Antineoplastic Agents 500. Narcistatin[†,1]

George R. Pettit,* Noeleen Melody, Michael Simpson, Michael Thompson, Delbert L. Herald, and John C. Knight

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-2404

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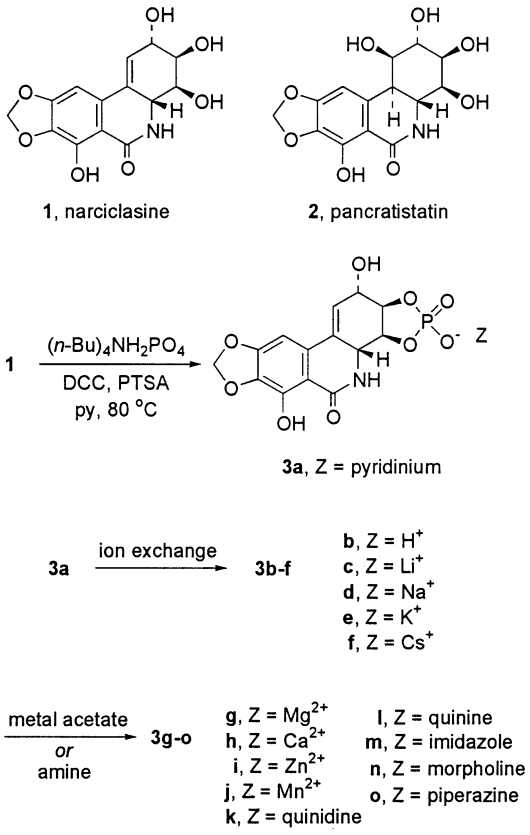
An efficient procedure was found for synthetic conversion of the sparingly soluble anticancer isocarbostyril narciclasine (**1**), a component of various *Narcissus* species, to a cyclic phosphate designated narcistatin (**3b**). The reaction between narciclasine, tetrabutylammonium dihydrogen phosphate, and *p*-toluenesulfonic acid in pyridine afforded pyridinium narcistatin (**3a**) in reasonable yields. Transformation of narcistatin (**3a**) to, for example, the water-soluble prodrug sodium narcistatin (**3d**) was easily achieved by cation exchange chromatography. Narcistatin (**3b**) and 15 salt derivatives were evaluated against a panel of human cancer cell lines, and the range (0.1−0.01) of GI_{50} values in μg/mL was found to parallel that shown by the parent narciclasine.

Over 30 species representing 11 genera (among 85 total) of the plant family Amaryllidaceae have been employed in traditional treatments for human cancer. Such applications of certain *Narcissus* species were recorded as early as 200 B.C.[2] The biologically active constituents of Amaryllidaceae species have been under investigation from at least 1877[3] following Gerrard's report on a component of *Narcissus pseudonarcissus* designated narcissia.[3a] Presently, some 48 alkaloids and carbostyrils bearing a variety of carbon skeletons have been isolated from *Narcissus* species.[4a] Of these, the isocarbostyrils narciclasine (**1**) and pancratistatin (**2**)[2,5] have been found to display the most promising in vivo antineoplastic activities,[5,6] and a selection of other amaryllidaceae alkaloids has been shown to provide cancer cell growth inhibitory activity.[4b]

Pancratistatin (**2**), which we first discovered[5] in *Pancratium littorale* (reidentified as *Hymenocallis littoralis*) and later[2] in *Narcissus* species, has been undergoing extended preclinical development. That very important initiative was greatly assisted by conversion of the sparingly soluble isocarbostyril to a 7-*O*-phosphate salt.[7] The antimitotic activity of narciclasine (**1**) has been known for over 35 years.[6] Subsequently, it was shown in U.S. National Cancer Institute research to be active against in vivo growth of the M5076 sarcoma and P388 lymphocytic leukemia.[6] In addition, it was found to inhibit protein synthesis in Erlich ascites cancer cells.[6] However, as with the closely related pancratistatin (**2**), the low solubility properties of narciclasine have contributed to the delay in its preclinical development. Most of our early investigations involving this potentially useful isocarbostyril have targeted its use as a starting point for a practical[5a] synthesis[5b] of pancratistatin (**2**) and for SAR purposes.[8] Now we are pleased to report a very convenient transformation of narciclasine (**1**) to water-soluble cyclic phosphate prodrugs (**3**).

