Synthesis of Unprotected (±)-Tunichrome An-1, a Tunicate Blood Pigment

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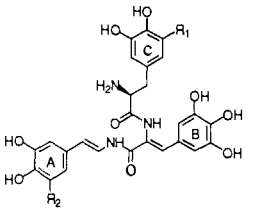
Abstract: The unstable, oxygen-sensitive tunicate blood pigment (±)-tunichrome An-1 (1a), which has been implicated in the 10^7-fold concentration of vanadium from the ocean in some tunicate species, has been synthesized in its *underivatized* form on a semipreparative scale; methods to isolate and purify the compound are also described. The synthesis employed *tert*-butyl carbamate (BOC) and *tert*-butyldimethylsilyl ethers (TBDMS) for N,O-protection; pure tunichrome An-1 was obtained in >90% yield by demasking with TFA and pyridine/48% aqueous HF followed by fractional precipitation; the synthetic and purification schemes should have general utility in the preparation of other structurally related marine natural products, which have been isolated and synthesized only as derivatives. Availability of underivatized tunichrome is essential for clarification of the biological role of tunichromes, including their reactions with vanadium.

Tunichromes (TC) (**1a-c**)[1,2] are a group of unstable poly-

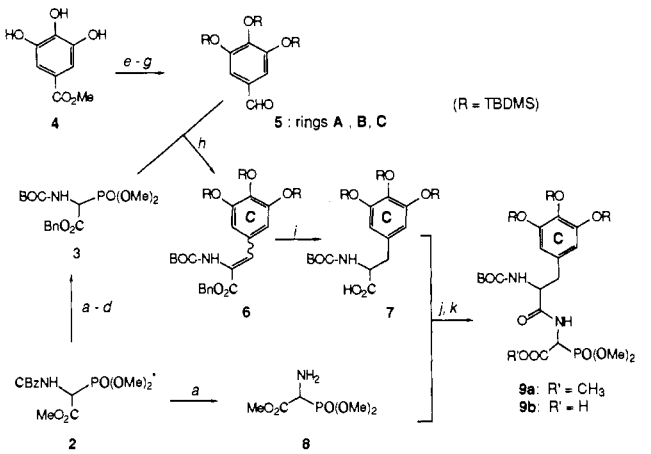


1a: An-1, $R_1 = R_2 = OH$
b: An-2, $R_1 = H$, $R_2 = OH$
c: An-3, $R_1 = R_2 = H$

phenolic blood pigments found in the vanadium assimilating[3-5] tunicate *Ascidia nigra* and others. In these tunicates vanadium is concentrated 10^7-fold from the ocean and is reduced from V(V) to primarily the V(III) state. Tunichromes have been implicated[6] in this V sequestering process because of their excellent properties as a ligand and reductant for V.[7a,b] However, this is a controversial matter, and little is known regarding the means and purpose for vanadium assimilation in the tunicate and higher animals,[1d] including man; the role of tunichromes, only recently structurally determined, is also totally unknown.[1b,c] The characterization of tunichrome/vanadium interactions and clarification of their biological roles requires a supply of underivatized tunichrome, but isolation from the natural source is not practical due to difficulties in purification which arise as a result of their air instability and tenacious adherence to solid-phase chromatographic material;[2] moreover, final HPLC purification is accompanied by a 90% loss.[2] The polyphenolic rings are responsible for the sensitivity of TC to O_2 and alkali, while the styrylamide and dehydroaminoacid moieties lead to hydrolytic instability.[8a-c]

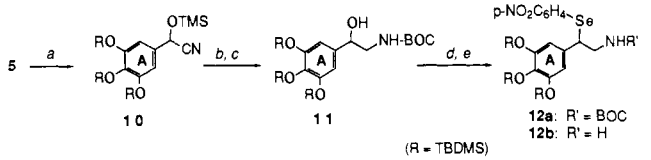
We report the synthesis of underivatized (±)-tunichrome An-1 (**1a**). The difficulty in the synthesis resided in the choice of synthetic steps compatible with protecting groups, removal of said moieties under neutral or weakly acidic conditions, and semipreparative scale isolation of product. In the scheme described, common building blocks are utilized for separate moieties, the

Scheme I



[a] (a) 5% Pd-C/H_2, MeOH; (b) (t-Bu-O-CO)$_2$O, CH_2Cl_2, 80% (from **2**); (c) 1.2 equiv of 1 N KOH, 15 min, 83%; (d) 1.05 equiv of BnBr, 1 equiv of DBU, CH_3CN, 2 h, 75%; (e) 4 equiv of TBDMSCl, 7 equiv of imidazole, DMF, 20 h, 93%; (f) 1 equiv of $LiAlH_4$, THF, reflux, 3 h, 79%; (g) 1.1 equiv of PCC, CH_2Cl_2, 4 h, 75%; (h) 1.1 equiv of NaH (97%), THF, then **5**, 5 h, 60%, $Z/E \approx 3:1$; (i) 5% Pd-C/H_2, MeOH, 12 h, 92%; (j) 1 equiv of DCC, CH_2Cl_2/DMF, 0° to room temperature, 4 h, 62% (from **2**); (k) 1.2 equiv of 1 N KOH, MeOH, 1.5 h, 94%.

Scheme II



[a] (a) 1.5 equiv of TMS cyanide, catalytic ZnI_2, 4 h, quantitative (crude); (b) 1.15 equiv of $LiAlH_4$, THF, room temperature, 3 h; (c) (t-Bu-O-CO)$_2$O, CH_2Cl_2, 30 min, 67% (from **5**); (d) 1.3 equiv of p-$NO_2C_6H_4SeCN$, n-Bu_3P, THF, 3 h, 75%; (e) 30% TFA/CH_2Cl_2, 0 °C to room temperature, 1 h, quantitative (crude).

yields of each steps are >60%, and the overall yield of final N,O-deprotection (BOC[11] and TBDMS[12]) and product isolation is >90%. The scheme should be applicable to other tunichromes and structurally-related marine natural products, the latter being isolated[9a,b] and synthesized[10a,b] as derivatives unsuited for the study of their biological roles.

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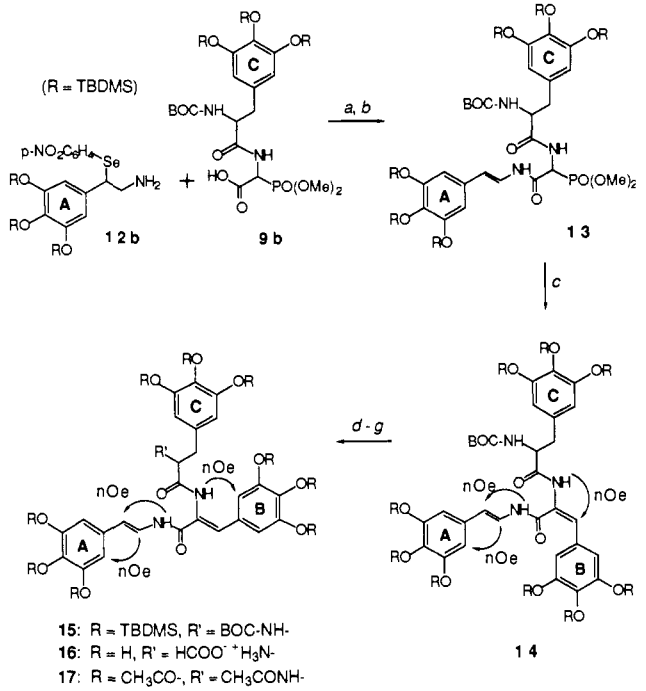
Scheme III



