Synthesis and Immunosuppressive Activity of Novel Prodigiosin Derivatives

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Prodigiosins (Ps) represent a family of naturally occurring red pigments characterized by a common pyrrolylpyrromethene skeleton. Some members of this family have been shown to possess interesting immunosuppressive properties exerted with a novel mechanism of action, different from that of currently used drugs. In fact, Ps inhibit phosphorylation and activation of JAK-3, a cytoplasmic tyrosine kinase associated with a cell surface receptor component called common γ-chain, which is exclusive of all IL-2 cytokine family receptors. Blocking common γ-chain transduction activity results in a potent and specific immunosuppressive activity. With respect to the interesting and unexploited immunomodulating properties of this family of compounds we initiated a medicinal chemistry program aimed at finding novel prodigiosin derivatives with improved immunosuppressive activity and lower toxicity. Utilizing an unprecedented and flexible way of assembling the prodigiosin frame, a number of new derivatives have been prepared and tested leading to the choice of 4-benzyloxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole (PNU-156804, **16**) as a lead immunosuppressant.

Introduction

Immunosuppression is required to reduce detrimental immune reactions and has a potential role in the therapy of autoimmune diseases. Main indications of immunosuppressive therapy are prevention and treatment of acute and chronic allogeneic organ transplant rejection and graft-versus-host disease (GVHD) resulting from transplantation of foreign organs or tissues, a practice that is becoming increasingly commonplace, with renal transplantation the most frequently performed transplant.

Although the use of cyclosporin A (CyA) has been a major advance in the progress of organ transplantation,[1,2] current immunosuppressive therapies[3] still have strong limitations because of low efficacy and relevant side effects on transplant recipients. Present strategies seek to use low-dose combinations of drugs able to target different crucial steps of lymphocyte proliferation, to improve rejection prophylaxis and reduce cumulative toxicity.

Prevention of solid organ transplant rejection is presently accomplished with the combination of steroids, a noncytotoxic immunosuppressant,[4] and a cytotoxic drug.[5] The above combination acts by inhibiting different steps in the cascade of events which lead to lymphocyte proliferation that represents the primary target of immunosuppression.

Even with the best treatment available, up to 50% of patients receiving a solid organ graft experience at least one acute rejection episode within 6−12 months after transplantation and GVHD still occurs in 40% of the patients undergoing allogeneic bone marrow transplantation.

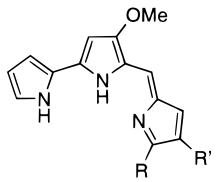

Figure 1.

Since toxicity and efficacy are unsolved problems in immunosuppression, a real breakthrough in the field would be a drug with a mechanism of action different from that of currently available immunosuppressive drugs, thus providing the rationale for its use in combination with current therapy, at lower doses and reduced cumulative toxicity.[6]

Prodigiosins (Ps) represent a family of naturally occurring red pigments[7] produced by microorganisms including *Streptomyces* and *Serratia* and characterized by the common pyrrolylpyrromethene skeleton shown in Figure 1. Prodigiosin[8] and some related compounds isolated in the 1960s have been originally studied as antibiotic and cytotoxic compounds but have never been developed due to their high systemic toxicity.[9]

During the past decade some members of this class, particularly undecylprodigiosin (Prodigiosin 25-C, UP),[10] methacycloprodigiosin,[11] and, more recently, prodigiosin[12] have been found to possess interesting immunosuppressive properties, acting through a novel mechanism of action, different from that of currently used drugs. Competitive antagonism studies have shown that Ps interact neither with the binding site for CyA nor with the common binding site for FK506 and Rapamycin on FK binding protein.

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Particularly, Ps do not prevent, unlike CyA, IL-2 production but are able to block its biological activity by inhibition of IL-2 signal transduction. Preliminary experiments showed that Ps inhibit phosphorylation and activation of JAK-3,[13,14] a cytoplasmic tyrosine kinase associated to a cell surface receptor component called common γ-chain, which is exclusive for all IL-2 cytokine family receptors. Blocking common γ-chain transduction could result in a potent and specific immunosuppressive activity.

Moreover, since Ps and CyA have different and complementary mechanisms of action they could be used in synergistic drug combination. This would potentiate the prevention and therapy of alloantigen-induced rejection and, more importantly, permit individual drug dose reductions with consequent broadening of the therapeutic window.

The interesting and unexploited immunomodulating properties of this family of compounds prompted us to start a medicinal chemistry program aimed at synthesizing novel prodigiosin derivatives with higher in vitro immunosuppressive activity/cytotoxicity ratio and a better pharmacotoxicological profile.

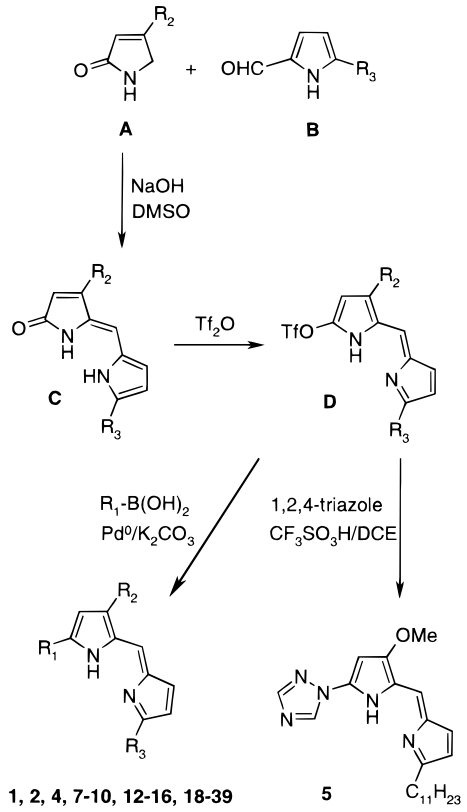
Chemistry

Prodigiosins are weakly basic (the pK_a of the free base in water/acetonitrile is 7.2) due to the presence of a pyrrolenine nitrogen atom in their structure. The 2,2′-bipyrrolylpyrromethene chromophore, characteristic of this class of compounds, is compatible with several geometrical isomers arising from the extensive conjugation in this system. In the presence of protic solvents Ps exist as a mixture of two conformers at equilibrium, related to the geometry of the exocyclic double bond. Kinetic studies[15] proved that the interconversion rate and the equilibrium distribution are greatly affected by nitrogen protonation. In particular, the interconversion rate increases considerably at higher pH and the free base (above pH 8) exists as one conformer. A structural model of the two conformers has been also proposed on the basis of two-dimensional nuclear magnetic resonance nuclear Overhauser enhancement spectroscopy data.

