Synthesis of Pyrrolostatin and Its Analogues

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Pyrroles are not only important building blocks in nature such as in hemes, chlorophyll, and various porphyrinoids,[1] but also commonly found as various kinds of derivatives in living cells to show biological activity.[2] Thus, there have been many synthetic studies on pyrrole derivatives. In 1993, Kato et al. isolated a novel lipid peroxidation inhibitor, pyrrolostatin (**1a**), from *Streptomyces chrestomyceticus*, which consists of a pyrrole-2-carboxylic acid with a geranyl group at the 4-position (Figure 1).[3] This novel pyrrole has in vitro inhibitory activity against lipid peroxidation, and its activity is comparable to that of vitamin E (α-tocopherol), a well-known antioxidant. As generation of free radicals has been suggested to play a major role in the progression of a wide range of pathological disturbances, there has been an explosion of research into free-radical-induced peroxidation of lipoprotein and its prevention by antioxidants.[4] The antioxidation activity of pyrrolostatin decreases dose-dependently when Fe^{2+} is added to the reaction mixture as a promoter of lipid peroxidation. Thus, the action mechanism is suggested as decreasing the concentration of free Fe^{2+} which catalyzes the decomposition of hydroperoxides to hydroxy and alkoxy radicals by chelation with Fe^{2+}. This indicates that pyrrolostatin (**1a**) would also prevent the Fenton reaction which is thought to be a certain and fatal pathway to produce a brutal hydroxyl radical from hydrogen peroxide in neuronal cells when hypoximia occurs in the event of ischemia attack.[5] Since we have been continuously interested in neuronal cell-protecting compounds, we have selected pyrrolostatin (**1a**) and its analogues **1b** and **1c** (Figure 1) as synthetic targets.

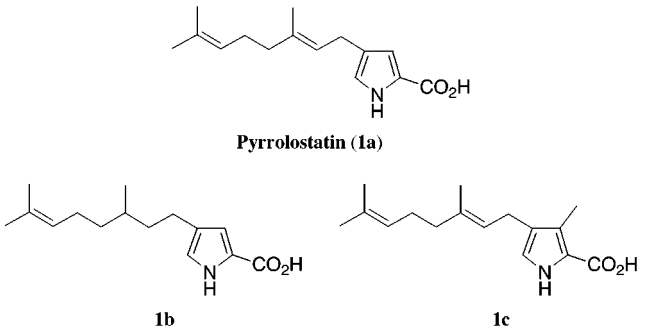

Figure 1. Structure of pyrrolostatin and its analogues.

Results and Discussion

Although there are many methods for pyrrole synthesis,[6] preparation of α-unsubstituted pyrroles such as pyrrolostatin (**1a**) and its analogues is not always simple. A recent method using isocyanoacetates and nitroalkenes or their equivalents (Barton−Zard reaction) is useful to prepare 5-unsubstituted pyrrole-2-carboxylates.[7] The substituents at the 3- and 4-positions are variously changed by choosing the starting nitroalkenes or β-nitroacetates.[8] For the synthesis of pyrrolostatin (**1a**) and its analogues **1b** and **1c**, β-nitroacetates **10a**−**c** are required (Scheme 1). They were prepared from readily available geraniol or citronellal as shown in Scheme 1. Homogeraniol **4** was prepared from geraniol by the reported procedure,[9] and **4** was converted to the corresponding iodide **5** in 90% yield by the reaction of the mesylate from **4** with sodium iodide. The iodide **5** was nitrated with $NaNO_2$ in DMSO in the presence of urea and phloroglucinol in 66% yield.[10] Nitro compound **7** was easily prepared from (±)-citronellal, namely, by treatment of citronellal with CH_3NO_2 and triethylamine followed by H_2SO_4, Ac_2O, and then $NaBH_4$ in DMSO, giving the nitro compound **7** in 60% yield.[11] The nitro compounds **6** and **7** were treated with formaldehyde in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) or Et_3N to afford β-nitro alcohols **8a** and **8b** in respective yields of 68% and 64%, and bishydroxymethylated byproducts. The reaction of **8** with acetaldehyde in the presence of Amberlyst A-21 gave the desired β-nitro alcohol **8c** as a sole product in 57% yield.[12] The β-nitro alcohols **8a**−**c** were quantitatively transformed to the key β-nitro acetates **10a**−**c** by acetylation with Ac_2O and pyridine (Scheme 2). As these compounds were rather labile under both acidic and basic conditions, they were used in the next step without purification.