Results and Discussion

Early experience[9] by one of us (G.R.P.) in nucleotide chemistry involving phosphate esters and cellular phosphatases combined with recent successes in the synthesis of phosphate[7,10] prodrugs[11] made such an approach most attractive for obtaining a water-soluble narciclasine prodrug. However, a selection of the more obvious methods[7,12] such as $POCl_3$[12a] or 2-cyanoethyl phosphate[12b] with dicyclohexylcarbodiimide (DCCI)[13] and various unprotected or protected (e.g., narciclasine 3,4-acetonide) strategies involving narciclasine (**1**) led only to unpromising mixtures. Eventually, we examined the use of the readily soluble tetrabutylammonium dihydrogen phosphate in pyridine as the phosphate source. Initially, the phosphate failed to couple with narciclasine in the presence of DCCI until 3 equiv of *p*-toluenesulfonic acid was employed to promote condensation, at which point precipitation of dicyclohexylurea (DCU) began. When the reaction mixture was heated to 80 °C, the pyridinium salt of narciclasine-3,4-cyclic phosphate **3a** (herein designated pyridinium narcistatin) precipitated. Following collection of precipitated DCU and



[†] Dedicated to the memory of Prof. Thomas A. Connors, an extraordinarily effective advocate of new anticancer drug discovery and development, who passed away February 3, 2002 (b. 1934).

* To whom correspondence should be addressed. Tel: (480) 965-3351. Fax: (480) 965-8558.

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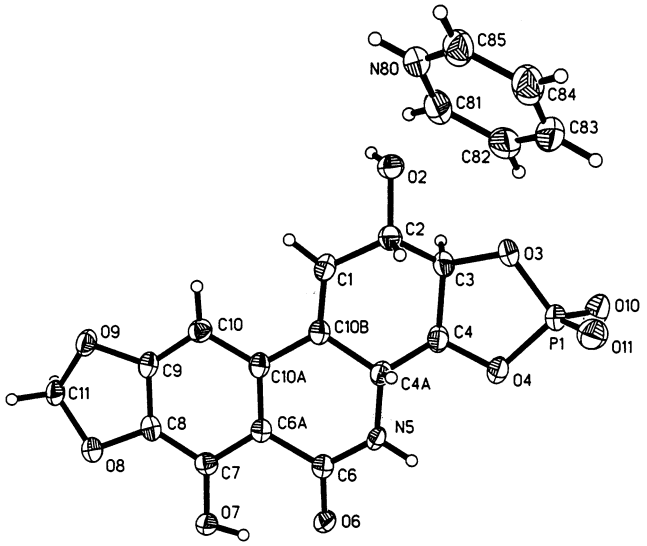

Figure 1. X-ray structure of pyridinium narcistation (**3a**).

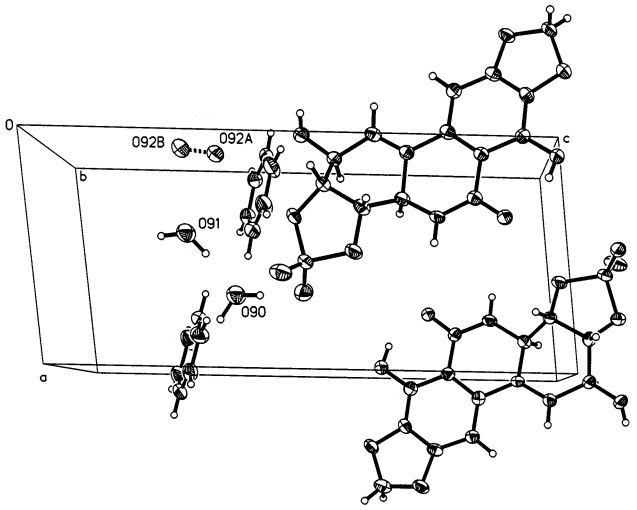

Figure 2. X-ray cell contents of pyridinium narcistatin hydrate (**3a**).

the narcistatin pyridinium salt, the solids were triturated with water to dissolve the cyclic phosphate (**3a**). Concentration of the water fraction afforded the pyridinium salt in 40% yield. The mother liquor was concentrated to a brown oil and added to a large volume of water; an immediate precipitate was observed. The solution was filtered, and the filtrate was found to be primarily unreacted narciclasine with some DCU as impurity. The reaction did not go to completion even after prolonged stirring and addition of more reagents.