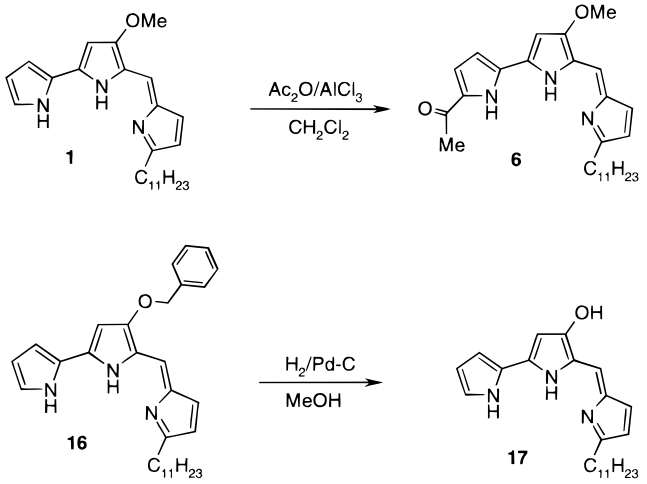
The chemistry involved in the preparation of the present compounds relies upon an unprecedented concept of assembling the prodigiosin frame. Different from all of the previous routes[16] that proceed from the dipyrrole unit, the present scheme provides first the pyrromethene to which different aromatic nuclei are connected at a final stage.[17] The flexibility achieved with such an approach opens the door to SAR studies on a wider class of derivatives otherwise hardly accessible. The structural features of the compounds in the present paper are reported in Tables 1−4.

The synthesis of most of the products (**1**, **2**, **4**, **7**−**10**, **12**−**16**, **18**−**39**) was accomplished by connecting pyrrolidinone of general formula A to formylpyrrole B via aldol condensation and adding the required third ring R_1 to "dimer" C by a Suzuki coupling reaction, using a protocol previously published from our labs (Scheme 1).[18] Formylpyrroles B were prepared by formylation of the corresponding alkylpyrroles under the well-established Vilsmeier conditions, while pyrrolidinones A were

Scheme 1



Scheme 2

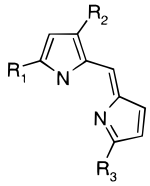


obtained from alkyl (*E*)-4-bromo-3-alkoxy-2-butenoates.[19] When R_2 is bigger than butoxy, pyrrolidinones A or C were prepared by transesterification of the corresponding methoxy analogues.[20]

For compound **5** the same synthetic route was adopted except for the last coupling step, simply performed by nucleophilic displacement of the triflate D with 1,2,4 triazole.[21]

Compounds **6** and **17** were obtained directly from preformed prodigiosins (Scheme 2) while compound **3** was obtained by aldol condensation between 5-undecyl-2-formylpyrrole and 4-methoxy-2-(*N*-ethyl)carboxamidopyrrole that, in turn, was prepared in seven steps[22] from 2-carboethoxy-3-hydroxy-5-methylpyrrole.[23] Finally, compound **11** has been prepared reacting 2-undecylpyrrole with 2,2′-bipyrrole-5-carboxaldehyde.[16c] The

Table 1. Structural and in Vitro Biological Data[a] for Compounds **1**−**10**



				IC_{50} (ng/mL)	
entry	R_1	R_2	R_3	Con A	cytotoxicity
1 (UP)	1*H*-pyrrol-2-yl	methoxy	undecyl	2.0 ± 0.3	106 ± 12
2	phenyl	methoxy	undecyl	>1000	>1000
3	ethylcarbamoyl	methoxy	undecyl	>1000	>1000
4	5-methyl-1*H*-pyrrol-2-yl	benzyloxy	methyl	4.1 ± 1.0	77 ± 13
5	1,2,4-triazol-1-yl	methoxy	undecyl	>1000	>1000
6	5-acetyl-1*H*-pyrrol-2-yl	methoxy	undecyl	100.0 ± 22	>1000
7	thiophen-2-yl	benzyloxy	undecyl	>1000	>1000
8	1*H*-indol-2-yl	methoxy	undecyl	250.0 ± 30	>1000
9	5-methoxy-1*H*-indol-2-yl	methoxy	undecyl	128.0 ± 30	>1000
10	5-chloro-1*H*-indol-2-yl	methoxy	undecyl	>1000	>1000

[a] Results are presented as the mean ± SD of at least three experiments assayed in triplicate.

latter was obtained by Vilsmeier formylation of 2,2′-bipyrrole, prepared from pyrrole and 2-pyrrolidinone.[24]

Biology

Consistent with the primary target of immunosuppressive drugs, the testing protocol has been designed to identify compounds with proven in vitro and in vivo ability to specifically inhibit activated lymphocyte proliferation.

The in vitro screening has been based on the capability of the new molecules, with respect to undecylprodigiosin (UP, **1**) chosen as reference standard, to inhibit lymphocyte proliferation induced by a polyclonal mitogen (concanavalin A, Con A) without affecting the viability of resting lymphocytes (RL) or tumor cell lines (K 562 and B 16). The concentration inhibiting 50% of cell proliferation (IC_{50}) in the different settings is calculated for the test compound and for UP. The ratio between mean IC_{50} values on tumor and resting lymphocyte cell viability (cytotoxicity) and the IC_{50} on the activated splenocyte proliferation produces the selectivity index (SI), defined as:

$$SI = \frac{[IC_{50}(K\ 562) + IC_{50}(B\ 16) + IC_{50}(RL)]/3}{IC_{50}(Con\ A)}$$

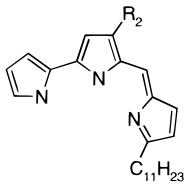
The most promising compounds, based on SI evaluation, have been tested in vivo on delayed type hypersensitivity reaction (DTH) to sheep red blood cells (SRBC) in mice, to determine the relationship between in vitro and in vivo active and toxic doses and to select those with the largest therapeutic window for further studies.

Results and Discussion

A first group of molecules (**1**−**10**; Table 1) was designed to evaluate the role played by pyrrole ring R_1, present in all the naturally occurring prodigiosins, in influencing the immunosuppressive activity of this class of compounds.

Replacement of pyrrole with a nitrogen-deprived aromatic ring, as in compounds **2** and **7**, produces a total drop of activity. On the other hand, derivative **3**, with a nitrogen in the proper position but not part of an aromatic ring, is equally inactive, suggesting that a nitrogen-containing heterocyclic ring might be essential for biological activity on the condition that an extensive conjugation of the π electron system along the whole tricyclic frame is permitted. In fact, triazole **5**, where such conjugation is prevented by a nitrogen located in the attachment point, is inactive, while indole **8**, fulfilling this prerequisite, still retains significative activity.

Table 2. Structural and in Vitro Biological Data[a] for Compounds **11**−**17**



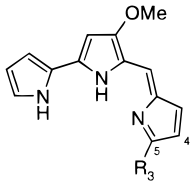
		IC_{50} (ng/mL)		
entry	R_2	Con A	cytotoxicity	SI
11	H	400.0 ± 88	1215 ± 214	3
1 (UP)	methoxy	2.0 ± 0.3	106 ± 12	53
12	ethoxy	1.8 ± 0.5	193 ± 30	107
13	*n*-propoxy	5.6 ± 0.9	322 ± 94	58
14	isopropoxy	2.6 ± 0.5	282 ± 26	108
15	*n*-butoxy	11.4 ± 2.2	1513 ± 446	133
16	benzyloxy	24.2 ± 3.5	3740 ± 503	155
17	hydroxy	>1000	>1000	

[a] Results are presented as the mean ± SD of at least three experiments assayed in triplicate.