β-Nitro acetates **10** were treated with ethyl isocyanoacetate in THF in the presence of DBU to give pyrrole-2-carboxylates (Table 2). Although the Barton−Zard reaction normally gives pyrrole-2-carboxylates in more than 60% yield,[5,6] ethyl pyrrole-2-carboxylate **11a** was

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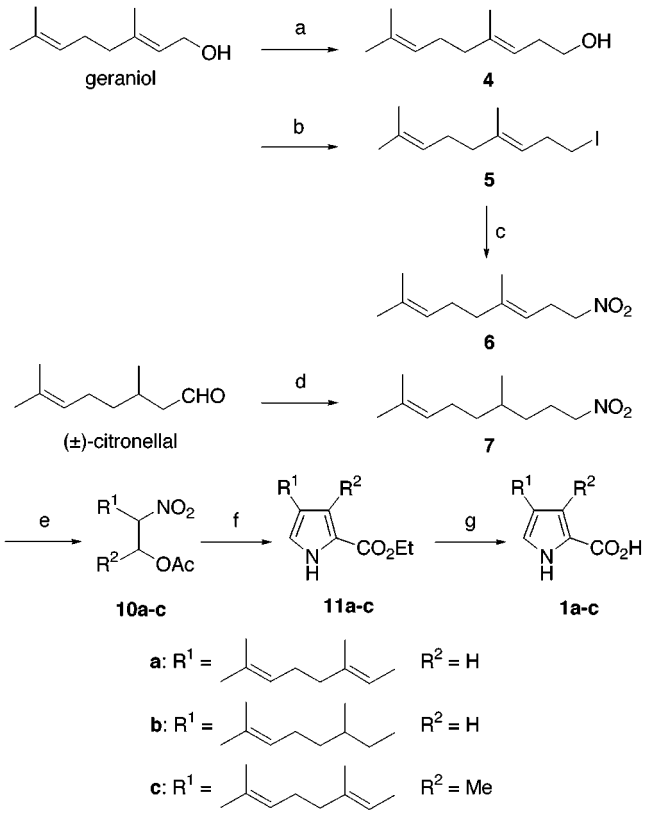
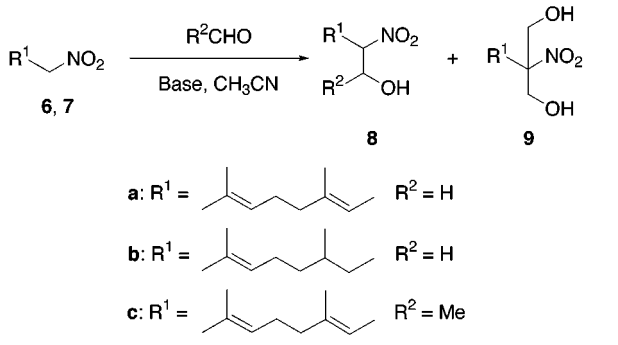
Scheme 1. Synthesis of Pyrolostatin (1a) and Its Analogues[a]



[a] Reagents and conditions: (a) $(COCl)_2$, DMSO, CH_2Cl_2; $CH_2{=}PPh_3$, THF; $(Sia)_2BH$; H_2O_2, NaOH, THF, (ref 9); (b) MsCl, Et_3N; NaI, acetone, 90%; (c) $NaNO_2$, urea, phlorogrucinol, DMSO, 66%; (d) CH_3NO_2, Et_3N; Ac_2O, H_2SO_4; $NaBH_4$, DMSO, 60%; (e) HCHO or CH_3CHO, base; Ac_2O, pyridine; (f) $CNCH_2CO_2Et$, base; (g) $LiOH{\cdot}H_2$, aqueous dioxane.

Table 1. Preparation of β-Nitro Alcohols



run	substrate	R^2CHO	base	products (yield, %)
1	**6**	HCHO	Et_3N	**8a** (68), **9a** (5)
2	**7**	HCHO	DBU	**8b** (64), **9b** (12)
3	**6**	CH_3CHO	A-21[a]	**8c** (57)

[a] Amberlyst A-21 was used.

obtained in only 3% yield under the standard conditions used in the previous papers using DBU in THF.[8] Despite our various efforts including the use of a strong nonionic base such as (*tert*-butylimino)tri(pyrrolidino)phosphorane (BTPP) instead of DBU (run 4), the yield was only improved to 18% (run 5). On the other hand, **11b** and **11c** were obtained in good yields under the standard conditions. Final conversion to **1a**−**c** was accomplished by hydrolysis with $LiOH{\cdot}H_2O$ in aqueous dioxane. Pyrrolostatin (**1a**) was obtained as a pale yellow crystal in 73% yield. The structure of synthesized pyrrolostatin (**1a**) was confirmed by the comparison with the reported spectral data.[3]