[a] (a) 1 equiv of DCC, EtOAc, 0 °C to room temperature, 5 h, 67%; (b) 5.2 equiv of $NaIO_4$, dioxane/H_2O, 4.5 h, 61%; (c) 1.1 equiv of LDA/THF, −78 °C, then 1 equiv of **5** in THF, warm to room temperature, 4 h, 82%; (d) *hν*, 300 nm, hexane, 16 h, **15**/**14** (2:1); (e) 20% TFA/CH_2Cl_2, 0 °C to room temperature, 1 h; (f) 190 equiv of 2:1 (mol) pyridine/48% aqueous HF, 5.5 h; (g) fractional precipitation,[23] lyophilize from aqueous HCOOH, 90% (from **15**).

A key starting material, phosphonoglycinate **3**[13] was prepared from the known methyl carbobenzoxyphosphonoglycinate **2**[14] (Scheme I). Horner–Emmons condensation of glycinate **3** with TBDMS-gallaldehyde **5** gave dehydrohydroxy-Dopa **6** (60%) as a 3:1 *Z*/*E* mixture, readily separable by column chromatography. Hydrogenation/hydrogenolysis of (*Z*)-**6** afforded moiety C **7**, 92%. Condensation with DCC of acid **7** and glycinate **8**, obtained by catalytic hydrogenation[14] of **2**, yielded the phosponodipeptide methyl ester **9a**, diastereomeric mixture, 62%. Saponification of ester **9a** gave phosphonodipeptide **9b**, 94%.

Moiety A (Scheme II) was formed stereoselectively by selenoxide elimination, a technique employed[10a,b] in the synthesis of related (but derivatized) natural products. The β-amino selenide was constructed as follows. Reaction of aldehyde **5** with TMS cyanide gave TMS cyanohydrin[15] **10** (quantitative); this was reduced with LAH to the crude amino alcohol[16] which was immediately acylated with di-*tert*-butyl dicarbonate to provide *N*-BOC carbinol **11**, 67% overall yield from **5**. Selenide **12a** was obtained upon treatment of carbinol **11** with 4-nitrophenyl selenocyanate[17] and tri-*n*-butylphosphine,[18] 70% yield; reaction of **12a** with TFA gave β-amino selenide **12b**.

The final steps are shown in Scheme III. Phosphonodipeptide **9b** was coupled with amino selenide **12b** to provide the selenyldipeptide, diastereomeric mixture, 67%. Oxidation/elimination of the selenide with $NaIO_4$[10a] afforded styrylamide **13** in 61% yield, J_{vic} = 14.5 Hz (E geometry). Condensation of **13** with aldehyde **5** (LDA, −78 °C) provided protected An-1's **14**(E)/**15**(Z), 82%, but unexpectedly[14] with an *E*/*Z* ratio of 25/1. Synthetic conditions to reverse the ratio were not found; instead, irradiation at 300 nm isomerized the product to a 1:2 mixture of **14**/**15**, which was separated by preparative TLC; HR-FAB-MS[19] of **15**, 1682.986 (calcd for $C_{85}H_{159}N_3Si_9O_{13}$ + H, 1682.9870). The geometries of both olefinic double bonds were established by the NOE results depicted in structures **14** and **15**. UV[20] and ^{13}C NMR[21] data were also in accord with the assignment of B unit geometries: **14** λ_{max} (hexane) 314 nm (ε 15000) and **15** λ_{max} 328 nm (ε 34000).

Checking numerous conditions for deprotection with models as well as with **15** showed that it could be effected by treatment of **15** with dry 20% TFA/CH_2Cl_2 (removal of BOC) followed by excess pyridine/48% HF (removal of TBDMS); all deprotection and product isolation operations were performed under Ar in degassed solvents. Removal of pyridine/HF to obtain free tunichrome encountered difficulties: phenylboronic acid affinity chromatography,[22] employed for Dopa and catecholamines, was satisfactory at analytical scale but was unsuited for the present 5–10-mg scale purification; centrifugal partition chromatography and LH-20 chromatography, which played crucial roles in the tunichrome isolation,[2] were not suited here due to requirement of larger sample amounts (CPC >50 mg) or poor recovery (LH-20 ca. 40%). However, the unstable product could be isolated successfully from pyridine/HF by fractional precipitation with a CH_2Cl_2 based solvent system; this nondestructive purification proceeded with high recovery and led to completion of the synthesis. After precipitation, the bright yellow An-1 was lyophilized from dilute aqueous formic acid to provide the yellow formate salt **16**, 90% yield from **15**, having NMR and UV spectra in agreement with those of naturally derived An-1 formate.[1] ^1H NMR, UV, DCI-MS, molecular formula ($C_{46}H_{45}N_3O_{21}$, HR-FAB-MS), and HPLC (coinjection) of (±)-An-1 peracetate **17** (Ac_2O/pyridine) were virtually identical with natural An-1 decaacetate.[1]

The present scheme is being employed for the preparation of other tunichrome analogues, including Mm-1 and Mm-2[1b] containing glycine and leucine moieties, respectively, instead of the hydroxy-Dopa unit C. Synthetic An-1 **1a** and analogues, natural and unnatural, should play crucial roles in clarification of numerous ambiguities encountered in the chemistry of tunichrome and vanadium, e.g., chelation/redox chemistry and the metabolic fate of tunichrome.[1b]

Experimental Section

General Procedures. Solvents employed were reagent or HPLC grade. In reactions requiring anhydrous conditions, solvents were dried by distillation under Ar from the appropriate drying agent (THF, Na/benzophenone; CH_2Cl_2, CaH_2; CH_3CN, CaH_2). Peroxide-free dioxane was obtained by fractional distillation from CuCl under Ar.

All chromatography solvents were HPLC grade, except ethyl acetate which was reagent grade. When required, solvents were degassed by repeated evacuation and release of vacuum under Ar and were thereafter maintained under an Ar atmosphere. Thin-layer chromatography was performed on glass plates with UV fluorescent indicator (Merck, Silica Gel 60 F254). Preparative TLC was performed on 20 × 20 cm silica plates (Silica GF) from Analtech. Column chromatography employed 32-63 mesh silica from ICN. HPLC employed a Dupont Instruments pump, a Scheffel detector/monochromater operated at 320 nm, and a Perkin-Elmer miniature high resolution cartridge column (3μ silica).

NMR spectroscopy was performed on a Bruker WM-250 spectrometer operating at 250.13 and 62.9 MHz for observation of hydrogen and carbon, respectively. Chemical shifts are reported relative to the isotopic impurity peak for a given solvent ($CDCl_3$, 7.24 ppm; MeOH-d_4, 3.30 ppm; pyridine-d_5, 8.71 ppm). IR spectra were obtained on a Perkin-Elmer 983 infrared spectrometer. DCI mass spectra were measured on

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a Nermag R 10-10C spectrometer using NH_3 as carrier gas unless otherwise mentioned. FAB mass spectra were recorded on a VG Analytical 7070 EQ spectrometer using Xe for bombardment gas; glycerol, thioglycerol, or *m*-nitrobenzyl alcohol was used as matrix. High resolution FAB-MS were recorded on Hitachi M-80B and Finnigen MAT 90 spectrometers. UV spectra were obtained with a Perkin-Elmer Lambda Array 3840 spectrometer.