Another parameter which seems to play an important role is the electronic density of R_1. The presence of electron-donating substituents enhances potency (**9** vs **8** and **4** vs **33**), whereas electron-withdrawing substituents induce the opposite effect (**10** vs **8** and **6** vs **1**).

A second group of derivatives (**11**−**17**; Table 2) has been prepared in order to understand the importance of the alkoxy R_2, which is methoxy in all the natural Ps. Removal of alkoxy, with consequent reduction of basicity, causes a drastic drop of activity, as shown by compound **11**. Substitution of methoxy with a larger alkoxy (see **12**−**16**) leads to a progressive reduction of activity that is overbalanced by a more marked decrease of cytotoxicity, thus producing a more favorable SI

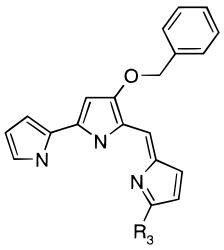
Table 3. Structural and in Vitro Biological Data[a] for Compounds **18**−**31**



entry	R_3	IC_{50} (ng/mL) Con A	IC_{50} (ng/mL) cytotoxicity	SI
18	pentyl	20.0 ± 3.7	79 ± 9	4
19	heptyl	5.6 ± 1.4	163 ± 11	29
20	decyl	1.8 ± 0.2	156 ± 49	87
1 (UP)	undecyl	2.0 ± 0.3	106 ± 12	53
21	tridecyl	1.4 ± 0.3	282 ± 59	201
22	pentadecyl	16.3 ± 2.8	1238 ± 375	76
23	phenethyl	7.5 ± 1.6	299 ± 39	40
24	4,5-butylidene	18.5 ± 4.5	120 ± 5	6
25	6-fluorohexyl	7.4 ± 1.8	172 ± 36	23
26	7-cyanoheptyl	16.2 ± 3.0	235 ± 23	14
27	6-hydroxyhexyl	56.4 ± 11.2	787 ± 183	14
28	5-carboxypentyl	>1000	>1000	
29	5-methylcarboxypentyl	>1000	>1000	
30	5-morpholinocarboxamidopentyl	>1000	>1000	
31	10-carboxydecyl	>1000	>1000	

[a] Results are presented as the mean ± SD of at least three experiments assayed in triplicate.

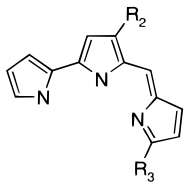
Table 4. Structural and in Vitro Biological Data[a] for Compounds **32**−**39**



entry	R_3	IC_{50} (ng/mL) Con A	IC_{50} (ng/mL) cytotoxicity	SI
32	H	15.3 ± 3.2	181 ± 46	12
33	methyl	7.9 ± 2.0	83 ± 20	10
34	pentyl	4.9 ± 1.2	83 ± 18	17
35	heptyl	3.6 ± 0.9	178 ± 48	49
36	decyl	13.0 ± 0.6	970 ± 259	75
16	undecyl	24.2 ± 3.5	3740 ± 503	155
37	tridecyl	262.0 ± 66	2780 ± 577	11
38	cyclohexylmethyl	2.1 ± 0.4	151 ± 44	72
39	benzyl	6.5 ± 1.5	279 ± 14	43

[a] Results are presented as the mean ± SD of at least three experiments assayed in triplicate.

Table 5. In Vivo (Mice) DTH Assay Data[a] for Compounds **12**, **13**, **15**, **16**, and **21**



entry	R_2	R_3	ED_{50} (mg/kg ip die × 6)	toxicity (mg/kg ip)[b]	therapeutic index
1 (UP)	methoxy	undecyl	0.6 ± 0.1	1.0 ± 0.2	1.7
12	ethoxy	undecyl	1.2 ± 0.2	2.0 ± 0.2	1.7
13	*n*-propoxy	undecyl	1.0 ± 0.2	2.0 ± 0.5	2.0
15	*n*-butoxy	undecyl	1.5 ± 0.3	3.0 ± 0.4	2.0
16	benzyloxy	undecyl	1.1 ± 0.2	5.0 ± 0.8	4.5
21	methoxy	tridecyl	0.8 ± 0.1	1.5 ± 0.4	1.8

[a] Results are presented as the mean ± SD of at least three experiments with 10 animals/group. [b] Minimal daily dose × 6 causing toxicity symptoms (weight loss, hair ruffling, and occasionally death).

(compound **16**, with a SI of 155 is the best representative of this series). Particularly interesting is the case of compound **17**, where R_2 is a free hydroxyl group (norundecylprodigiosin). Analogously to what was reported on norprodigiosin,[25] this compound is neutral since, as a tautomeric ketone, it is a vinylogous amide instead of a basic pyrrolenine. As for compound **11**, the reduction of basicity might explain the lack of activity of **17**.

Derivatives **18**−**39** (Tables 3 and 4) differ in the nature of the side chain R_3. In the series of compounds **18**−**22** and **34**−**37** the linear aliphatic chain has been gradually extended from five to 15 carbon atoms in order to find the optimal length. In the methoxy series (Table 3), the highest SI is achieved when R_3 has 13 carbon atoms, while in the benzyloxy series (Table 4) 11 carbon atoms seems to be the best length. Shortening or removal of the side chain (compounds **32**, **33**) does not induce a marked reduction of activity, rather a decrease of SI, clearly leading to more cytotoxic derivatives. The introduction of an alicyclic or aromatic appendage in the side chain (**38**, **39**), as well as the conversion of the linear aliphatic side chain into a condensed ring (**24**), is of no benefit, and any further investigation along this line has been abandoned. Finally, insertion of various chemical functionalities such as fluoro, cyano, and hydroxy in the side chain R_3 (**25**−**27**, Table 3) is detrimental for activity and SI. Whenever a carboxy group is present in R_3 (**28**−**31**, Table 3), activity is completely lost.

On the basis of the above considerations, a restricted list of derivatives has been selected for in vivo evaluation on delayed type hypersensitivity reaction (DTH) in mice (Table 5). We addressed our attention to the series of compounds **12**−**16**, containing the highest number of derivatives with SI values considerably better than

UP. Compound **21** has also been included for its good SI that, unfortunately, did not translate into in vivo efficacy.

As discussed in the in vitro type activity, substitution of methoxy with larger alkoxy residues (**12**−**16** series) produces a progressive reduction of both activity and cytotoxicity, leading to more favorable SI. Interestingly, the same trend is maintained in the DTH assay, with **16** having a therapeutic index (toxicity/ED_{50}) almost 3-fold that of UP (4.5 vs 1.7), which makes it the most promising compound as immunosuppressive agent.