Scheme 2. Mechanism of Pyrrole Cyclization

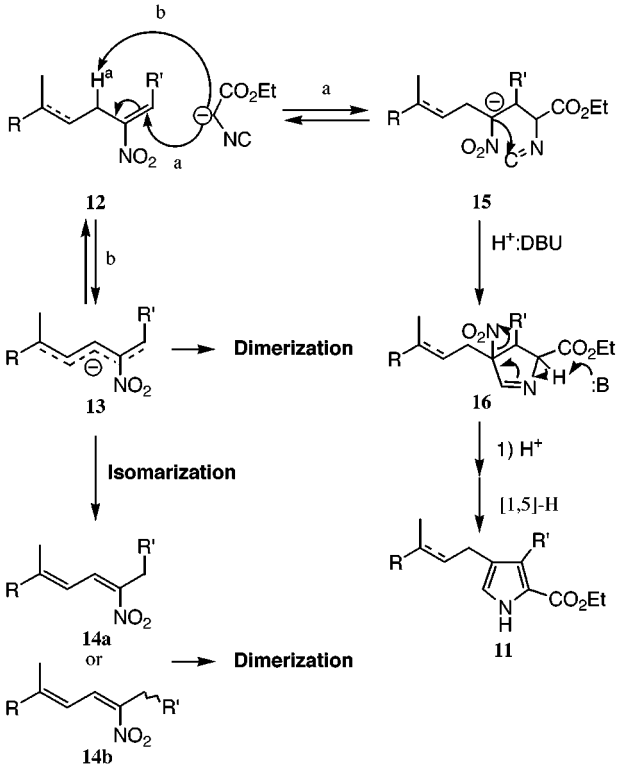


Table 2. Preparation of Pyrrole-2-carboxylates 11a−c from β-Nitro Acetates 10a−c

run	base	solvent	time, h	temp, °C	product (yield, %)
1	DBU	THF	24	rt	**11a** (3)
2	DBU	THF	18	0	**11a** (6)
3	DBU	THF	18	−78	**11a** (10)
4	BTPP[a]	THF	18	0	**11a** (8)
5	DBU	THF/tBuOH	26	rt	**11a** (18)
6	DBU	THF	18	rt	**11b** (61)
7	DBU	THF	27	rt	**11c** (60)

[a] BTPP = (*tert*-butylimino)tri(pyrrolidino)phosphorane.

The pyrrole formation by Barton−Zard reaction is summarized in Scheme 2. When substituents of nitroalkenes are simple alkyl groups, this reaction gives the desired pyrroles in good yield as in the case of **10b**. However, the reaction is complicated in the case of **10a**. In the case of **10a** and **10c**, pentadienyl anions **13** would be formed by treatment with a base, and they may give conjugated nitroalkenes **14** which would be highly reactive toward the Diels−Alder or the Michael reaction to give the dimeric products. Thus, the yield of **11a** is poor due to the side reactions. In the case of **10c**, the terminal methyl group would disfavor the isomerization process leading to **12** by the steric effect. Moreover, the rate-determining cyclization step from **15** to **16** would be facilitated by the presence of the methyl group to give **11c** in better yield than **11a**.

Various kinds of radical inhibitors have been extensively studied so far.[4] Among them, vitamin E's (α-, β-, γ-, and δ-tocopherol) are the most active inhibitors for the autoxidation of lipids. It is interesting that pyrrolostatin **1a** exhibits inhibitory activity against lipid

peroxidation similar to that of vitamin E. Although synthesis of **1a** is rather difficult, its analogues **1b** and **1c** are more easily prepared. So biological activity including the antioxidation ability of **1b** and **1c** is of interest for study. Preliminary experiment shows that rate constants for hydrogen abstraction of **1a**, **1b**, and **1c** by phenoxy radical are 2×10^{-1}, 4×10^{-2}, and 3×10^{-2} M^{-1} s^{-1}, respectively. These values are much smaller than the rate of hydrogen abstraction of vitamin E by phenoxy radicals.[4] The mechanism of antioxidation of **1a** may be different from that of vitamin E.