When anhydrous conditions are specified in a procedure, the glassware was oven dried, cooled in a dessicator, and then flame dried under a steady stream of argon and cooled. Glassware was equipped with rubber septa; reagent transfers were performed with syringe techniques. Reagents employed were of the highest purity available; if necessary purification by distillation or recrystallization was performed prior to use.

***N*-BOC-phosphonoglycinate (Scheme I, Steps a and b)**,[14] (a) *N*-CBz-phosphonoglycinate **2** was hydrogenated with 5% Pd–C in MeOH at 1 atm until the reaction was judged complete by TLC (usually about 45 min). The methanolic solution of the free amine was concentrated in vacuo for the next step. (b) The crude product from the above step was acylated with di-*tert*-butyl dicarbonate: (3.12 g, 79%, lit. 80%) mp 59.5–60 °C, lit 47–48 °C; TLC 2:1 EtOAc/hexane, white spot (vanillan) R_f 0.20; CI-MS (NH_3) 298 (M + 1), 315 (M + 18); ^{1}H NMR ($CDCl_3$) δ5.33 (d, J = 9.3 Hz, 1 H, NH), 4.84 (dd, J = 9.3, 22.6, 1 H, α-H), 3.75–3.84 (m, 9 H, OMe's), 1.42 (s, 9 H, *t*-Bu); IR (KBr) cm^{-1} 3260 (br), 1758, 1709, 1544, 1249, 1031, 831, 795.

***N*-BOC-(dimethylphosphono)glycine (Scheme I, Step c)**, *N*-BOC-phosphonoglycinate, the product from step b (3.02 g, 10.17 mmol), was stirred for 15 mins in 11.2 mL of 1 N KOH (11.19 mmol). The reaction is nearly complete after ca. 5 min as monitored by TLC. The reaction mixture was acidified to pH 3 by dropwise addition of 6 N HCl resulting in precipitation of the acid as a white semisolid. The mixture was diluted with 25 mL of EtOAc and transferred to a separatory funnel. The two phases were separated, and the aqueous phase was extracted 2 × 25 mL with EtOAc. The combined organic phases were washed with saturated NaCl solution and then dried over Na_2SO_4. Concentration in vacuo afforded 2.40 g (83%) of acid as a white solid: mp 154–155 °C; CI-MS 284 (M + 1), 301 (M + 18); ^{1}H NMR ($CDCl_3$) δ5.53 (d, J = 9.1 Hz, 1 H, NH), 4.91 (dd, J = 9.1, 22.7 Hz, 1 H, α), 3.85 (apparent triplet, J = 11.1, 6 H, OMe's), 1.43 (s, 9 H, t-Bu).

***N*-BOC-(dimethylphosphono)glycine Benzyl Ester 3 (Scheme I, Step d)**, The product from step c (2.40 g, 8.48 mmol) was suspended in 5 mL of dry CH_3CN under Ar. DBU (1.29 g, 8.48 mmol, dried over KOH) was dissolved in 0.5 mL of dry CH_3CN and added to the stirring suspension. Benzyl bromide (1.52 g, 8.90 mmol) was dissolved in 0.5 mL of dry CH_3CN and added to the clear reaction mixture; the mixture was stirred at room temperature for 2 h at which time TLC showed complete disappearance of the starting acid. The reaction mixture was diluted with 40 mL of 2:1 water/brine solution and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with 20 mL of 1 N HCl, water, and brine and then dried over Na_2SO_4. Concentration in vacuo afforded 3.03 g of the crude benzyl phosphoryl glycinate **3** as a viscous oil. The crude product was purified by column chromatography (1:1 H/EtOAc, 200 × 40 mm silica column) to afford 2.36 g (75%) of **3**, oil: TLC 2:1 EtOAc/hexane, off-white spot (vanillan) R_f 0.52; CI-MS 374 (M + 1), 391 (M + 18), 335 (M + NH_4 − 56, base); ^{1}H NMR ($CDCl_3$) δ 7.35 (br m, 5 H, phenyl), 5.35 (d, J = 9.3 Hz, 1 H, NH), 5.23 (ABq, ΔAB = 30.6 Hz, J = 12.4 Hz, 2 H, CH_2), 4.88 (dd, J = 9.3, 22.6 Hz, 1 H, α), 3.74 (d, J = 7.3 Hz, 3 H, OMe), 3.69 (d, J = 7.3 Hz, 3 H, OMe), 1.42 (s, 9 H, *t*-Bu); IR (film on NaCl plate), cm^{-1} 3300 (br), 2960, 2930, 1735, 1708, 1492, 1155, 1049, 1030.

Methyl Tris-*O*-TBDMS-gallate (Scheme I, Step e), By a modification of the literature proceedure,[12] methyl gallate (18.42 g, 0.1 mol), *tert*-butyldimethylsilyl chloride (60.3 g, 0.4 mol), and imidazole (49 g, 0.72 mol) were stirred in 300 mL of DMF for about 20 h. The reaction mixture was cast into 500 mL of 5% aqueous $NaHCO_3$ and extracted with hexane (3 × 150 mL). The combined organic layers were washed with 100 mL of brine and dried over Na_2SO_4. Concentration in vacuo afforded a pale green oil which was diluted with about 200 mL of methanol and placed in the refrigerator (ca. 4 °C) for 2 h. After this period, 42.7 g of white crystals were collected by filtration; mp 69–70 °C. A second crop (6.1 g, mp 68–68.5 °C) was obtained by concentration of the mother liquors and seeding with a crystal from the previous crop. The combined yield was 48.8 g (93%): TLC 2:1 hexane/EtOAc, UV, R_f 0.94; CI-MS 527 (M + 1), 544 (M + 18); ^{1}H NMR ($CDCl_3$) δ 7.18 (s, 2 H, Ar), 3.83 (s, 3 H, OMe), 0.96 (s, 9 H, Si-*t*-Bu), 0.92 (s, 18 H, Si-*t*-Bu), 0.21 (s, 12 H, Si-CH_3), 0.11 (s, 6 H, Si-CH_3); IR (KBr) cm^{-1} 2955, 2925, 1718, 1490, 1428, 1224, 1101, 840, 826.