Conclusions

A number of totally synthetic derivatives of UP, a potent naturally occurring immunosuppressant with a novel mechanism of action, have been synthesized.

This work was aimed at finding novel compounds having a higher selectivity index (in vitro cytotoxicity/immunosuppressive activity ratio) with respect to that of the reference standard UP.

Modifications at three different sites of the molecule lead to the following SAR considerations:

(1) a nitrogen containing heterocycle R_1, allowing an extensive π-electron conjugation with the rest of the molecule, is crucial for biological activity. Interestingly, the pyrrole present in all natural Ps turns out to be the best.

(2) alkoxy R_2 is also important. Generally larger alkoxy groups lead to more favorable selectivity indexes.

(3) although not essential for immunosuppressive activity, side chain R_3 can be tailored to achieve the best selectivity index. Replacement of the linear aliphatic chain with variously functionalized chains is detrimental for activity.

With respect to in vitro results, a limited number of derivatives has been also tested in vivo on DTH assay. As a result, 4-benzyloxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole (**16**, PNU-156804) has been selected for further characterization as a potential immunosuppressant.[26]

Experimental Section

A. Chemistry. Elemental analyses were performed on Carlo Erba EA1108 or EA1110 instruments and C, H, and N results were within ±0.4% of theoretical values, unless otherwise noted. ^{1}H NMR spectra were recorded on Varian XL-200, XL-400, or XL-500 spectrometers, using the solvent as internal standard; chemical shifts are expressed in ppm (δ). Where not otherwise noted, mass spectra were obtained by fast atom bombardment (FAB) technique on a Finnigan-Mat TSQ 700 (triple quadrupole) instrument. Electrospray ionization (ESI) spectra were run on Finnigan-Mat LCQ while field desorption (FD) spectra were obtained on Varian Variamat 311A. Column chromatographic separations were carried out on 40/60 μm silica gel (Merck) or on 63/200 μm aluminum oxide 90 (Merck, activity II−III). Thin-layer chromatography was performed on Merck silica gel 60 plates coated with a 250 μm layer with fluorescent indicator. Components were visualized by UV light (λ = 254 nm). All experiments dealing with moisture-sensitive compounds were conducted under dry argon. Starting materials, unless otherwise specified, were commercially available, of the best grade, and used without further purification.

General Procedure for the Synthesis of Prodigiosins (Scheme 1). To a solution of 2-formyl-5-alkylpyrrole (16 mmol)[16d] and commercially available 4-alkoxy-3-pyrrolin-2-one (2 equiv) in DMSO (50 mL) was added 2 N sodium hydroxide (45 mL) and the mixture was stirred at 60 °C for 8 h. After dilution with water (200 mL) the suspension was extracted with dichloromethane (600 mL). The organic phase was shaken with water and brine, dried, and evaporated to dryness. The crude material was taken up in hexane and filtered to give the desired 4-alkoxy-5-(5-alkyl-1*H*-pyrrol-2-ylmethylidene)-1,5-dihydropyrrol-2-one. To a solution of the former pyrrolone (2.9 mmol) in dichloromethane (50 mL) at 0−5 °C was added trifluoromethansulfonic anhydride (3.5 mmol) dropwise under argon. After stirring at this temperature for 30 min. the reaction mixture was poured into a 2% aqueous $NaHCO_3$ solution and extracted with ethyl acetate (2 × 50 mL). The collected organic extracts were shaken with brine, dried, and evaporated to dryness. The crude material was chromatographed on silica gel eluting with hexane/ethyl acetate 85:15 to give the desired 2-trifluoromethansulfonyloxy-4-alkoxy-5-[(5-alkyl-2*H*-pyrrol-2-ylidene)methyl]-1*H*-pyrrole.

An oxygen-free solution of the trifluoromethanesulfonate (0.88 mmol) in dioxane (30 mL) was treated in sequence with (1-*tert*-butoxycarbonylpyrrol-2-yl)[27] or (thiophen-2-yl)[16f] or (indol-2-yl)[28] boronic acid (3.5 mmol), potassium carbonate (7 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.044 mmol) and heated to 90 °C with stirring for 4−6 h. After cooling, the reaction mixture was poured into ice−water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was shaken with water and brine, dried, filtered, and evaporated to dryness. The residue was purified over a short alumina column using hexane/ethyl acetate 4:1 as eluant. The collected fractions were concentrated, treated with a solution of hydrochloric acid in diisopropyl ether, and evaporated to dryness at room temperature to yield the desired prodigiosine hydrochlorides. Unoptimized percentage yields, referring to the last coupling step, are given for each product.

4-Methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (1): 73%; ^{1}H NMR (400 MHz, $CDCl_3$) 0.9 (m, 3H, Me), 1.1−1.5 (m, 16H), 1.8 (m, 2H), 2.95 (t, J = 7.5 Hz, 2H), 4.05 (s, 3H, MeO), 6.0 (d, J = 1.8 Hz, 1H), 6.2 (dd, J = 1.8 and 3.9 Hz, 1H), 6.5 (m, 1H), 6.85 (dd, J = 3.9 and 2.6 Hz, 1H), 7.0 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 12.5−12.7 (2 bs, 2H), 12.9 (bs, 1H); MS *m*/*z* 394 $[M + H]^+$. Anal. ($C_{25}H_{35}N_3O$·HCl) C, H, N.

5-Phenyl-3-methoxy-2-[(5-undecyl-2*H*-pyrrol-2-ylidene)-methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (2): 57%; ^{1}H NMR (400 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.8 (m, 18H), 2.76 (m, 2H), 3.93 (s, 3H), 6.04 (d, J = 3.8 Hz, 1H), 6.1 (s, 1H), 6.6 (d, J = 3.8 Hz, 1H), 6.85 (m, 1H), 6.92 (s, 1H), 7.4−8.0 (m, 5H); MS−FD *m*/*z* 404 $[M + H]^+$. Anal. ($C_{27}H_{36}N_2O$·HCl) C, H, N.

5′-Methyl-4-benzyloxy-5-[(5-methyl-2*H*-pyrrol-2-ylidene)-methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (4): 42%; ^{1}H NMR (200 MHz, $CDCl_3$) 2.45 (s, 3H), 2.55 (s, 3H), 5.32 (s, 2H), 5.95 (m, 1H), 6.1 (m, 1H), 6.15 (m, 1H), 6.75 (m, 1H), 6.85 (m, 1H), 6.9 (s, 1H), 7.3−7.5 (m, 5H), 12.45−12.7 (2 bs, 3H); MS *m*/*z* 344 $[M + H]^+$. Anal. ($C_{22}H_{21}N_3O$·HCl) C, H, N.