Experimental Section

General Procedures. Dichloromethane and DMSO were distilled from CaH_2 under an inert atmosphere. *N*,*N*-Dimethylformamide was stirred with NaOH for several hours and distilled from CaH_2 under a reduced pressure. Tetrahydrofuran was distilled from sodium benzophenone ketyl under an inert atmosphere. Acetone was distilled from Drierite under an inert atmosphere. Chloroform was washed with water to remove EtOH, dried with Na_2SO_4, and distilled from CaH_2 under an inert atmosphere. Other commercially available materials were used without further purification. Ethyl isocyanoacetate was prepared from ethyl *N*-formylglycinate using $POCl_3$ and triethylamine.[13]

Homogeraniol (**4**) was prepared from geraniol according to the literature procedure as a yellow liquid: bp 174−176 °C (0.1 Torr) (lit.[9] bp 150 °C (0.02 Torr)).

(*E*)-9-Iodo-2,6-dimethylnona-2,6-diene (5). To a stirred solution of homogeraniol (**4**; 7.25 g, 43.26 mmol) and Et_3N (6.18 mL, 44.3 mmol) in anhydrous $CHCl_3$ (150 mL) was added dropwise MsCl (2.63 mL, 44.3 mmol) at 0 °C. After 1 h, water (100 mL) and hexane (200 mL) were added. The organic layer was separated, and the aqueous layer was extracted with hexane (3 × 50 mL). The combined organic layer was washed with water (2 × 50 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated to give 7.08 g of crude (*E*)-4,8-dimethylnona-3,7-dienyl methanesulfonate as a yellow liquid.

To a solution of the crude mesylate (7.08 g) in anhydrous acetone (150 mL) was added NaI (17.69 g, 118 mmol) under a nitrogen atmosphere. After the suspension was refluxed for 1 h, the mixture was cooled to room temperature, and then water and hexane were added. The organic layer was separated, and the aqueous layer was extracted with hexane (100 mL). The combined organic layer was washed with water (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. Hexane (100 mL) was added to the residue, and the mixture was filtered through a Celite pad, which was thoroughly washed with hexane (2 × 100 mL). Removal of the hexane by a rotary evaporator gave 7.27 g of crude **5** in 90% yield: yellow liquid; ^{1}H NMR δ 1.60 (s, 3H), 1.61 (s, 3H), 1.63 (s, 3H), 2.05 (m, 4H), 2.58 (m, 2H) 3.10 (m, 2H), and 5.10 (m, 2H); EI/MS 278 (M^+, 6) and 109 (40). This material was used in the next step without further purification.

(*E*)-2,6-Dimethyl-9-nitronona-2,6-diene (6). To a stirred solution of $NaNO_2$ (3.8 g, 44.43 mmol), phulorogrucinol (3.48 g, 27.99 mmol), and urea (3.48 g) in anhydrous DMSO (22 mL) was added the iodide **5** (7.27 g, 26.13 mmol) with cooling by a water bath. After 15 h, water (30 mL) and hexane (30 mL) were added. The organic layer was separated, and the aqueous layer was extracted with hexane (3 × 100 mL). The combined organic layer was washed with water (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (5% EtOAc/hexane). The title compound **6** was obtained from the first fraction (3.39 g, 66%), and homogeraniol **4** was obtained in 31% yield from the second fraction. Data for **6**: colorless oil; IR (NaCl) 1552 and 1455 cm^{-1}; ^{1}H NMR δ 1.65 (s, 3H), 1.70 (s, 3H), 1.73 (s, 3H), 2.09 (m, 4H) 2.76 (m, 2H), 4.39 (m, 2H), and 5.12 (m, 2H); ^{13}C NMR δ 16.0, 17.6, 25.6, 26.3, 31.8, 39.5, 75.1, 117.1, 123.6, 131.6, and 140.3; EI/MS 197 (M^+, 17) and 109 (100); HRMS calcd for M^+ ($C_{11}H_{19}NO_2$) 197.1416, found 197.1405.