Tris-*O*-TBDMS-trihydroxybenzyl Alcohol (Scheme I, Step f). Methyl tris-*O*-TBDMS-gallate (42.7 g, 81.2 mmol) was dissolved in 100 mL of dry THF and added to a suspension of 3.1 g (82 mmol) of lithium aluminum hydride in 300 mL of THF at a rate sufficient to maintain a gentle reflux. The reaction mixture was refluxed for 3 h; TLC indicated complete dissappearence of starting material. The reaction mixture was let cool to room temperature and diluted with 200 mL of diethyl ether. The reaction mixture was stirred rapidly, and ca. 10 mL of 1 N NaOH was added dropwise until the color turned from grey to white. The heterogeneous mixture was filtered through celite, and the resulting filtrate was partitioned against brine (3 × 100 mL) and dried over Na_2SO_4. Concentration in vacuo gave 31.9 g (79%) of the alcohol as a pale green oil which was sufficiently pure to be employed in the next step without further purification: TLC 2:1 hexane/EtOAc, black spot (vanillan) R_f 0.81; CI-MS 499 (M + 1), 481 (M − 17); ^{1}H NMR ($CDCl_3$) δ 6.47 (s, 2 H, Ar), 4.46 (d, J = 5.9 Hz, 2 H, CH_2), 1.42 (t, J = 5.9 Hz, 1 H, OH), 0.97 (s, 9 H, Si-*t*-Bu), 0.91 (s, 18 H, Si-*t*-Bu), 0.18 (s, 12 H, Si–CH_3), 0.09 (s, 6 H, Si–CH_3); IR (KBr pellet) cm^{-1}: 3410 (br), 2930, 1580, 1100, 840, 830.

Tris-TBDMS-gallaldehyde (5) (Scheme I, Step g), Pyridinium chlorochromate (3.27 g, 15.16 mmol) was suspended in 40 mL of dry CH_2Cl_2 and cooled to 0 °C. The above mentioned TBDMS-trihydroxybenzyl alcohol (6.95 g, 13.96 mmol) was dissolved in 40 mL of dichloromethane and then added to the stirring PCC suspension in 1 portion. The reaction mixture was let warm to room temperature and stirred for 4 h. Diethyl ether (200 mL) was added to the reaction mixture which was then stirred for 5 min and filtered through Celite. The filtrate was concentrated in vacuo; the resulting dark oil was diluted with 2:1 dichloromethane/diethyl ether and passed through a short pad of silica and concentrated. The resulting light brown solid was passed through a column of silica (50 g) in 7:1 hexane/EtOAc, concentrated, and recrystallized from methanol to afford 5.20 g (75%) of **5** as a white solid: mp 67–69 °C; TLC 9:1 H/EtOAc, R_f 0.84, green spot (vanillan); CI-MS 497 (M + 1), 439 (M − 57); ^{1}H NMR ($CDCl_3$) δ 9.70 (s, 1 H, CHO), 7.00 (s, 2 H, Ar), 0.97 (s, 9 H, Si-*t*-Bu), 0.93 (s, 18 H, Si-*t*-Bu), 0.23 (s, 12 H, Si-CH_3), 0.13 (s, 6 H, Si-CH_3); IR (KBr pellet) cm^{-1} 3060, 2950, 2930, 2780, 2700, 1695, 1255, 1100. Anal. Calcd for $C_{25}H_{48}O_4Si_3$: C, 60.4; H, 9.7. Found: C, 60.3; H, 9.6.

(*E*/*Z*)-*N*-BOC Dehydroamino Acid Ester 6 (Scheme I, Step h), Phosphonate **3** (2.36 g, 6.33 mmol) was dissolved in 5 mL of dry THF and added dropwise under Ar to a stirred suspension of 97% NaH (0.177 g, 7,15 mmol) in 10 mL of THF. The mixture was stirred at room temperature for 20 min, until hydrogen evolution had ceased. A solution of gallaldehyde **5** (2.98 g, 6.00 mmol) in 10 mL of THF was added to the reaction mixture dropwise, and then the reaction mixture was stirred 5 h. The reaction was quenched with a few drops of H_2O and then concentrated in vacuo. The light yellow/green residue was purified by column chromatography (silica, 15:1 H/EtOAc) to provide 2.68 g (60%) of (*E*/*Z*)-**6**. Previous reactions performed at the 1.25 mmol scale afford an *E*/*Z* ratio ≈ 1:3: TLC (15:1 hexane/EtOAc) (*E*)-**6**, R_f 0.39 (brown, vanillan); (*Z*)-**6**, R_f = 0.24 (yellow, vanillan). ***Z* isomer**: DCI-MS 744 (M + 1), 688 (M + 1 − 56); ^{1}H NMR ($CDCl_3$) δ 7.42–7.3 (m, 5 H, phenyl), 7.02 (s, 1 H, vinyl), 6.78 (s, 2 H, Ar), 5.83 (br s, 1 H, NH), 5.24 (s, 2 H, CH_2), 1.39 (s, 9 H, *t*-Bu), 0.96 (s, 9 H, Si-*t*-Bu), 0.91 (s, 18 H, Si-*t*-Bu), 0.19 (s, 12 H, Si-CH_3), 0.10 (s, 6 H, Si-CH_3); IR (film on NaCl plate) cm^{-1} 3300 (br), 2960, 2932, 2865, 1750 (br), 1254, 1091, 840, 827. ***E* isomer** DCI-MS 744 (M + 1), 762 (M + 18); ^{1}H NMR (acetone-d_6) δ 8.20 (br s, 1 H, NH), 7.31 (s, 5 H, phenyl), 6.60 (s, 2 H, Ar), 6.50 (s, 1 H, vinyl), 1.41 (s, 9 H, *t*-Bu), 1.00 (s, 18 H, Si-*t*-Bu), 0.95 (s, 9 H, Si-*t*-Bu), 0.24 (s, 12 H, Si-CH_3), 0.14 (s, 6 H, Si-CH_3); IR (film on NaCl) cm^{-1} 3340/(br), 3000, 2928, 2855, 1726, 1705, 1353, 1250, 1086, 781.

***N*-BOC-TBDMS-5-hydroxy-Dopa (7) (Scheme I, Step i)**, (*Z*)-**6** (0.173 g, 0.233 mmol) was hydrogenated at 1 atm with 20 mg of 5% Pd–C in 5.0 mL of methanol for 12 h. The TLC behavior of the reaction intermediates suggests cleavage of the benzyl ester occurs faster than the hydrogenation as evidenced by initial (over a few hours) formation of a polar intermediate which was UV active; at the end of 12 h reaction time detection by UV was difficult. The reaction mixture was filtered through Celite and concentrated in vacuo to afford 140 mg (92%) of **7** as a foamy white glass. This material was employed in the next step without further purification. TLC EtOAc, tailing oval R_f 0.47, white (vanillan); FAB-MS (*m*-nitrobenzyl alcohol) 655 (M^+), 678 (M + Na); ^{1}H NMR ($CDCl_3$) δ 6.30 (s, 2 H, Ar), 4.85 (d, J = 7.3 Hz, 1 H, NH), 4.43 (m, 1 H, a), 2.92 (m, 2 H, br s), 1.40 (s, 9 H, *t*-Bu), 0.96 (s, 9 H, Si-*t*-Bu), 0.90 (s, 18 H, Si-*t*-Bu), 0.18 (s, 12 H, Si-CH_3), 0.08 (s, 6 H, Si-CH); IR (film on NaCl) cm^{-1} 3200 (br, weak), 2923, 2855, 1714, 1491, 1254, 1088, 838, 830.