2-[(1,2,4)Triazol-1-yl)-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole[21] (5): ^{1}H NMR (200 MHz, $CDCl_3$)) 0.9 (m, 3H), 1.2−1.5 (m, 16H), 1.7 (m, 2H), 2.7 (t, J = 7.5 Hz, 2H), 3.95 (s, 3H), 6.05 (m, 2H), 6.65 (m, 1H), 7.0 (m, 1H), 8.1 (s, 1H), 8.95 (s, 1H), 11.0 (bs, 1H); MS *m*/*z* 396 $[M + H]^+$. Anal. ($C_{23}H_{33}N_5O$) C, H, N.

2-(Thiophen-2-yl)-4-benzyloxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (7): 53%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 16H), 1.8 (m, 2H), 3.05 (t, J = 7.6 Hz, 2H), 5.3 (s, 2H), 6.2 (s, 1H), 6.35 (m, 1H), 7.0 (m, 1H), 7.2 (s, 1H), 7.3−7.5 (m, 5H), 7.6 (m, 1H), 9.05 (m, 1H), 13.6 (bs, 1H), 14.2 (bs, 1H); MS *m*/*z* 487 $[M + H]^+$. Anal. ($C_{31}H_{38}N_2OS$·HCl) C, H, N.

2-(1*H*-Indol-2-yl)-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (8): 79%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.87 (m, 3H), 1.1−1.9 (m, 18H), 3.0 (m, 2H), 4.06 (s, 3H), 6.3 (dd, J = 1.7, 4.1 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 6.95 (dd, J = 2.4, 4.1 Hz, 1H), 7.0−7.4 (m, 3H), 7.13 (s, 1H), 7.61 (m, 2H), 12.4, 13.2, 13.3 (3 bs, 3H); MS *m*/*z* 444 $[M + H]^+$. Anal. ($C_{29}H_{37}N_3O$·HCl) C, H, N.

2-(5-Methoxy-1*H*-indol-2-yl)-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (9): 44%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (m, 3H), 1.2−1.5 (m, 16H), 1.8 (m, 2H), 3.0 (m, 2H), 3.9 (s, 3H), 4.05 (s, 3H), 6.3 (m, 2H), 6.9−7.05 (m, 4H), 7.1 (m, 1H), 7.5 (m, 1H), 12.3 (bs, 1H), 13.05−13.15 (m, 2H); MS *m*/*z* 474 $[M + H]^+$. Anal. ($C_{30}H_{39}N_3O_2$·HCl) C, H, N.

2-(5-Chloro-1*H*-indol-2-yl)-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (10): 35%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (m, 3H), 1.2−1.5 (m, 16H), 1.8 (m, 2H), 3.0 (m, 2H), 4.05 (s, 3H), 6.3 (m, 2H), 6.9−7.1 (m, 3H), 7.25 (m, 1H), 7.5 (m, 2H), 12.5,(bs, 1H), 13.1 (bs, 1H), 13.2 (bs, 1H); MS *m*/*z* 478 $[M + H]^+$. Anal. ($C_{29}H_{36}ClN_3O$·HCl) C, H, N.

4-Ethoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (12): 35%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (m, 3H), 1.1−1.9 (m, 21H), 2.95 (m, 2H), 4.25 (q, J = 7.0 Hz, 2H), 6.05 (d, J = 1.8 Hz, 1H), 6.21 (dd, J = 1.5 Hz, J = 4.0 Hz, 1H), 6.37 (m, 1H), 6.86 (dd, J = 4.0 Hz, J = 2.4 Hz, 1H), 6.94 (m, 1H), 7.03 (s, 1H), 7.25 (m, 1H), 12.6−13.0 (2 bs, 3H); MS *m*/*z* 408 $[M + H]^+$. Anal. ($C_{26}H_{37}N_3O$·HCl) C, H, N.

4-Propoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (13): 40%: ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (t, J = 6.6 Hz, 3H), 1.07 (t, J = 7.5 Hz, 3H), 1.1−1.5 (m, 16H), 1.76 (m, 2H), 1.90 (m, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.12 (t, J = 6.5 Hz, 2H), 6.04 (d, J = 1.6 Hz, 1H), 6.19 (dd, J = 4.0, 1.2 Hz, 1H), 6.35 (m, 1H), 6.84 (dd, J = 4.0, 1.9 Hz, 1H), 6.92 (m, 1H), 7.0 (s, 1H), 7.23 (m, 1H), 12.5−13.0 (bs, 3H); MS *m*/*z* 422 $[M + H]^+$. Anal. ($C_{27}H_{39}N_3O$·HCl) C, H, N.

4-Isopropoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (14): 39%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.3 (m, 16H), 1.45 (2s, 6H), 1.7 (m, 2H), 2.9 (m, 2H), 4.6 (m, 1H), 6.0 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m,1H), 6.9 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 7.45 (m, 5H), 12.7 (bs, 2H), 12.8 (bs, 1H); MS *m*/*z* 422 $[M + H]^+$. Anal. ($C_{27}H_{39}N_3O$·HCl) C, H, N.

4-Butoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (15): 54%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (t, J = 6.8 Hz, 3H), 1.0 (t, J = 7.3 Hz, 3H), 1.1−1.6 (m, 18H), 1.6−1.9 (m, 4H), 2.9 (t, J = 7.3 Hz, 2H), 4.15 (t, J = 6.7 Hz, 2H), 6.05 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 12.7 (2 bs, 2H), 12.85 (bs, 1H); MS *m*/*z* 436 $[M + H]^+$. Anal. ($C_{28}H_{41}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (16): 64%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 16H), 1.75 (m, 2H), 2.95 (m, 2H), 5.2 (s, 2H), 6.15 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 7.45 (m, 5H), 12.6−12.8 (2 bs, 2H), 12.95 (bs, 1H); MS *m*/*z* 470 $[M + H]^+$. Anal. ($C_{31}H_{39}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-pentyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (18): 35%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.3−1.5 (m, 4H), 1.8 (m, 2H), 2.95 (t, J = 7.5 Hz, 2H), 4.0 (s, 3H), 6.1 (s, 1H), 6.1 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.9 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 12.7 (bs, 2H), 13.0 (bs, 1H); MS *m*/*z* 310 $[M + H]^+$. Anal. ($C_{19}H_{23}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-heptyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (19): 46%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.25−1.45 (m, 8H), 1.8 (m, 2H), 2.95 (m, 2H), 4.05 (s, 3H), 6.1 (m, 1H), 6.25 (m, 1H), 6.4 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 12.65−12.75 (2 bs, 2H), 12.95 (bs, 1H); MS *m*/*z* 338 $[M + H]^+$. Anal. ($C_{21}H_{27}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-decyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (20): 48%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (t, J = 7.7 Hz, 3H), 1.2−1.5 (m, 14H), 1.75 (m, 2H), 2.95 (t, J = 7.7 Hz, 2H), 4.05 (s, 3H), 6.1 (s, 1H), 6.25 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 12.54−12.62 (2 bs, 2H), 12.93 (bs, 1H); MS *m*/*z* 380 $[M + H]^+$. Anal. ($C_{24}H_{33}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-tridecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (21): 60%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (t, J = 7.7 Hz, 3H), 1.1−1.5 (m, 20H), 1.7 (m, 2H), 2.9 (t, J = 7.7 Hz, 2H), 4.0 (s, 3H), 6.05 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.8 (m, 1H), 6.9 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 12.55−12.7 (bs, 2H), 12.9 (bs, 1H); MS *m*/*z* 422 $[M + H]^+$. Anal. ($C_{27}H_{39}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-pentadecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (22): 52%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 24H), 1.8 (m, 2H), 2.90 (t, J = 7.5 Hz, 2H), 4.05 (s, 3H), 6.1 (s, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.0 (s, 1H), 7.3 (m, 1H), 12.7 (bs, 2H), 12.9 (bs, 1H); MS *m*/*z* 450 $[M + H]^+$. Anal. ($C_{29}H_{43}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(5-phenethyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (23): 62%; ^{1}H NMR (200 MHz, $CDCl_3$) 3.05−3.3 (m, 4H), 4.0 (s, 3H), 6.15 (m, 2H), 6.4 (m, 1H), 6.8 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.1−7.4 (m, 6H), 12.65−12.95 (3 bs, 3H); MS *m*/*z* 344 $[M + H]^+$. Anal. ($C_{22}H_{21}N_3O$·HCl) C, H, N.

