2), 6.21 (d, 1, C_7-H), 6.27 (d, 1, C_5-H), 7.23 (dd, 1, C_3-H), 7.86 (d, 1, C_4-H), and 8.46 (d, 1, C_2-H).

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This product was used for the preparation of **2a** without further purification.

4′-*N*-(D-Valylleucyllysyl)primaquine (2a). Run 1. A solution of **8a** (5.4 g, 0.0068 mol) in 30 mL of 50% trifluoroacetic acid in methylene chloride was stirred at room temperature for 30 min. The mixture was evaporated to dryness under reduced pressure at room temperature and triturated with ether. After filtration the slightly reddish colored residue was washed with ether and dried to give deprotected primaquine peptide derivative **2a**. The solid residue was dissolved in MeOH and treated with 85% phosphoric acid (1.4 g). The resulting pale white solid was collected and recrystallized from H_2O–ethanol to give 2.58 g (44%) of **2a** diphosphate: mp 125–128 °C dec; ^{1}H NMR (D_2O) δ 0.53 [d, 6, $C(CH_3)_2$], 0.80 [m, 6, $C(CH_3)_2$ valyl], 3.49 (s, 3, OCH_3), 6.41 (s, 1, C_7-H), 6.51 (s, 1, C_5-H), 7.42 (dd, 1, C_3-H), 8.05 (d, 1, C_4-H), and 8.51 (d, 1, C_2-H). Anal. ($C_{32}H_{53}N_7O_4 \cdot 2H_3PO_4 \cdot H_2O$) C, H, N.

Run 2. Removal of the Boc group from **8a** was carried out as described for run 1. Thus, 5.6 g of **8a** gave 2.70 g (73%) of the free base. A solution of the free base in 10 mL of MeOH was treated with 1.03 g of 85% H_3PO_4 to give 2.85 g of **2a** diphosphate. The sample was purified by recrystallization from H_2O–ethanol to give 2.3 g (65%): mp 125–128 °C dec.

4′-*N*-[Cbz-D-alanylleucyl(N^e-Boc-lysyl)]primaquine (8b). To a stirred solution of 4 (0.88 g, 0.0018 mol), **7b**[9] (0.71 g, 0.0018 mol), and HOBT (0.26 g, 0.0018 mol) in 3 mL of DMF at 0 °C was added dropwise a solution of DCC (0.42 g, 0.002 mol) in 5 mL of methylene chloride. After 4.5 h the reaction mixture was diluted with 25 mL of ethyl acetate. The resulting precipitate was separated by filtration. The residue obtained on evaporation of the filtrate was purified by column chromatography (silica gel, 60 g). The column was eluted with 10% ethyl acetate in methylene chloride to give 0.94 g (64%) of **8b**. Recrystallization from ethyl acetate–hexanes gave 0.63 g of **8b** as pale yellow crystals: mp 55–56 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 [d, 6, $C(CH_3)_2$], 1.24 (d, 3, CH_3), 1.42 [s, 9, $C(CH_3)_3$], 3.87 (s, 3, OCH_3), 5.01 (s, 2, CH_2O), 6.28 (d, 1, C_7-H), 6.34 (d, 1, C_5-H), 7.25 (s, 5, C_6H_5), 7.30 (dd, 1, C_3-H), 7.92 (d, 1, C_4-H), 8.52 (d, 1, C_2-H).

This product was used in the preparation of **2b** without further purification.