Phosphonodipeptide Methyl Ester 9a (Scheme I, Step j). *N*-CBz-phosphonoglycinate **2** (71 mg, 0.214 mmol) was deprotected by hydrogenolysis at 1 atm with 10 mg of 5% Pd/C in 2 mL of methanol over 2 h. The reaction mixture was filtered through Celite, concentrated in vacuo, and used immediately for acylation with protected hydroxy-Dopa **7**. Acid **7** (140 mg, 0.214 mmol) was dissolved in 1.0 mL of dry CH_2Cl_2

and added to the crude free amine which was dissolved in 1.0 mL of 1:1 CH_2Cl_2/DMF. The stirred mixture was cooled to 0 °C, and 44 mg (0.214 mmol) of solid dicyclohexylcarbodiimide was added in 1 portion. The reaction mixture was let warm to room temperature and stirred for 4 h. One volume of EtOAc was added to the reaction mixture, and the precipitated DCU was removed by filtration. The filtrate was concentrated in vacuo, dissolved in 10 mL of EtOAc, and washed with 5.0 mL of $NaHSO_4$ and 5.0 mL of saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. Concentration in vacuo afforded a light brown foam. The crude product was purified by column chromatography (3:2 EtOAc/hexane, 100 × 20 mm silica) to afford 111 mg of phosphoryldipeptide methyl ester **9a** (mixture of diastereomers, 62%) as a clear colorless glass. The diastereomeric mixture was carried through the synthetic sequence: TLC 3:2 EtOAc/hexane, white oval (vanillan) R_f 0.32; FAB-MS 835 (M + 1), 777 (M − 57), 819 (M − 15); high resolution FAB-MS (glycerol) calculated for $C_{37}H_{71}O_{11}N_2PSi_3$ 835.2144, found 835.2155; ^{1}H NMR ($CDCl_3$) d 7.11 (br s, 1 H, NH), 6.30 (s, 2 H, Ar), 5.20 (m, 1 H, phosphonate α), 4.69 (m, 1 H, BOC-NH), 4.23 (s, 1 H, α), 3.80 (m, 9 H, OMe's), 3.01 (m, 1 H, β), 2.67 (m, 1 H, β), 1.37 (s, 9 H, *t*-Bu), 0.95 (s, 9 H, Si-*t*-Bu), 0.90 (s, 18 H, Si-*t*-Bu), 0.17 (s, 12 H, Si-CH_3), 0.08 (s, 6 H, Si-CH_3); IR (KBr pellet) cm^{-1} 3300 (br), 3050, 2950, 2930, 1755, 1685, 1255, 1045.

Phosphonodipeptide 9b (Scheme I, Step k). Ester **9a** (111 mg, 0.133 mmol) was dissolved in 5.0 mL of methanol and 0.16 mL of 1.0 N aqueous KOH was added to the solution at room temperature. The reaction mixture was stirred 1.5 h, during which time a yellow color developed. The reaction mixture was concentrated in vacuo then dissolved in 10 mL of 1:1 EtOAc/H_2O. The aqueous layer was brought to pH 3 with 1 N $NaHSO_4$ and then transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine and then dried over Na_2SO_4. Concentration in vacuo afforded 103 mg (94%) of **9b** as a colorless glass: FAB-MS (*m*-nitrobenzyl alcohol) 821 (M + 1), 843 (M + Na); ^{1}H NMR ($CDCl_3$) δ 7.70–7.50 (2 br d's, 1 H, phos. NH), 6.38, 6.36 (overlap. s's, 2 H, Ar), 5.35 (m, 1 H, phos. a), 5.08, 4.95 (m's, 1 H, BOC-NH), 4.37 (m, 1 H, a), 3.87 (m, 6 H, OMe's), 3.08 (m, 1 H, b), 2.70 (m, 1 H, b), 1.40 (s, 9 H, *t*-Bu), 1.01 (s, 9 H, Si-*t*-Bu), 0.95 (s, 18 H, Si-*t*-Bu), 0.22 (s, 12 H, Si-CH_3), 0.13 (s, 6 H, Si-CH_3); IR (KBr pellet) cm^{-1} 3330 (br), 1720, 1690, 1493, 1255, 1050, 840, 830.

TMS Cyanohydrin 10 (Scheme II, Step a). Protected gallaldehyde **5** (1.50 g, 3.02 mmol) was stirred with trimethylsilyl cyanide (600 μL, 4.50 mmol) and a catalytic amount of anhydrous ZnI_2 for 4 h at room temperature. The reaction mixture was concentrated in vacuo with a vacuum pump to remove excess TMS-CN. An aliquot of the crude product was analyzed by ^{1}H NMR and showed complete disapperance of starting aldehyde. This material was sufficiently pure to proceed to the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 6.58 (2, 2 H, Ar), 5.30 (s, 1 H, CH), 0.97 (s, 9 H, Si-*t*-Bu), 0.92 (s, 18 H, Si-*t*-Bu), 0.20 (s, 12 H, Si-CH_3), 0.18 (s, 9 H, TMS), 0.10 (s, 6 H, Si-CH_3); IR (film on NaCl) cm^{-1} 2950, 2925, 2240 (weak), 1580, 1255, 1087, 783.

***N*-BOC Amino Carbinol 11 (Scheme II, Steps b and c).** (Step b). A solution of TMS cyanohydrin **10** (3.02 mmol) in 5.0 mL of dry THF was added dropwise to a stirred suspension of lithium aluminum hydride (0.132 g, 3.47 mmol) in 25 mL of THF at room temperature under Ar. The mixture was stirred 3 h and then quenched with 1.0 mL of EtOAc. The bulk of the THF was carefully removed on a rotary evaporator, and the resulting thick slurry was resuspended in 50 mL of diethyl ether. NaOH (1 N) was added dropwise (ca. 0.5 mL) to the stirring mixture until a granular white precipitate formed which was then removed by filtration through Celite. The filtrate was dried over Na_2SO_4 and concentrated in vacuo to afford 1.35 g of the amino alcohol as an off-white solid. (Step c). The solid was dissolved in 20 mL of dry dichloromethane, and 660 mg (3.02 mmol) of di-*tert*-butyl dicarbonate was added with stirring in 1 portion. The mixture was stirred for 30 min and then concentrated in vacuo. The resulting oil was purified by column chromatography (6:1 hexane/EtOAc, silica, 180 × 30 mm) to afford 1.267 g (67% from 5) of **11** as a foamy white glass: TLC 6:1 H/EtOAc, brown spot (vanillan) R_f 0.15; DCI-MS 628 (M + 1), 645 (M + NH_4), 610 (M − 18); ^{1}H NMR ($CDCl_3$) d 6.46 (s, 2 H, Ar), 4.78 (br s, 1 H, NH), 4.60 (br m, 1 H, CH), 3.40–3.30 (m, 1 H, CH2), 3.25, 3.10 (m, 1 H, CH_2), 1.42 (s, 9 H, t-Bu), 0.92 (s, 9 H, Si-*t*-Bu), 0.88 (s, 18 H, Si-*t*-Bu), 0.18 (s, 12 H, Si-CH_3), 0.09 (s, 6 H, Si-CH_3); IR (KBr) 3490 (br), 2925, 2855, 1693, 1250, 1074, 895, 830, 780. Anal. Calcd for $C_{31}H_{61}NO_6Si_3$: C, 59.3; H, 9.8; N, 2.2. Found: C, 59.1; H, 9.9; N, 2.2.