4-Methoxy-5-[(4,5,6,7-tetrahydro-2*H*-indol-2-ylidene)-methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (24): 80%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.4−1.9 (m, 4H), 2.5 (t, J = 6.2 Hz, 2H), 3.0 (t, J = 6.1 Hz, 2H), 4.0 (s, 3H), 6.1 (s, 1H), 6.35 (m, 1H), 6.6 (s, 1H), 6.9 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 12.6 (m, 3H); MS *m*/*z* 294 $[M + H]^+$. Anal. ($C_{18}H_{19}N_3O$·HCl) C calcd 65.54, found 64.98; H; N calcd 12.74, found 12.03.

4-Methoxy-5-[[5-(6-fluoro-hex-1-yl)-2*H*-pyrrol-2-ylidene]-methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (25): 35%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (m, 2H), 1.3−1.7 (m, 6H), 2.75 (m, 2H), 4.05 (s, 3H), 4.36 (t, J = 6.1 Hz, 1H), 4.48 (t, J = 6.1 Hz, 1H), 6.4 (m, 1H), 6.45 (m, 1H), 6.8 (s, 1H), 7.25 (s, 1H), 7.5 (m, 3H), 12.4−12.6 (m, 3H); MS *m*/*z* 342 $[M + H]^+$. Anal. ($C_{20}H_{24}FN_3O$·HCl) C, H, N.

4-Methoxy-5-[[5-(7-cyano-hept-1-yl)-2*H*-pyrrol-2-ylidene]-methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (26): 48%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.3−1.9 (m, 10H), 2.35 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 3.45 (s, 3H), 4.05 (s, 3H), 6.1 (m, 1H), 6.2 (m, 1H), 6.4 (m, 1H), 6.85 (m,1H), 6.95 (m, 1H), 7.0 (s, 1H), 7.3 (m, 1H), 12.7 (2 bs, 2H), 12.9 (bs, 1H); MS *m*/*z* 363 $[M + H]^+$. Anal. ($C_{22}H_{26}N_4O$·HCl) C, H, N.

4-Methoxy-5-[[5-(6-hydroxy-hex-1-yl)-2*H*-pyrrol-2-ylidene]methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (27): 35%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.2−1.5 (m, 6H), 1.7 (m, 2H), 2.8 (t, J = 7.7 Hz, 2H), 3.3 (m, 2H), 4.05 (s, 3H), 4.3 (m, 1H), 6.4 (m, 2H), 6.8 (m, 1H), 7.25 (s, 1H), 7.5 (m, 3H), 12.4 (bs, 1H), 12.8 (m, 2H); MS *m*/*z* 340 $[M + H]^+$. Anal. ($C_{20}H_{25}N_3O_2$·HCl) C calcd 64.23, found 64.86; H; N.

4-Methoxy-5-[(5-(5-carboxy-pent-1-yl)-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (28): 36%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.2−2.1 (m, 6H), 2.35 (t, J = 7.5 Hz, 2H), 2.95 (t, J = 7.5 Hz, 2H), 4.05 (s, 3H), 6.1 (s, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.9 (m, 1H), 7.0 (s, 1H), 7.25 (s, 1H), 12.5−12.9 (m, 3H); MS *m*/*z* 354 $[M + H]^+$. Anal. ($C_{20}H_{23}N_3O_3$·HCl) C calcd 61.61, found 60.54; H; N calcd 10.77, found 9.01.

4-Methoxy-5-[(5-(5-carboxy-pent-1-yl)-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, methyl ester, hydrochloride (29): 32%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.2−1.9 (m, 6H), 2.35 (t, J = 7.5 Hz, 2H), 2.95 (t, J = 7.5 Hz, 2H), 3.65 (s, 3H), 4.05 (s, 3H), 6.1 (m, 1H), 6.2 (m, 1H), 6.4 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 12.6−12.8 (m, 3H); MS *m*/*z* 368 $[M + H]^+$. Anal. ($C_{21}H_{25}N_3O_3$·HCl) C, H, N.

4-Methoxy-5-[[5-(5-morpholinecarboamide-pent-1-yl)-2*H*-pyrrol-2-ylidene]methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (30): 38%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.1−1.8 (m, 6H), 2.3 (t, J = 7.3 Hz, 2H), 2.7 (t, J = 7.4 Hz, 2H), 3.2−3.6 (m, 8H), 4.0 (s, 3H), 6.4 (m, 2H), 6.8 (s, 1H), 7.2 (s, 1H), 7.5 (m, 3H), 12.2−12.8 (2 bs, 3H); MS *m*/*z* 423 $[M + H]^+$. Anal. ($C_{24}H_{30}N_4O_3$·HCl) C, H, N.

4-Methoxy-5-[[5-(10-carboxy-dec-1-yl)-2*H*-pyrrol-2-ylidene]methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (31): 30%; ^{1}H NMR (200 MHz, $CDCl_3$) 1.2−1.5 (m, 12H), 1.5−1.9 (2m,

4H), 2.3 (t, $J = 7.4$ Hz, 2H), 2.9 (t, $J = 7.5$ Hz, 2H), 4.0 (s, 3H), 6.1 (s, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.0 (s, 1H), 7.25 (m, 1H), 12.7 (bs, 2H), 12.9 (m, 1H); MS *m/z* 424 $[M + H]^+$. Anal. ($C_{25}H_{33}N_3O_3$·HCl) C; H calcd 7.45, found 8.01; N.