Examination of the ^{1}H NMR (DMSO-d_6) spectrum of the pyridinium salt **3a** showed a multiplet corresponding to the signals for four protons at 4.42−4.31 ppm and a doublet of doublets corresponding to the signal for one proton at 4.15 ppm. Assuming four ring hydrogens resonating in this region, the signal for H-1 was assigned downfield at 6.5 ppm. Only one of the signals corresponded to a hydroxyl group. A D_2O experiment resulted in a considerable change in the splitting pattern of the multiplets at 4.3 and 8.60 ppm, suggesting loss of the OH signal and NH-5 signal, respectively. Other signals at 13.66 and 9.00 were also absent from the D_2O-treated spectrum owing to deuterium exchange with OH-7 and pyridinium NH. The ^{31}P NMR (DMSO-d_6) spectrum gave one signal at 20.3 ppm, suggesting only one phosphorus atom. This together with the ^{1}H NMR data suggested the formation of the cyclic phosphate. However, despite extensive 2D NMR experiments, the position of the phosphate could not be established unambiguously. Consequently, narciclasine pyridinium salt (**3a**) was recrystallized from pyridine−water and examined by X-ray crystallography to establish the 3,4-cyclic phosphate structure. The resulting structure of **3a** is depicted in Figure 1. In addition to two pyridinium cations and two cyclic phosphate anions, the unit cell was found to contain three molecules of water solvate, as shown in Figure 2.

To extend the narcistatin cation series, phosphoric acid **3b** was prepared by dissolving the pyridinium narcistatin in water and passing it through a column containing Dowex 50W X8 200 cation-exchange resin (hydrogen form). A solution of the pyridinium narcistatin in water was also used to prepare the lithium (**3c**), sodium (**3d**), potassium (**3e**), and cesium (**3g**) salts of narcistatin by passage through a Dowex 50W X2 column bearing the respective cations. The magnesium (**3g**), calcium (**3h**), zinc (**3i**), and manganese (**3j**) salts were obtained by suspending phosphoric acid **3b** in methanol−water (3:2) and adding 0.5 equiv of the respective metal acetate in water. The resulting opaque solution was stirred for several days as the salt precipitated from solution. These dication salts proved to be only sparingly soluble in water. A selection of ammonium salts were prepared by allowing phosphoric acid **3b** to react with the respective amine (1.2 equiv) at room temperature. The reaction mixture was concentrated, and product precipitated to give ammonium salts **3k**−**o**.

Narciclasine cyclic phosphate prodrugs **3a**−**o** were evaluated against a minipanel of human cancer cell lines and murine P388 lymphocytic leukemia. Results of the cancer cell line evaluation of narcistatins **3a**−**o** appear in Table 1. The GI_{50} 0.1−0.02 μg/mL strong activity range parallels that already reported for the parent, narciclasine (**1**).[5a]

In summary, the very successful conversion of narciclasine to a water-soluble (60 mg/mL for sodium salt **3d**) cyclic phosphate prodrug will now allow this potentially useful *Narcissus* anticancer component to be further developed.

Experimental Section

Narciclasine (**1**) was isolated from *Hymenocallis littoralis* (Jacq.) Salisb (Amaryllidaceae), grown by our group in Tempe, Arizona.[2] Reagents were purchased from Aldrich Chemical unless otherwise noted and used as received. Solvents were distilled prior to use, and pyridine preceding distillation was dried over potassium hydroxide pellets. Dowex 50X8-200 and Dowex 50WX2 cation-exchange resins (H^+ form) were washed with methanol, 1 N hydrochloric acid, and deionized water. The cation forms of the resin were obtained by washing with a 1 N solution of the appropriate base followed by deionized water. DEAE Sephadex A-25 weak anion-exchange resin (acetate form) was purchased from the Sigma-Aldrich Company and was washed with 1 N triethylammonium bicarbonate (TEAB) solution and then equilibrated with 10 mN TEAB buffer solution.