***N*-BOC Amino Selenide 12a (Scheme II, Step d).** 4-Nitrophenyl selenocyanate was prepared by the literature method,[16] (mp 134–6 °C lit. mp 135 °C). Carbinol **11** (0.165 g, 0.263 mmol) and tri-*n*-butylphosphine (85 μL, 0.342 mmol) were dissolved in 2.0 mL of dry THF under Ar. 4-Nitrophenyl selenocyanate (78 mg, 0.342 mmol) was dissolved in 1.0 mL of THF and added dropwise to the solution of **11** and tri-*n*-butylphosphine over 15 min. The ruby red solution was stirred at room temperature for 3 h. The mixture was concentrated in vacuo and purified by preparative TLC (6:1 hexane/EtOAc, 2 × 1000 μm silica plates) to afford 0.160 g (75%) of **12a** as a yellow glass: TLC 6:1 hexane/EtOAc yellow spot (vis), brown (vanillan), R_f 0.38; FAB-MS (glycerol) 813 (M + 1), 755 (M − 57), 609 (M − 203 (SeAr)); ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 8.7 Hz, 2 H, SeAr), 7.54 (d, J = 8.7 Hz, 2 H, SeAr), 6.42 (s, 2 H, Ar), 4.60 (br s, 1 H, NH), 4.44 (br t, J = 7.3 Hz, 1 H, Se-CH), 3.72 (m, 1 H, CH_2), 3.40 (m, 1 H, CH_2), 1.40 (s, 9 H, *t*-Bu), 0.95 (s, 9 H, Si-*t*-Bu), 0.88 (s, 18 H, Si-*t*-Bu), 0.16 (s, 12 H, Si-CH_3), 0.09 (s, 6 H, Si-CH_3); IR (film on NaCl plate) cm^{-1} 3350 (br), 3090, 2930, 1715, 1570, 1340, 1090, 840.

Amino Selenide 12b (Scheme II, Step e). *N*-BOC amino selenide **12a** (0.113 g, 0.139 mmol) was dissolved in 1.0 mL of dry dichloromethane and cooled to 0 °C. Freshly distilled trifluoroacetic acid (0.25 mL) was added dropwise to the chilled solution. The mixture was let warm to room temperature and stirred for 1 h; TLC showed disappearance of the BOC-protected material and formation of a strongly ninhydrin active product. The deep yellow reaction mixture was concentrated in vacuo by using a rotary evaporator equipped with a vacuum pump. The yellow residue was taken up in 20 mL off EtOAc, washed with saturated $NaHCO_3$ solution (3 × 15 mL), and then dried over $MgSO_4$. Concentration in vacuo afforded 98.7 mg (ca. 100%) of the crude BOC-deprotected **12b** which was employed without further purification in the next step.

Styrylamide 13 (Scheme III, Steps a and b). (Step a). Amino selenide **12b** (98.7 mg, 0.139 mmol), acid **9b** (114 mg, 0.139 mmol), and ca. 100 mg of anhydrous Na_2SO_4 were combined in 300 μL of dry EtOAc and cooled to 0 °C. Solid dicyclohexylcarbodiimide (29 mg, 0.139 mmol) was added to the stirring solution in 1 portion. The mixture was let warm to room temperature; stirring was continued for a total reaction time of 5 h. The DCU precipitate was removed from the reaction mixture by filtration through a short plug of glass wool. The filtrate was concentrated in vacuo and purified by preparative TLC (2:1 hexane/EtOAc, 2 × 100 m silica plates) to afford 140 mg (67%) of the selenyl dipeptide (mixture of diastereomers) as a yellow glass: TLC 2:1 hexane/EtOAc, yellow oval (vis), brown (vanillan), R_f 0.23; FAB-MS (glycerol) 1516 (M + 1), 1459 (M + 1 − 57), 1313 (M + 1 − 203 (SeAr)); ^{1}H NMR ($CDCl_3$) δ 8.2–7.95 (m, 2 H, SeAr), 7.56–7.48 (m, 2 H, SeAr), 7.15 (m, 1 H, phos. NH), 7.08 (m, 1 H, selenide NH), 6.40 (s, 2 H, Ar(selenide)), 6.32 (s, 2 H, Ar(hydroxy-Dopa)), 5.05–4.86 (m, 1 H, phos. α), 4.70 (br s, 1 H, BOC-NH), 4.50 (br t, J = 7.3 Hz, 1 H, Se-CH), 4.25–4.10 (m, 1 H, hydroxy-Dopa α), 3.85–3.60 (m, 8 H, OMe's and CH_2), 3.14–3.02 (m, 1 H, β), 2.68–2.50 (m, 1 H, β), 1.38 (br overlapping singlets, 9 H, *t*-Bu), 0.98–0.85 (overlapping singlets, 54 H, Si-*t*-Bu's), 0.20–0.08 (overlapping singlets, 36 H, Si-CH_3's); IR (film on NaCl plate) cm^{-1} 3300 (br), 3060, 2925, 2855, 1715, 1675, 1490, 1255, 838, 830. Step b. The above selenide (215 mg, 0.142 mmol) was dissolved in 5.0 mL of 1,4-dioxane. $NaIO_4$ (152 mg, 0.71 mmol) dissolved in 6.0 mL of 2:1 dioxane/water was added to the selenide solution in 1 portion. Within a minute of the addition of fluffy white solid formed. The mixture was stirred for 4.5 h at room temperature; although TLC (3% MeOH/CH_2Cl_2) showed a small amount of starting material, the reaction was stopped at this time due to competitive formation of side products. The reaction mixture was taken up in 40 mL of EtOAc + 10 mL of water and transferred to a separatory funnel. The layers were separated, and the organic phase was washed with water (3 × 15 mL) and then brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was diluted with cyclohexane (2 × 20 mL) and concentrated to remove dioxane azeotropically. Preparative TLC (3% MeOH/CH_2Cl_2, 2 × 1000 μ silica plates, developed 2×) afforded 114 mg (61%) of **13** as a clear colorless glass: TLC 3% MeOH/CH_2Cl_2, brown oval (vanillan) R_f 0.49 (starting selenide R_f 0.59); FAB-MS (*m*-nitrobenzyl alcohol) 1313 (M + 1), 1335 (M + Na); ^{1}H NMR ($CDCl_3$) δ 8.90–8.70 (m, 1 H, styryl NH), 7.21–7.11 (m, 1 H, C=CH—N), 7.06–6.98 (m, 1 H, phos. NH), 6.43 (s, 2 H, styryl Ar), 6.33, 6.31 (overlapping singlets, 2 H, hydroxy-Dopa Ar), 6.19–6.08 (overlapping doublets, J = 14.6 Hz, 1 H, ArCH=C), 5.22–5.05 (m, 1 H, α phos.), 4.75 (br s, 1 H, BOC-NH), 4.14 (m, 1 H, hydroxy-Dopa α), 3.9–3.7 (m, 6 H, OMe's), 3.10 (m, 1 H, β), 2.78–2.49 (m, 1 H, β), 1.38 (overlapping singlets, 9 H, *t*-Bu), 0.96–0.90 (overlapping singlets, 54 H, Si-*t*-Bu), 0.18–0.09 (overlapping singlets, 36 H, Si-CH_3).