4-Benzyloxy-5-[(2H-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (32): 33%; ^{1}H NMR (200 MHz, $CDCl_3$) 5.25 (s, 2H), 6.15 (m, 1H), 6.4 (m, 2H), 6.85 (m, 1H), 7.0 (m, 1H), 7.15 (s, 1H), 7.3 (m, 1H), 7.4−7.6 (m, 5H), 7.5 (m, 1H), 12.7−12.95 (three bs, 3H); MS *m/z* 316 $[M + H]^+$. Anal. ($C_{20}H_{17}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-methyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (33): 38%; ^{1}H NMR (400 MHz, $CDCl_3$) 2.56 (s, 3H), 5.22 (s, 2H), 6.15 (d, $J = 1.7$ Hz, 1H), 6.2 (dd, $J = 1.8$ and 3.9 Hz, 1H), 6.35 (m, 1H), 6.85 (dd, $J = 3.9$ and 2.4 Hz, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 7.45 (m, 5H), 12.65 (bs, 1H), 12.8 (bs, 1H), 12.85 (bs, 1H); MS *m/z* 330 $[M + H]^+$. Anal. ($C_{21}H_{19}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-pentyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (34): 55%; ^{1}H NMR (400 MHz, $CDCl_3$) 0.9 (m, 3H), 1.38 (m, 4H), 1.78 (m, 2H), 2.95 (t, $J = 7.6$ Hz, 2H), 5.22 (s, 2H), 6.15 (d, $J = 1.8$ Hz, 1H), 6.2 (dd,, $J = 1.8$ and 4.0 Hz, 1H), 6.36 (m, 1H), 6.84 (dd, $J = 4.0$ and 3.0 Hz, 1H), 6.94 (m, 1H), 7.06 (s, 1H), 7.24 (m, 1H), 7.44 (m, 5H), 12.68 (bs, 1H), 12.75 (bs, 1H), 12.95 (bs, 1H); MS *m/z* 386 $[M + H]^+$. Anal. ($C_{25}H_{27}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-heptyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (35): 36%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (m, 3H), 1.2−1.4 (m, 8H), 1.75 (m, 2H), 2.9 (t, $J = 7.7$ Hz, 2H), 5.2 (s, 2H), 6.1 (m, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.8 (m, 1H), 6.9 (m, 1H), 7.05 (s, 1H), 7.2 (m, 1H), 7.4 (m, 5H), 12.6−12.75 (2 bs, 2H), 12.9 (bs, 1H); MS *m/z* 414 $[M + H]^+$. Anal. ($C_{27}H_{31}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-decyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (36): 41%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 14H), 1.8 (m, 2H), 2.95 (m, 2H), 5.2 (s, 2H), 6.15 (s, 1H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.9 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 7.45 (m, 5H), 12.6−12.8 (2 bs, 2H), 12.95 (bs, 1H); MS *m/z* 456 $[M + H]^+$. Anal. ($C_{30}H_{37}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-tridecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (37): 41%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 20H), 1.75 (m, 2H), 2.95 (m, 2H), 5.2 (s, 2H), 6.1 (m, 1H), 6.25 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.05 (s, 1H), 7.25 (m, 1H), 7.45 (m, 5H), 12.6−12.8 (2 bs, 2H), 12.95 (bs, 1H); MS *m/z* 498 $[M + H]^+$. Anal. ($C_{33}H_{43}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-cyclohexylmethyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (38): 36%; ^{1}H NMR (200 MHz, $CDCl_3$) 0.9−1.95 (m, 11H), 2.8 (m, 2H), 5.2 (s, 2H), 6.2 (m, 1H), 6.35 (m, 1H), 6.85 (m, 1H), 6.9 (m, 1H), 7.05 (s, 1H), 7.2 (m, 1H), 7.45 (m, 5H), 12.65−12.8 (2 bs, 2H), 12.95 (bs, 1H); MS *m/z* 412 $[M + H]^+$. Anal. ($C_{27}H_{29}N_3O$·HCl) C, H, N.

4-Benzyloxy-5-[(5-benzyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, hydrochloride (39): 36%; ^{1}H NMR (200 MHz, $CDCl_3$) 4.3 (s, 2H), 5.2 (s, 2H), 6.1 (m, 1H), 6.2 (m, 2H), 6.35 (m, 1H), 6.75 (m, 1H), 6.95 (m, 1H), 7.0 (s, 1H), 7.15−7.45 (m, 11H), 12.75 (bs, 1H), 12.95 (m, 2H); MS *m/z* 406 $[M + H]^+$. Anal. ($C_{27}H_{23}N_3O$·HCl) C, H, N.

Direct Modification of Prodigiosins (Scheme 2). 5′-Acetyl-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)-methyl]-2,2′-bi-1*H*-pyrrole, Hydrochloride (6). To a solution of prodigiosin **1** (250 mg, 0.58 mmol) in dichloromethane (50 mL), acetic anhydride (0.11 mL, 1.16 mmol), and then $AlCl_3$ (330 mg, 2.46 mmol, in two portions) were added at room temperature, and the reaction mixture was stirred for 1 h. HCl (200 mL, 0.5 N)/ice and ethyl acetate (500 mL) were added, the mixture was filtered through Celite, and the organic layer was separated, washed with brine (3 × 50 mL), and dried. After removal of the solvent the residue (345 mg) was chromatographed over alumina with *n*-hexane/ethyl acetate 4:1 as eluant to yield a red solid (156 mg). The solid was dissolved in ethyl acetate (25 mL), 1.5 N HCl in diisopropyl ether (1 mL) was added, and the solution was concentrated to dryness at room temperature. The residue was stirred with *n*-pentane (15 mL), filtered, and washed with *n*-pentane to yield **6** as a red solid (163 mg, 0.35 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.9 (m, 3H), 1.2−1.5 (m, 16H), 1.8 (m, 2H), 2.65 (s, 3H), 3.0 (m, 2H), 4.0 (s, 3H), 6.15 (s, 1H), 6.3 (m, 1H), 6.8−6.95 (m, 3H), 7.15 (s, 1H), 12.9 (bs, 1H), 13.1 (bs, 1H), 13.5 (bs, 1H); MS *m/z* 436 $[M + H]^+$. Anal. ($C_{27}H_{37}N_3O_2$·HCl) C, H, N.

4-Hydroxy-5-[(5-undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, Hydrochloride (17). To a solution of benzyloxy prodigiosin **16** (200 mg, 0.395 mmol) in MeOH (100 mL) was added 5% Pd on carbon (80 mg) and the mixture was hydrogenated in a Parr apparatus for 6 h at room pressure and temperature. After filtration and solvent removal the crude product was flash-chromatographed on silica gel (eluant, *n*-hexane/ethyl acetate 3:2). The collected fractions were concentrated, treated with a solution of hydrochloric acid in diisopropyl ether, and evaporated to dryness at room temperature to yield the desired product **17** as a red solid (138 mg, 0.33 mmol, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) 0.90 (t, $J = 6.8$ Hz, 3H), 1.1−1.5 (m, 16H), 1.74 (m, 2H), 2.71 (t, $J = 7.8$ Hz, 2H), 4.9 (bs, 1H), 5.73 (s, 1H), 6.11 (m, $J = 2.7$ Hz, 1H), 6.38 (m, $J = 3.0$ Hz, 1H), 6.52 (t, $J = 2.6$ Hz, 1H), 6.58 (s, 1H), 6.73 (m, 1H), 7.04 (m, 1H), 9.2 (bs, 1H), 14.2 (bs, 1H); MS-ESI (+) *m/z* 380 $[M + H]^+$. Anal. ($C_{24}H_{33}N_3O$·HCl) C calcd 75.95, found 75.45; H; N calcd 11.07, found 10.28.