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Thin-layer chromatography was performed on Analtech silica gel GHLF plates, and the narciclasine-containing derivatives were visible as green-blue fluorescent spots under long-wave ultraviolet light and were rendered permanent by staining with iodine vapor. Phosphorus-containing compounds were detected using the modified Jungnickel's reagent (perchloric acid−malachite green−sodium molybdate) developed by Vaskovsky and Latshev.[13] Optical rotation values were recorded using a Perkin-Elmer 241 polarimeter. High-resolution FAB spectra were

Table 1. Solubilities and Human Cancer Cell Line and Murine P-388 Lymphocytic Inhibitory Activities of Cyclic Phosphates **3**−**16**

		ED_{50} (μg/mL)	GI_{50} (μg/mL)					
compound	solubilities[a] (mg/mL)	leukemia P388	pancreas-a BXPC-3	breast MCF-7	CNS SF 268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145
3a	7	1.91×10^{-1}	2.2×10^{-1}	2.7×10^{-1}	1.5×10^{-1}	2.7×10^{-1}	3.4×10^{-1}	1.7×10^{-1}
3b	4	2.75×10^{-1}	3.3×10^{-1}	3.5×10^{-1}	2.2×10^{-1}	4.7×10^{-1}	5.3×10^{-1}	1.6×10^{-1}
3c	>50	1.21×10^{-1}	2.5×10^{-1}	3.1×10^{-1}	1.7×10^{-1}	3.0×10^{-1}	2.6×10^{-1}	1.3×10^{-1}
3d	60	2.55×10^{-1}	3.2×10^{-1}	5.6×10^{-1}	2.3×10^{-1}	>1	4.5×10^{-1}	1.2×10^{-1}
3e	11	2.42×10^{-1}	3.6×10^{-1}	4.0×10^{-1}	1.9×10^{-1}	6.7×10^{-1}	5.6×10^{-1}	2.6×10^{-1}
3f	<13	1.83×10^{-1}	4.1×10^{-1}	6.2×10^{-1}	3.3×10^{-1}	>1	6.6×10^{-1}	1.3×10^{-1}
3g	<1.5	1.70×10^{-1}	1.9×10^{-1}	2.5×10^{-1}	1.4×10^{-1}	2.9×10^{-1}	3.1×10^{-1}	1.0×10^{-1}
3h	<1	2.23×10^{-2}	4.5×10^{-2}	5.9×10^{-2}	3.1×10^{-2}	1.2×10^{-1}	5.9×10^{-2}	9.3×10^{-3}
3i	1.7	2.87×10^{-2}	6.9×10^{-2}	1.4×10^{-1}	5.3×10^{-2}	2.1×10^{-1}	1.6×10^{-1}	1.6×10^{-2}
3j	<3	4.27×10^{-2}	4.9×10^{-2}	7.0×10^{-2}	4.0×10^{-2}	1.5×10^{-1}	1.3×10^{-1}	3.4×10^{-2}
3k	<1	2.71×10^{-1}	3.1×10^{-1}	5.0×10^{-1}	2.5×10^{-1}	7.7×10^{-1}	5.8×10^{-1}	2.2×10^{-1}
3l	<1	3.42×10^{-2}	5.1×10^{-2}	1.2×10^{-1}	4.5×10^{-2}	1.7×10^{-1}	1.2×10^{-1}	1.3×10^{-2}
3m	5.8	2.40×10^{-1}	4.5×10^{-1}	9.0×10^{-1}	3.8×10^{-1}	>1	>1	4.4×10^{-1}
3n	>13	2.32×10^{-1}	2.5×10^{-1}	4.8×10^{-1}	2.4×10^{-1}	>1	5.4×10^{-1}	1.4×10^{-1}
3o	1.9	3.78×10^{-2}	1.0×10^{-1}	1.7×10^{-1}	9.9×10^{-2}	2.4×10^{-1}	2.2×10^{-1}	3.2×10^{-2}

[a] Solubility values were obtained with 1 mL of distilled water at 25 °C.

obtained using a JEOL LCMate magnetic sector instrument either in the FAB mode, with a glycerol matrix, or by APCI with a poly(ethylene glycol) reference. All ^{1}H NMR spectra were obtained using a Varian Gemini 300 MHz instrument unless otherwise noted. The ^{13}C, ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMBC, ^{1}H−^{13}C HMQC, and ^{31}P NMR experiments were conducted employing a Varian Unity 500 MHz instrument.