2,6-Dimethyl-9-nitronon-2-ene (7). To a solution of triethylamine (2.1 mL, 15 mmol) in nitromethane (18.5 mL, 300 mmol) was added (±)-citronellal (23.1 g, 150 mmol) with cooling by a water bath, and the mixture was stirred for 1 day. Saturated aqueous NH_4Cl (20 mL) and diethyl ether (20 mL) were added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The combined etheral layer was washed with water (2 × 20 mL) and saturated brine (50 mL) and dried with Na_2SO_4. After evaporation of the solvent, the residue was dissolved in a mixture of acetic anhydride (150 mL) and pyridine (14.6 mL, 180 mmol). After 2.5 h, saturated aqueous $NaHCO_3$ (20 mL) and ethyl acetate (20 mL) were carefully added. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was dissolved in DMSO (150 mL), and $NaBH_4$ (14.6 mL, 180 mmol) was added. After 2.5 h, saturated aqueous $NaHCO_3$ (100 mL) and diethyl ether (100 mL) were added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 × 80 mL). The combined organic layer was washed with water (2 × 100 mL) and brine (100 mL), dried with Na_2SO_4, and concentrated to give the crude nitro compound **7**. The crude **7** was distilled (80 °C, 1 Torr) to give 12.46 g (42% from citronellole) as a pale yellow liquid: IR (NaCl) 1554 and 1438 cm^{-1}; ^{1}H NMR δ 0.91 (d, 3H, J = 6.8 Hz), 1.19 (m, 2H), 1.33 (m, 2H), 1.44 (m, 1H), 1.60 (s, 3H) 1.68 (s, 3H), 1.99 (m, 4H), 4.36 (t, 2H, J = 7.1), and 5.07 (m, 1H); ^{13}C NMR δ 17.7, 19.3, 25.1, 31.9, 33.3, 36.7, 76.0, 124.5, and 131.4; MS 199 (M^+, 12) and 109 (100); HRMS calcd for M^+ ($C_{11}H_{21}NO_2$) 199.1572, found 199.1561.

(*E*)-5,9-Dimethyl-2-nitrodeca-4,8-dien-1-ol (8a). To a stirred solution of nitro compound **6** (3.39 g, 17.18 mmol) in acetonitrile (7 mL) were added triethylamine (0.24 mL, 1.72 mmol) and 37% HCHO (1.40 g, 17.2 mmol) in acetonitrile (10 mL) at 0 °C. After 1 h, saturated aqueous NH_4Cl (50 mL) and hexane (50 mL) were added. The organic layer was separated, and the aqueous layer was extracted with hexane (2 × 30 mL). The combined organic layer was washed with water and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (10% EtOAc/hexane) to give **8a** (0.21 g, 68%) and (*E*)-2-(hydroxymethyl)-5,9-dimethyl-2-nitrodeca-4,8-dien-1-ol (**9a**) (0.21 g, 5%). The starting nitro compound **6** was recovered from the first fraction (0.52 g, 15%). Data for **8a**: colorless oil; IR (NaCl) 3396, 1558, 1456, and 1377 cm^{-1}; ^{1}H NMR δ 1.60 (s, 3H), 1.63 (s, 3H), 1.69 (s, 3H), 2.05 (m, 5H), 2.56 (m, 2H), 2.71 (m, 2H), 3.92 and 4.04 (ddd and m, 2H, J = 12.69, 2.93 and 0.34), 4.56 (ddd, 1H, J = 14.64, 7.33 and 4.04), and 5.05 (m, 2H); ^{13}C NMR δ 16.1, 17.7, 25.7, 26.3, 28.8, 39.6, 62.6, 88.7, 116.4, 123.6, 131.9, and 141.0; EI/MS 227 (M^+, 0.5) and 109 (100). Anal. Calcd for $C_{13}H_{21}NO_2 \cdot (1/2)H_2O$: C, 63.13. H, 9.71. N, 6.12. Found: C, 62.95; H, 9.38; N, 6.00. Data for **9a**: colorless oil; IR (NaCl) 3318, 1540, 1074, and 1051 cm^{-1}; ^{1}H NMR δ 1.60 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 2.09 (m, 4H), 2.43 (t, 2H, J = 6.83 Hz), 2.62 (d, 2H, J = 7.81), 3.99 (dd, 2H, J = 12.7 and 6.35), 4.19 (dd, 2H, J = 12.2 and 7.3), and 5.02 (m, 2H); ^{13}C NMR δ 16.1, 17.7, 25.7, 26.3, 28.8, 39.6, 62.6, 88.7, 116.4, 123.6, 131.9, and 141.0; EI/MS 257 (M^+, 0.5) and 109 (100). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.44; H, 9.10; N, 5.30.