Compounds 3 and 11. *N*-Ethyl-4-methoxy-5-[(5-undecyl-2*H*-pyrrol-2-yliden)methyl]-2,2′-bi-1*H*-pyrrole-2-carboxamide (3). A solution of 4-methoxy-2-(*N*-ethyl)carboxamidopyrrole (300 mg, 1.8 mmol) and 5-undecyl-2-formylpyrrole (492 mg, 1.98 mmol) in ethyl acetate (30 mL) was cooled to 15 °C. HCl (3.6 N) in diisopropyl ether (1.2 mL) was added and the dark red solution stirred for 30 min. The reaction mixture was poured into ice−water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The organic phase was shaken with $NaHCO_3$ solution, water, and brine, dried, filtered, and evaporated to dryness. The crude material was crystallized from diisopropyl ether/ethyl acetate to give **3** (150 mg, 0.37 mmol, 21%). ^{1}H NMR (200 MHz, $CDCl_3$) 0.9 (m, 3H), 1.15 (t, $J = 7.3$ Hz, 3H), 1.2−1.4 (m, 16H), 1.6 (m, 2H), 2.55 (t, $J = 7.5$ Hz, 2H), 3.4 (m, 2H), 3.9 (s, 3H), 6.05 (d, $J = 3.8$ Hz, 1H), 6.2 (s, 1H), 6.7 (d, $J = 3.8$ Hz, 1H), 7.1 (s, 1H), 7.4 (bs, 1H); MS *m/z* 400 $[M + H]^+$. Anal. ($C_{24}H_{37}N_3O_2$) C, H, N.

5-[(5-Undecyl-2*H*-pyrrol-2-ylidene)methyl]-2,2′-bi-1*H*-pyrrole, Hydrochloride (11). A solution of 2,2′-bipyrrole-5-carboxaldehyde (80 mg, 0.5 mmol) and 2-undecylpyrrole (132 mg, 0.6 mmol) in ethanol (10 mL) was cooled to 15 °C, treated with 37% HCl (0.25 mL), and stirred for 45 min. The reaction mixture was poured into ice−water (100 mL) and extracted with methylene chloride (3 × 30 mL). The organic phase was shaken with water and brine, dried, filtered, and evaporated to dryness. The crude material was washed with hexane to give **11** (138 mg, 0.38 mmol, 75%).^{1}H NMR (200 MHz, $CDCl_3$) 0.85 (t, $J = 6.6$ Hz, 3H), 1.1−1.5 (m, 16H), 1.77 (m, 2H), 2.98 (t, $J = 7.6$ Hz, 2H), 6.28 (dd, $J = 4.1$, 1.7 Hz, 1H), 6.35 (m, 1H), 6.81 (dd, $J = 4.7$, 1.8 Hz, 1H), 6.90 (s, 1H), 6.97 (m, 2H), 7.11(dd, $J = 4.7$, 2.2 Hz, 1H), 7.23 (m, 1H), 12.56 (bs, 1H), 13.16 (bs, 1H), 13.4 (bs, 1H); MS *m/z* 364 $[M + H]^+$. Anal. ($C_{24}H_{33}N_3$·HCl) C, H, N.

B. Biology. For the in vitro assays, stock solutions of the tested compounds at 2 mg/mL were prepared in DMSO and then diluted at the moment of use in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 10% inactivated fetal calf serum (complete medium). For DTH assay the tested compounds were administered dissolved in Cremophor ELP/ethanol (6.5:3.5 v:v).

Proliferation of Murine Splenocytes Induced by the Mitogen Concanavaline A. Spleens were aseptically removed from C57B1/6 mice and a cell suspension prepared in complete medium. Cell viability was evaluated by trypan blue exclusion.

Splenocytes were seeded in triplicate in 96-well tissue culture plates (Nunclon) at 4×10^5 cells/well in the presence or absence of the stimulus (Con A 1.7 μg/mL) and of different concentrations of the test compound. Cells were harvested after 72 h of incubation (37 °C, 5% CO_2) with [^{3}H]thymidine and pulsed (Amersham, 0.2 μCi/well) during the last 18 h, and the [^{3}H]thymidine uptake was quantified in a Packard liquid scintillation counter (Top Count) as an index of DNA proliferation. The percentage of inhibition of cell proliferation was calculated by the formula

$$\% \text{ inhibition} = [(\text{cpm control} - \text{cpm drug})/\text{cpm control}] \times 100$$

The activity is expressed as IC_{50} (concentration able to inhibit lymphocyte proliferation by 50%).

Drug Cytotoxicity Evaluation. To test drug cytotoxicity murine resting lymphocytes (4×10^5cells/well), murine B16 melanoma cells (1×10^5 cells/well) and human K562 erytroleukemia cells (1×10^5 cells/well) were seeded in triplicate in 96-well tissue culture plates in the presence or absence of a range of concentrations of the test compound, and cell viability was assessed after 48 h culture with the MTT assay.[29]

Briefly, 20 μL/well of a MTT solution (0.5% in PBS) was added to the cultures for the last 4 h of incubation. Subsequently, medium was removed by aspiration and 200 μL/well of 0.04 N HCl−2-propanol was added to dissolve the MTT formazan produced. After agitation, plates were placed in a microplate reader (Lambda Reader, Perkin-Elmer) and the absorption (OD) was measured at a wavelength of 570 nm and a reference wavelength of 690 nm.

The inhibition of cell viability was calculated by the formula

$$\% \text{ inhibition} = [(\text{OD control wells} - \text{OD treated wells})/\text{OD control}] \times 100$$

The cytotoxicity is expressed as IC_{50} (concentration able to inhibit cell viability by 50%).

Comparative in Vivo Activity Evaluation by DTH Assay. According to the test, sheep red blood cells (SRBC) (1×10^5 cells) suspended in 500 μL of saline, were injected iv into the tail vein of female C57 Bl/6 mice (8−9 weeks old). Five days later 1×10^8 SRBC suspended in 50 μL saline were injected into the left hind footpad. The increase in footpad thickness was measured with a dial micrometer 24 h after challenge. The test compounds were given daily by ip route for 6 days at different doses starting on the day of priming. Activity is expressed as ED_{50} (dose able to reduce by 50% the thickness increase compared to controls). Toxicity is expressed as minimal daily dose causing toxicity symptoms (weight loss, hair ruffling, occasionally death).

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Supporting Information Available: Elemental analyses for **1**−**39**. This material is available free of charge via the Internet at http://pubs.acs.org.

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