Pyridinium Narcistatin (3a). Narciclasine **1** (1.0 g, 3.4 mmol) was added to pyridine (50 mL), and the solution was heated to 80 °C. Next, tetrabutylammonium dihydrogen phosphate (5.13 g, 15.11 mmol, 4.4 equiv), dicyclohexylcarbodiamide (5.0, 24.5 mmol, 7.0 equiv), and *p*-toluenesulfonic acid (3.0 g, 15.8 mmol, 4.63 equiv, added slowly) were added. After 2 g of the sulfonic acid was added, a precipitate began to separate. The reaction mixture was stirred under argon at 80 °C for 2.5 h. The precipitate was collected and washed with methanol to remove pyridine. The precipitated cyclic phosphate (**3a**) was separated from the DCU by washing with water (200 mL). The aqueous filtrate was concentrated to an off-white solid and dried (vacuum) overnight to yield 0.59 g, 40.4%. The mother liquor was concentrated to a brown oil, and water (750 mL) was added. An immediate precipitate was observed, which was collected and dried to 0.75 g of white solid. The ^{1}H NMR (DMSO-d_6) showed this material to be recovered starting material with a small amount of DCU impurity. Recrystallization of phosphate **3a** from pyridine−water gave crystals that were used for X-ray crystallography. $[\alpha]^{26}_D$ −6.4° (*c* 0.44, DMSO); mp 275 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 13.66 (s, 1H), 9.00 (s, 1H), 8.60 (m, 3H), 7.9 (t, J = 7.5 Hz, 1H), 7.5 (m, 2H), 7.04 (s, 1H), 6.5 (s, 1H), 6.06 (d, J = 3 Hz, 2H), 4.42−4.31 (m, 4H), 4.15 (dd, J_{14}, 6.5 Hz, 1H); ^{13}C NMR (DMSO, 500 MHz) δ 167.7, 152.6, 148.6(2), 145.2, 137.4(2), 133.5, 128.5, 126.9, 125.3, 124.4, 104.3, 102.1, 94.3, 76.9, 76.7, 70.4, 53.9; ^{31}P (DMSO-d_6, 200 MHz) 20.3 (s, 1P); found by HRAPCl (negative ions) MS 368.0179, calc for $C_{14}H_{11}O_9NP$ 368.0171.

Crystal Structure of Pyridinium Narcistatin (3a). X-ray Crystal Structure Determination. Pyridinium narcistatin hydrate (**3a**): A thin plate (~0.07 × 0.35 × 0.54 mm), grown from a pyridine−water solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 123 K with a Bruker SMART 6000 diffractometer system using Cu Kα radiation. A sphere of reciprocal space was covered using the multirun technique.[14] Thus, six sets of frames of data were collected with 0.396° steps in ω, and a last set of frames with 0.396° steps in φ, such that 91.7% coverage of all unique reflections to a resolution of 0.84 Å, was accomplished.

Crystal Data: $C_{14}H_{11}NO_9P \cdot C_5H_6N \cdot 1^1/_2H_2O$ (hydrate), M_r = 475.34, triclinic, *P*1, a = 7.4949(1) Å, b = 8.0371(1) Å, c = 16.9589(2) Å, α = 85.248(1)°, β = 83.243(1)°, γ = 79.383(1)°, V = 994.60(2) A^3, Z = 2, ρ_c = 1.577 Mg/m^3, μ(Cu Kα) = 1.837 mm^{-1}, λ = 1.54178 Å, F(000) = 494.

A total of 7587 reflections were collected, of which 4733 reflections were independent reflections (R(int) = 0.0273). Subsequent statistical analysis of the data set with the XPREP[15] program indicated the space group was *P*1. Final cell constants were determined from the set of the 4564 observed (>2σ(I)) reflections, which were used in structure solution and refinement. An absorption correction was applied to the data with SADABS.[16] Structure determination and refinement were readily accomplished with the direct-methods program SHELXTL.[17] All non-hydrogen atom coordinates were located in a routine run using default values for that program. The remaining H atom coordinates were calculated at optimum positions, except for water hydrogen atoms, which were located via difference maps. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement procedure. The H atoms were included, and their U_{iso} thermal parameters were fixed at either 1.2 or 1.5 (depending on atom type) times the value of the U_{iso} of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for **3a** was 0.0393 for observed data and 0.0403 for all data. The goodness-of-fit on F^2 was 1.053. The corresponding Sheldrick R values were wR_2 of 0.1074 and 0.1099, respectively. The final model used for pyridinium narcistatin **3a** is shown in Figure 1. In addition to the parent molecules (i.e., two narcistatin anions and two pyridinium cations) in the unit cell, three molecules of water solvate were also present. One of these water molecules was disordered over two sites, each of which were given 0.5 site occupancies. A final difference Fourier map showed minimal residual electron density, the largest difference peak and hole being +0.350 and −0.255 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits.