(2*R,5*R**)- and (2*R**,5*S**)-5,9-Dimethyl-2-nitrodec-8-en-1-ol (8b) and (*E*)-2-(hydroxymethyl)-5,9-dimethyl-2-nitrodeca-8-en-1-ol (9b)** were obtained in 64% and 12% yields, respectively, from the reaction of **6** and 37% HCHO. Data for **8b**: colorless oil; IR (NaCl) 3432, 1554, 1468, and 1378 cm^{-1}; ^{1}H NMR δ 0.89 (d, 3H, J = 4.0), 1.18 (m, 2H), 1.26 (m, 2H), 1.36 (m, 1H), 1.61 (m, 3H), 1.69 (m, 3H), 1.96 (m, 4H). 2.12 (t, 1H, J = 6.1), 3.93 and 4.02 (m, 2H), 4.57 (m, 1H), and 5.07 (t, 1H, J = 8.2); ^{13}C NMR δ 17.6, 19.1, 19.2, 25.3, 63.1, 89.7, 124.4, and 131.5; EI/MS 229 (M^+, 14) and 109 (100); HRMS calcd for M^+ ($C_{11}H_{21}NO_2$) 229.1678, found 229.1665. Data for **9b**: colorless oil; IR (NaCl) 3307, 1535, 1455, and 1355 cm^{-1}; ^{1}H NMR δ 0.87 (d, 3H, J = 6.7), 1.22 (m, 5H), 1.59 (s, 3H), 1.68 (s, 3H), 1.90 (m, 4H), 2.65 (br s, 2H), 3.99 and 4.23 (d, d, 4H, J = 12.5, and 12.2),

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and 5.06 (t, 1H, $J = 1.4$); ^{13}C NMR δ 17.5, 19.1, 25.2, 25.6, 29.8, 30.2, 32.2, 36.4, 68.9, 94.0, 124.3, and 131.3; EI/MS 210 (M^+, 34) and 109 (100). Anal. Calcd for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72. N, 5.40. Found: C, 59.88; H, 9.64; N, 5.40.

(2*R,3*R**)- and (2*R**,3*S**)-6,10-Dimethyl-3-nitrodeca-5,9-dien-2-ol (8c).** To a stirred solution of nitro compound **6** (3.39 g, 17.18 mmol) and distilled acetaldehyde (1.14 mL, 11.4 mmol) was added Amberlyst A21 (4 g) at 0 °C. After 16 h the reaction mixture was filtrated through a Celite pad and washed with hexane (3 × 10 mL). The hexane layer was washed with water (2 × 10 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (10% EtOAc/hexane). The title compound **8c** was obtained (2.36 g, 57%): colorless oil; IR (NaCl) 341.5, 1552, 1450, and 1376 cm^{-1}; ^{1}H NMR δ 1.30 (m, 3H), 1.59 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 2.03 and 2.25 (m, 4H), 2.5 and 2.77 (m, 2H), 4.13 and 2.23 (m, 1H), 4.39 (m, 1H), and (m, 2H); ^{13}C NMR δ 16.1, 17.7, 19.1, 19.9, 25.6, 29.3, 39.6, 67.8, 93.5, 116.5, 123.7, 131.8, and 140.8. Anal. Calcd for $C_{13}H_{23}NO\cdot(1/2)H_2O$: C, 64.43; H,9.98; N, 5.78. Found: C, 63.98; H, 9.56; N, 5.75.

(*E*)-5,9-Dimethyl-2-nitrodeca-4,8-dienyl Acetate (10a). To a stirred solution of nitroalcohol **8a** (2.68 g, 11.69 mmol) in acetic anhydride (12 mL) was added pyridine (0.95 mL, 11.69 mmol) with cooling to 0 °C. After the reaction mixture was stirred at rt for 2 h, aqueous saturated $NaHCO_3$ (20 mL) and hexane (20 mL) were added. The mixture was extracted with hexane (2 × 10 mL). The combined organic layers were washed with water (2 × 10 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated to give 3.04 g of the crude **10a** in 98% yield: orange liquids; ^{1}H NMR δ 1.59 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 2.03 (m, 4H), 2.06 (s, 3H), 2.54 and 2.69 (m, 2H), 4.42 (m, 2H), 4.68 (m, 1H), and 5.09 (m, 2H); EI/MS 269 (M^+, 0.5) and 109 (100). This material was used for the next step without further purification.

(2*R,5*R**)- and (2*R**,5*S**)-5,9-Dimethyl-2-nitrodeca-8-enyl acetate (10b)** was obtained in 98% yield from the reaction of **9b** and acetic anhydride in the presence of pyridine: yellow oil; IR (NaCl) 1714, 1532, and 1370; EI/MS 271 (M^+, 2) and 109 (100). This material was used for the next step without further purification.