4′-*N*-(D-Alanylleucyllysyl)pyrimaquine (2b). A solution of **8b** (7.44 g, 0.095 mol) in 100 mL of 50% trifluoroacetic acid in CH_2Cl_2 was stirred at room temperature for 1 h. The deep red solution was evaporated under reduced pressure at 20–25 °C and dried under vacuum. The residue was triturated with ether and the ether solution decanted. The solid residue was dissolved in 150 mL of MeOH and hydrogenated in the presence of 10% Pd/C (0.75 g) for 2 h. The catalyst was separated by filtration, and the residue after evaporation of the solvent was chromatographed on silica gel. Elution with $CHCl_3$–MeOH–NH_4OH (80:18:4) gave 3.75 g (71%) of **2b**. This was dissolved in 10 mL of MeOH and treated with 1.5 g of 85% H_3PO_4 to give 3.54 g of a pale cream colored solid. The solid was recrystallized from H_2O–EtOH to give 3.32 g of **2b** phosphate salt: mp 123–128 °C dec; ^{1}H NMR (D_2O) δ 0.86 (d, 6, isopropyl), 1.28 (d, 3, $CHCH_3$), 3.87 (s, 3, OCH_3), 6.37, (s, 1, C_7-H), 6.47 (s, 1, C_5-H), 7.41 (dd, 1, C_3-H), 7.96 (d, 1, C_4-H), and 8.50 (d, 1, C_2-H). Anal. ($C_{30}H_{49}N_7O_4 \cdot 2H_3PO_4 \cdot 2.5H_2O$) C, H, N.

4′-*N*-[Cbz-L-valylleucyl(N^e-Boc-lysyl)]primaquine (8c). The intermediate **8c** was prepared as described for **8b**. Thus, N^e-Boc-lysylprimaquine (7.27 g, 0.15 mol) was condensed with Cbz-Val-Leu-OH (**7c**)[19] (5.4 g, 0.15 mol) to give 9.97 g (81%) of **8c** after purification by column chromatography: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9, *t*-Bu), 3.82 (s, 3, OCH_3), 5.04 (s, 2, $PhCH_2$), 6.37 (d, 1, C_7-H), 6.43 (d, 1, C_5-H), 7.35 (s, 5, C_6H_5), 7.43 (dd, 1, C_3-H), 7.73 (d, 1, C_4-H), and 8.32 (d, 1, C_2-H).

4′-*N*-(Valylleucyllysyl)primaquine (2c). Removal of the Cbz and Boc groups was accomplished as described for **2b**. Thus, 9.5 g (0.012 mol) of **8c** was hydrogenated in the presence of 900 mg of 10% Pd/C in methanol. After removal of the catalyst and the solvent, the residue was treated with 50 mL of trifluoroacetic acid in 50 mL of CH_2Cl_2. The resulting product was purified by column chromatography to give 5.10 g (71%) of **2c**.

A solution of the free base in 15 mL of MeOH was treated with 0.98 g of 85% H_3PO_4. The solution was diluted with absolute ethanol to induce crystallization. The cooled mixture was filtered to give 3.85 g of **2c** diphosphate. The sample was recrystallized from H_2O–EtOH to yield 2.93 g of pure **2c** as the diphosphate salt: mp 194–196 °C; ^{1}H NMR (D_2O) δ 0.81 [d, 6, $CH(CH_3)_2$], 1.00 [d, 6, valyl $CH(CH_3)_2$], 1.28 (d, 3, $CHCH_3$), 3.91 (s, 3, OCH_3), 6.47 (d, 1, C_7-H), 6.62 (d, 1, C_5-H), 7.48 (dd, 1, C_3-H), 8.12 (d, 1, C_4-H), and 8.53 (d, 1, C_2-H). Anal. ($C_{32}H_{53}N_7O_4 \cdot 2H_3PO_4 \cdot 0.5\ H_2O$) C, H, N.

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Registry No. 1·diphosphate, 84902-21-6; **2a** (free base), 112925-64-1; **2a**·TFA, 112925-65-2; **2a**·diphosphate, 112925-66-3; **2b** (free base), 112925-67-4; **2b**·diphosphate, 112925-68-5; **2b**·TFA (N^α-Cbz derivative), 112945-63-8; **2c** (free base), 112925-69-6; **2c**·diphosphate, 112925-70-9; **3** (diastereomer 1), 112925-71-0; **3** (diastereomer 2), 112925-72-1; **4** (diastereomer 1), 112925-73-2; **4** (diastereomer 2), 112925-74-3; **7a**, 74201-99-3; **7b**, 30611-60-0; **7c**, 17708-79-1; **8a**, 112925-75-4; **8b**, 112925-76-5; **8c**, 112925-77-6; Cbz-Lys(BOC)-OH, 2389-60-8.

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