Narcistatin (3b). A solution of pyridinium narcistatin (**3a**, 0.05 g) in water (2 mL) was obtained by heating (water bath) at 60 °C. The solution was allowed to cool prior to passing through a column prepared from Dowex 50X8-200 cation-exchange resin (hydrogen form). A suspension began to form in the column as the phosphoric acid (**3b**) formed. The column was eluted with water, and phosphoric acid **3b** eluted as a milky white suspension. The combined fractions containing phosphoric acid **3b** were freeze-dried to afford the product as a colorless solid (36 mg, 86%); mp 175 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz), δ 13.65 (s, 1H), 9.02 (s, 1H), 7.06 (s, 1H), 6.48 (s, 1H), 6.17 (d, J_{ab} = 10.2 Hz, 1H), 6.06 (m, 2H), 4.46−4.30 (m, 3H), 4.18 (m, 1H); calc for $C_{14}H_{13}NO_9P$ 370.0328; found by HR (APCI) $[M + H]^+$ 370.0361.

General Procedure for Preparation of Narcistatin Prodrugs 3c−**f.** Pyridinium narcistatin (**3a**, 50 mg) was dissolved in water (35 mL) and the solution passed through a column (1 × 20 cm) of Dowex 50W-X2 bearing the respective cation. The UV-active fractions were combined and freeze-dried to give the corresponding narcistatin salt as a colorless solid

unless otherwise recorded. The solubility of each in water (mg/mL) now follows: **3c**, >50 mg; **3d**, 60 mg; **3e**, 11 mg; **3f**, <13 mg.

Lithium narcistatin (3c): yield, 65 mg, 77%; mp 220 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 13.79 (s, 1H), 8.71 (s, 1H), 7.07 (s, 1H), 6.49 (s, 1H), 6.13 (m, 2H), 4.36 (m, 2H), 4.04 (m, 1H), 3.93 (m, 1H); ^{13}C NMR (DMSO-d_6, 300 MHz), 167.6, 152.5, 145.2, 133.3, 129.1, 127.3, 125.6, 104.3, 101.9, 94.2, 75.2, 74.6, 70.4, 53.8.

Sodium narcistatin (3d): colorless solid, 38 mg, 87%); $[\alpha]^{25}_D$ −6.33 (*c* 0.3, DMSO); mp 275 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 13.72 (s, 1H) 8.63 (s, 1H), 6.99 (s, 1H), 6.41 (s, 1H), 6.05 (m, 2H), 5.77 (bs, 1H), 4.26 (m, 2H), 3.4 (m, 1H), 3.83 (m, 1H); ^{13}C NMR (DMSO-d_6, 500 MHz), 167.6, 152.5, 145.2, 133.3, 129.1, 127.3, 125.5, 104.3, 101.9, 94.2, 75.2, 74.5, 70.4, 53.9; ^{31}P (DMSO-d_6, 200 MHz) 16.98.

Potassium narcistatin (3e): off-white solid, 59 mg, 80%, mp 250 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.74 (s, 1H), 8.65 (s, 1H), 6.98 (s, 1H), 6.40 (s, 1H), 6.04 (d, J_{ab} = 2.4 Hz, 2H), 5.74 (bs, 1H), 4.25 (m, 2H), 3.9 (m, 1H), 3.78 (m, 1H).

Cesium narcistatin (3f): off-white solid, 51 mg, 91%, mp 245 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.74 (s, 1H), 8.65 (s, 1H), 6.98 (s, 1H), 6.40 (s, 1H), 6.04 (m, 2H), 5.74 (bs, 1H), 4.25 (m, 2H), 3.92 (m, 1H), 3.79 (m, 1H).