(2*R,3*R**)- and (2*R**,3*S**)-6,10-Dimethyl-3-nitrodeca-5,9-dien-2-yl acetate (10c)** was obtained in 98% yield from the reaction of **9c** and acetic anhydride in the presence of pyridine: yellow oil; ^{1}H NMR δ 1.33 (m, 3H), 1.56 (s, 3H), 1.59 (s, 3H), 1.68 (s, 3H), 2.22 (m, 4H), 2.08 (s, 3H), 2.48 and 2.69 (m and m, 2H), 4.52 and 4.63 (m and m, 1H), 5.03 (m, 2H), and 5.26 (m, 1H). This material was used for the next step without further purification.

Ethyl 4-(3,7-Dimethylocta-2,6-dienyl)pyrrole-2-carboxylate (11a). To a stirred solution of β-nitro acetate **10a** (3.04 g, 11.29 mmol) in anhydrous 1:1 THF/tBuOH (28 mL) were added dropwise ethyl isocyanoacetate (1.23 mL, 11.29 mmol) and DBU (3.38 mL, 22.58 mmol) with cooling to 0 °C, and the resulting mixture was stirred for 26 h. Then water (30 mL) and EtOAc (30 mL) were added. The mixture was extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (2 × 20 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (10%, EtOAc/hexane) to give 2.36 g of **11a** in 18% yield: yellow oil; IR (NaCl) 3315 and 1681 cm^{-1}; ^{1}H NMR δ 1.32 (t, 3H, $J = 7.1$), 1.57 (s, 3H), 1.61 (s, 3H), 1.68 (s, 3H), 2.10 (m, 4H), 3.18 (d, 2H, $J = 7.1$), 1.57 (s, 3H), 1.61 (s, 3H), 1.68 (s, 3H), 2.10 (m, 4H), 3.18 (d, 2H, $J = 7.3$), 4.30 (q, 2H, $J = 7.3$), 5.10 and 5.33 (dd, dd, 1H, 1H, $J = 7.3$, 7.6, and $J = 7.3$, 5.3), 6.74 (s, 1H), 6.74 (s, 1H), and 8.87 (br s, 1H); ^{13}C NMR δ 14.5, 16.0, 17.7, 25.3, 25.4, 26.6, 39.6, 60.2, 114.78, 120.4, 120.7, 123.1, 124.3, 125.8, 131.4, 135.7, and 161.2; EI/MS 275 (M^+, 100); HRMS calcd for M^+ ($C_{17}H_{25}NO_2$) 275.1885, found 275.1884.

Ethyl 4-(3,7-dimethyloct-2-enyl)pyrrole-2-carboxylate (11b) was obtained in 61% yield from the reaction of **10b** in the presence of DBU in THF: IR (NaCl) 3312 and 1682 cm^{-1}; yellow oil; ^{1}H NMR δ 1.91 (d, 3H, $J = 6.10$), 1.30 (m, 2H, 2H and 1H), 1.35 (t, 3H, $J = 7.17$), 1.60 (s, 3H), 1.68 (s, 3H), 1.96 (m, 2H), 2.47 (m, 2H), 4.30 (q, 2H, $J = 7.0$), 5.09 (m, 1H), 6.73 (d, 1H, $J = 3.7$), 6.75 (d, 1H, $J = 2.1$), and 9.62 (br s, 1H); ^{13}C NMR δ 14.3, 17.5, 19.3, 24.0, 25.3, 31.8, 36.8, 38.1, 60.0, 114.7, 120.4, 124.3, 124.8, 126.6, 130.9, and 161.2; EI/MS 277 (M^+, 48) and 153 (100); HRMS calcd for M^+ ($C_{17}H_{27}NO_2$) 277.2042, found 277.2027.

Ethyl 3-methyl-4-(3,7-dimethylocta-2,6-dienyl)pyrrole-2-carboxylate (11c) was obtained in 60% yield from the reaction of **10c** in the presence of DBU in THF: yellow oils; IR (NaCl) 3322 and 1670 cm^{-1}; ^{1}H NMR δ 1.35 (t, 3H, $J = 7.2$), 1.60 (s, 3H), 1.68 (s, 3H), 1.72 (s, 3H), 2.08 (m, 4H), 2.28 (s, 3H), 3.11 (d, 2H, $J = 7.3$), 4.31 (q, 2H, $J = 14.6$, 7.3), 5.10 (m, 1H), 5.26 (m, 1H), 6.63 (s, 1H), and 8.75 (br s, 1H); ^{13}C NMR δ 10.3, 14.5, 16.0, 17.7, 23.9, 25.7, 26.6, 39.6, 59.8, 119.7, 122.7, 123.4, 124.2, 124.3, 125.0, 131.4, 135.6, and 161.8; EI/MS 289 (M^+, 86) and 174 (100); HRMS calcd for M^+ ($C_{18}H_{27}NO_2$) 289.2042, found 289.2034.