(*E*)/(*Z*)-*N*-BOC-nonakis-TBDMS-An-1 (14)/(15) (Scheme III, Step c). A stock solution of 0.5 N lithium diisopropyl amide in THF was prepared by dropwise addition of 1.47 mL (2.5 mmol) of 1.7 N *n*-BuLi (hexane) to a −78 °C solution of dry diisopropylamine (385 μL, 2.75 mmol) in 3.14 mL of dry THF. After completion of the addition the mixture was stirred for 10 mins. Phosphonate **13** (108 mg, 82.3 μmol) was dissolved in 400 μL of THF and cooled to −78 °C. The LDA

solution (180 μL, 90.5 μmol, 1.1 equiv was added to the phosphonate dropwise at −78 °C, resulting in the immediate formation of a red-orange colored solution. The reaction mixture was stirred for 5 min and then 41 mg (82.3 μmol) of protected gallaldehyde **5** in 100 μL of THF was added in 1 portion. The reaction mixture was warmed to room temperature and stirred for 4 h; TLC monitoring of the reaction indicated no further disappearance of gallaldehyde. The reaction mixture was concentrated in vacuo, and the residue was applied to a 150 × 15 mm silica column. The column was flushed with 100 mL of 15:1 hexane/EtOAc to remove unreacted aldehyde, leaving the bright yellow tunichrome on the column. The TC was eluted from the column with 9:1 H/EtOAc to afford, after concentration, 113 mg (82%) of *E*/*Z* tunichrome; TLC (10:1 hexane/EtOAc) showed primarily the *E* isomer at R_f 0.34 and a trace of the *Z* isomer at R_f 0.40; under longwave UV irradiation the *E* and *Z* isomers fluoresce with orange and yellow colors, respectively. ^{1}H NMR ($CDCl_3$) integration of unique aryl singlets for each isomer of the mixture showed the *E* isomer to predominate about 25:1. Preparative TLC (10:1 hexane/EtOAc) affords the pure *E* isomer: FAB-MS (*m*-nitrobenzyl alcohol/DMSO) 1683 (M + 1); UV (hexane) λ_{max} = 314 nm, *e* = 15 000; ^{1}H NMR ($CDCl_3$) δ 8.76 (br s, 1 H, 19N-H), 8.19 (s, 1 H, 12-H), 7.46 (d, *J* = 10.7, 1 H, 9N-H), 7.11 (dd, *J* = 10.7, 14.5, 1 H, 8-H), 6.43 (s, 2 H, Ar B), 6.33 (s, 2 H, Ar C), 6.32 (s, 2 H, Ar A), 5.39 (d, *J* = 14.5, 1 H, 7-H), 4.81 (br d, *J* = 7.2, 1 H, BOC-NH), 4.37 (m, 1 H, 21-H), 2.96 (m, 2 H, 22,22′-H), 1.42 (s, 9 H, *t*-Bu), 1.00–0.87 (m, 81 H, Si-*t*-Bu), 0.20–0.07 (m, 54 H, Si-CH_3).

(*Z*)-*N*-BOC-nonakis-TBDMS-An-1 (15) (Scheme III, Step 3). **(*E*)-14** (37 mg, 22 μmol) was dissolved in 20 mL of HPLC grade hexane in a Pyrex round-bottomed flask and deoxygenated for 15 min by rapid passage of Ar through the solution. The solution was irradiated at 300 nm with a Rayonet RPR-3000 Å fluorescent lamp fixed at a distance of about 7 cm from the reaction vessel and surrounded with aluminum foil, which served as a crude reflector. The reaction, monitored by TLC, ran for a total of 16 h over which time the mixture deepened in yellow color. TLC indicated a mixture of *E* and *Z* isomers with the *Z* isomer in preponderance. The hexane was removed in vacuo to afford a viscous yellow oil which was purified by preparative TLC (10:1 H/EtOAc, 1000 μ plate) to afford 12 mg of the starting *E* isomer and 21 mg (57%) of the desired **(*Z*)-15** (84% based on recovered *E* isomer): TLC 10:1 H/EtOAc, yellow fluorescent spot, R_f 0.40; FAB-MS (*m*-nitrobenzyl alcohol/DMSO) 1683 (M + 1); UV (hexane) λ_{max} = 328 nm, ε 34 000; ^{1}H NMR ($CDCl_3$) δ 9.26 (d, *J* = 10.2 Hz, 1 H, 9N-H), 7.59 (s, 1 H, 12-H), 7.39 (dd, *J* = 10.2, 14.6 Hz, 1 H, 8-H), 7.18 (s, 1 H, 19N-H), 6.63 (s, 2 H, ArB), 6.47 (s, 2 H, ArA), 6.32 (s, 2 H, ArC), 6.29 (d, *J* = 14.6 Hz, 1 H, H7), 4.80 (br d, *J* = 2.0 Hz, 1 H, BOC-NH), 4.00 (m, 1 H, 21-H), 3.29 (m, 1 H, 22-H), 2.59 (m, 1 H, 22′-H), 1.40 (s, 9 H, *t*-Bu), 1.0–0.8 (m, 81 H, Si-*t*-Bu), 0.18–0.04 (m, 56 H, Si-CH_3); IR (film on NaCl plate) cm^{-1} 3300 (br), 2930, 2858, 1693, 1565, 1487, 1254, 1086, 835, 828.

N-Deprotection of (*Z*)-15 (Scheme III, Step e). **(*Z*)-15** (18 mg, 11 μmol) was dissolved n 1.0 mL of dry CH_2Cl_2 under Ar and cooled to 0 °C. Freshly distilled TFA (0.25 mL) was added to the solution dropwise over 5 min; the initially yellow reaction mixture rapidly turned yellow-orange with addition of TFA. The mixture was warmed to room temperature and stirred for about 1 h, over which time the orange color faded and TLC indicated complete disappearance of starting material. The reaction mixture was frozen in a dry ice/acetone bath and placed on a rotary evaporator equipped with an efficient pump; the mixture was then concentrated to afford a dark yellow glass [TLC 5:1 H/EtOAc showed one yellow (vis) tailing band at R_f 0.5]. Under longwave UV irradiation, this band gave an orange fluorescense; a very small amount of streaking fluorescent blue material was observed at lesser R_f. The crude material was carried immediately to the next step.

Cleavage of TBDMS Groups (Scheme I, Step f). In the following work involving the deprotected An-1, care was taken to prevent contact of the product TC with the atmosphere and trace metals (HPLC grade, degassed solvents; degassed, glass distilled water; and scrupulously cleaned glassware were employed). The crude trifluoroacetate salt of the N-deprotected nonakis-TBDMS An-1 was transfered to a polypropylene test tube in about 0.5 mL of CH_2Cl_2, concentrated to dryness under a gentle stream of Ar, and then placed on a vacuum pump for a few minutes. Meanwhile, a solution of distilled pyridine (0.335 mL), 48% aqueous HF (0.086 mL), and ca. 10 μL of *tert*-butyl sulfide in a polypropylene test tube was degassed for 30 mins by passage of Ar through the solution. With a sufficient length of Teflon tubing and a plastic syringe, the pyridine/HF solution was transferred to the N-deprotected tunichrome residue. The mixture was stirred for 5.5 h under Ar; over this period the yellow color of the reaction mixture intensified slightly.