(*E*)-4-(3,7-Dimethylocta-2,6-dienyl)pyrrole-2-carboxylic Acid (12a, Pyrrolostatin). To lithium hydroxyde monohydrate (0.35 g, 8.57 mmol) in water (4.5 mL) was added ethyl pyrrole-2-carboxylate **10a** (2.36 g, 8.57 mmol) in dioxane (4.5 mL), and the resulting mixture was warmed at 40 °C under a nitrogen atmosphere. The solution was stirred for 19 h, and then buffer solution (pH 3.5), diethy ether, and NaCl were added. The aqueous layer was extracted with diethyl ether (10 mL). The organic layer was washed with water (2 × 20 mL) and saturated brine (50 mL) and dried with Na_2SO_4. After evaporation of the solvent, the residue was purified by recrystallization from diethyl ether/hexane to give 1.55 g of pyrrolostatin (**12a**) in 73% yield as a pale yellow crystal: mp 117−119 °C; IR (KBr) 3353, 2917, and 1686 cm^{-1}; ^{1}H NMR δ 1.59 (s, 3H), 1.66 (s, 3H), 1.67 (s, 3H), 2.07 (m, 4H), 3.15 (d, 2H, $J = 6.84$), 5.10 (m, 1H), 5.31 (m, 1H), 6.65 (d, 1H, $J = 1.5$), and 6.69 (d, 1H, $J = 1.0$); ^{13}C NMR δ 16.0, 17.7, 25. 2, 25.7, 26.6, 39.6, 116.8, 121.4, 121.9, 122.8, 124.2, 126.4, 131.4, 135.9, and 165.2; EI/MS 257 (M^+, 52) and 69 (100). Anal. Calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; O, 5.66. Found: C, 72.68; H, 8.48; O, 5.70. **12b** and **12c** were obtained by the same procedure.

4-(3,7-Dimethylocta-2-enyl)pyrrole-2-carboxylic acid (12b): mp 115−117 °C; IR (KBr) 3372 and1688 cm^{-1}; ^{1}H NMR δ 0.92 (d, 3H, $J = 6.35$), 1.18 (m, 2H), 1.45 (m, 1H and 2H), 1.60 (s, 3H), 1.68 (s, 3H), 1.98 (m, 2H), 2.48 (m, 2H), 5.10 (t, 1H, $J = 6.9$), 6.80 (s, 1H), 6.90 (s, 1H), 9.15 (br s, 1H), and 11.00 (br s, 1H); ^{13}C NMR δ 17.7, 24.2, 25.8, 32.0, 37.0, 38.2, 117.0, 121.3, 122.0, 124.9, 127.6, 131.1, and 165.3. Anal. Calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.20; H, 9.11; N, 5.66.

3-Methyl-4-(3,7-dimethylocta-2,6-dienyl)pyrrole-2-carboxylic acid (12c): mp 121−122 °C; IR (KBr) 3367, 2968, 2858, 2632, 1678, 1469, 1332, and 1394 cm^{-1}; ^{1}H NMR δ 1.60 (s, 3H), 1.68 (s, 3H), 2.06 (m, 4H), 2.31 (s, 3H) 3.12 (d, 2H, $J = 7.0$), 5.10 (m, 1H), 5.26 (t, 1H, $J = 6.1$), 6.70 d, 1 H, $J = 3.1$), and 8.80 (br s, 1H); ^{13}C NMR δ, 10.3, 16.0, 17.6, 17.7, 23.4, 23.7, 23.9, 25.7, 26.5, 26.6, 32.0, 39.7, 118.5, 121.2, 122.5, 123.2, 124.2, 124.3, 125.5, 128.2, 131.4, 135.9, and 166.4; EI/MS 261 (M^+, 100) and 148 (94). Anal. Calcd for $C_{16}H_{23}NO_2\cdot(1/2)H_2O$: C, 73.20; H, 9.20; N, 5.34. Found: C, 72.80; H, 8.90; N, 5.36.

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