Purification of Deprotection Reaction Mixture by Precipitation, 16 (Scheme III, Step g). All solvents in the following procedure were carefully degassed prior to use. Centifugations were performed in the original polypropylene reaction vessel. The crude reaction mixture from above was diluted dropwise with ca. 2.0 mL of 2:1 dichloromethane/*i*-PrOH. During the addition the mixture became cloudy, and finally a dark yellow precipitate collected at the bottom of the tube. Hexane (1.0 mL) was added to the stirred mixture to precipitate more TC; a pale yellow supernate remained, which was drawn off. The residue was dissolved in 0.5 mL of MeOH; 1.5 mL of dichloromethane was added slowly to the stirred yellow methanolic TC solution to provide a voluminous fluffy yellow precipitate. The mixture was centrifuged for about 10 min to pellet the bright yellow tunichromes. The pale yellow supernate was drawn off, and the yellow pellet dissolved in 0.4 mL of MeOH. Another 1.5 mL of dichloromethane was added to reprecipitate the TC, and the mixture was centrifuged. The yellow pellet was then stirred in 2.0 mL of dichloromethane and centrifuged, and the supernate was removed. The bright yellow tunichrome was pumped to remove remaining dichloromethane. The dry yellow solid was analyzed by UV spectroscopy in methanol; based on the reported[1a] ε value of 19 600 at 340 nm the yield for the two step deprotection and isolation was 90% [^{1}H NMR (MeOH-d_4, [Mg/mL] ≈ 13) δ 7.29 (d, *J* = 14.6, 1 H, 8-H), 7.00 (s, 1 H, 12-H), 6.64 (s, 2 H, Ar), 6.38 (s, 4 H, Ar's), 6.19 (d, *J* = 14.6, 1 H, 7-H), 4.15 (m, 1 H, 21-H), 3.35 (m, 1 H 22-H), 2.73 (m, 1 H, 22′-H)]. The tunichrome was lyophilized from 0.5% aqueous formic acid to afford the formate salt: ^{1}H NMR (pyridine-d_5, [Mg/mL] ≈ 13) δ 10.59 (d, *J* = 10.3, 1 H, 9N-H), 8.78 (s, 1 H, formyl-H), 8.06 (dd, *J* = 10.3, 14.8, 1 H, 8-H), 7.62 (s, 1 H, 12-H), 7.34 (s, 2 H, Ar), 7.00 (s, 2 H, Ar), 6.86 (s, 2 H, Ar), 6.42 (d, *J* = 14.8, 1 H, 7-H), 4.18 (dd, *J* = 4, 10, 1 H, 21-H), 3.68 (dd, *J* = 4, 14, 1 H, 22-H), 3.05 (dd, *J* = 10, 14, 1 H, 22′-H); IR (KBr) cm^{-1} 3280 (br), 1670, 1615, 1516, 1448, 1310, 1296, 1201, 1033, 625. Attempts to obtain a molecular ion with SIMS-MS (ethanolamine) and FAB-MS (glycerol, thioglycerol, and *m*-nitrobenzyl alcohol) failed.

(±)-An-1 Peracetate 17. Synthetic An-1 **16** (5 mg) was stirred in 1:1 pyridine/acetic anhydride for 1 h, following the literature proceedure.[1a] TLC analysis (4% *i*-PrOH/CH_2Cl_2) showed that the reaction proceeded to form a complex mixture of products, as previously reported.[1a] The mixture was concentrated, and tunichrome peracetate was purified by preparative TLC (4% *i*-PrOH/CH_2Cl_2, 250 μ silica plate). This material was identical with natural An-1 peracetate by TLC: R_f 0.22, 4% *i*-PrOH/CH_2Cl_2. HPLC comparison of natural and synthetic materials showed identical retention times for the two compounds and produced one sharp peak upon co-injection with a retention time of 4.0 min (Perkin-Elmer Cartridge, 4.5 × 30 mm, 3 m silica; 2% *i*-PrOH/$CHCl_3$, flow = 1.5 mL/min, detection at 320 nm). Spectroscopic data for the synthetic An-1 acetate **17** demonstrated that it was identical with the naturally derived material: DCI-MS (CH_4) 976 (M + 1); DCI-MS (NH_3) 976 (M + 1), 993 (M + 18); high resolution FAB-MS (*m*-nitrobenzyl alcohol) calculated for $C_{45}{}^{13}CH_{45}N_3O_{21}$ 976.2579, found 976.2583; ^{1}H NMR ($CDCl_3$) δ 9.17 (d, *J* = 11, 1 H, 9N-H), 7.73 (s, 1 H, 19N-H), 7.49 (dd, *J* = 11, 15, 1 H, 8-H), 7.40 (s, 1 H, 12-H), 7.15 (s, 2 H, ArB), 7.06 (s, 2 H, ArA), 7.00 (s, 2 H, ArC), 6.63 (d, *J* = 6, 1 H, NHAc), 6.32 (d, *J* = 15, 1 H, 7-H), 4.31 (ddd, *J* = 4, 6, 11, 1 H, 21-H), 3.31 (dd, *J* = 4, 14, 1 H, 22-H), 3.05 (dd, *J* = 11, 14, 1 H, 22′-H), 2.3–2.3 (m, 27 H; OAc's), 1.96 (s, 3 H, N-$COCH_3$).

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Registry No. **1a**, 121209-14-1; **2**, 100945-15-1; **3**, 121056-85-7; **4**, 99-24-1; **5**, 121056-86-8; (*Z*)-**6**, 121056-87-9; (*E*)-**6**, 121056-99-3; **7**, 121056-88-0; **8**, 100945-13-9; [(±)-*R**,*R**]-**9a**, 121057-00-9; [(±)-*R**,*S**]-**9a**, 121057-01-0; [(±)-*R**,*R**]-**9b**, 121057-02-1; [(±)-*R**,*S**]-**9b**, 121124-05-8; **10**, 121056-90-4; **11**, 121056-91-5; **12a**, 121056-92-6; **12b**, 121056-93-7; [(±)-*R**,*R**]-**13**, 121057-03-2; [(±)-*R**,*S**]-**13**, 121124-06-9; **14**, 121072-72-8; **15**, 121056-94-8; **15** (R = TBDMS, R′ = NH_2·F_3CCOOH), 121072-74-0; **16**, 121249-15-8; **17**, 121153-19-3; (±)-3,4,5-(TBDMSO)$_3$$C_6H_2CH(OH)CH_2NH_2$, 121056-89-1; (±)-(BOC)NHCH(COOMe)P(O)(OMe)$_2$, 121056-95-9; (±)-(BOC)NHCH(COOH)P(O)(OMe)$_2$, 121056-96-0; 3,4,5-(TBDMSO)$_3$$C_6H_2COOMe$, 121056-97-1; 3,4,5-(TBDMSO)$_3$$C_6H_2CH_2OH$, 121056-98-2; 3,4,5-(TBDMSO)$_3$$C_6H_2CH$(4-$O_2NC_6H_4Se$)$CH_2NHCOCH$[P(O)(OMe)$_2$]$NHCOCH$[NH(BOC)]-$CH_2C_6H_2$-3,4,5-(TBDMSO)$_3$, 121057-04-3.