An alternative method was also developed to isolate narcistatin sodium salt **3d**. Narciclasine, tetrabutylammonium dihydrogen phosphate, DCCI, and pyridinium *p*-toluenesulfonate were allowed to react at room temperature for 2 days. The reaction was monitored by TLC using the solvent system 4:3:2:1 butanol−methanol−water−concentrated aqueous ammonia. Two major fluorescent spots were evident, narciclasine at R_f 0.65 and product at a higher R_f 0.69. Even after 4 days of stirring, the reaction was incomplete. The reaction mixture was added to water, the DCU was collected, the mother liquor was evaporated to half its volume, and 2 N aqueous ammonia was added at regular intervals to maintain a pH of 8−9. The solution was passed through a column (15 × 15 cm) of Dowex 50 (pyridinium form) in order to remove the unreacted narciclasine. Narciclasine remained bound to the resin, while the charged phosphate passed through unchanged. The column was then washed with methanol, and the unreacted narciclasine was recovered. The cyclic phosphate was separated from contaminating inorganic phosphate by anion exchange chromatography using DEAE-Sephadex and gradient elution with aqueous triethylammonium bicarbonate. The triethylammonium salt was converted to the sodium salt by passage through a Dowex 50 column (Na^+ form). ^{31}P NMR confirmed the presence of a phosphate group. The yield from this reaction was 43%. Comparison of the ^{1}H NMR of this product in D_2O with the narcistatin sodium salt **3d** prepared from the pyridinium narcistatin **3a** by the method outlined above showed them to be identical. However, this method proved less practical and did not significantly improve the yield.

General Procedure for Preparation of Narcistatin Divalent Cation Salts 3g−j. The experiment leading to magnesium salt **3g** provides the general method and relative quantities of reactants and solvents. In each case, the respective metal acetate was employed.

Magnesium Narcistatin (3g). To a mixture of phosphoric acid (**3b**, 50 mg, 0.135 mmol) and methanol−water (3:2) was added a solution of magnesium acetate (15 mg, 0.0675 mmol, 0.5 equiv) in water (1 mL). The mixture became opaque immediately upon addition of the metal acetate and was stirred for 3 days while further precipitation occurred. The solution was concentrated to a white residue, and water−methanol was added (1.4 mL). The precipitate was collected and dried: gray solid, mp 210 °C dec, very insoluble in water, soluble in DMSO; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.69 (s, 1H), 8.73 (s, 1H), 6.99 (s, 1H), 6.43 (s, 1H), 6.14 (m, 1H), 6.05 (s, 2H), 5.82 (bs, 1H), 4.41−4.31 (m, 2H), 4.03−3.95 (m, 2H). Each of the divalent cation salts proved to be only sparingly soluble in water.

Calcium narcistatin (3h): gray solid; 30 mg, mp 195 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.68 (s, 1H), 8.69 (s, 1H), 7.0 (s, 1H), 6.43 (s, 1H), 6.14 (d, J = 12.9 Hz, 1H), 6.05 (m, 2H), 4.29 (m, 2H), 4.02 (m, 1H), 3.94 (m, 1H).

Zinc narcistatin (3i): yield of gray solid, 23 mg, mp 200 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.64 (s, 1H), 8.81 (s, 1H), 6.92 (s, 1H), 6.38 (s, 1H), 6.16 (m, 1H), 6.03 (s, 2H), 5.94 (bs, 1H), 4.31 (m, 2H), 4.20−4.17 (m, 1H), 4.07 (m, 1H).

Manganese Narcistatin (3j). For this experiment, 41 mg of narcistatin (**3b**) was treated with manganese acetate (16 mg, 0.065 mmol. 0.5 equiv) in water (1 mL) to afford 35 mg of gray solid, mp 165 °C (dec): ^{1}H NMR (DMSO-d_6, 300 MHz). The salt, while quite soluble in DMSO-d_6, did not give a useful spectrum.

General Procedure for Obtaining Ammonium Salts 3k−o. Phosphoric acid **3b** (0.25 g) was dissolved in methanol−dichloromethane−water (3:1:1) (10 mL). A 2 mL aliquot of the phosphoric acid solution was added to each of the five flasks containing 1.2 equiv of the respective amine and the reaction mixture stirred for 24 h at room temperature. A precipitate separated from the reaction mixture with the quinine and imidazole examples. The solvent was concentrated and the residues were reprecipitated from water−methanol to yield each of the ammonium salts **3k−o**).

Quinidinium narcistatin (3k): cream-colored solid; 34 mg, mp 205 °C (dec, 220 °C melts); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.71 (s, 1H), 8.68 (bs, 2H), 7.90 (d, J = 8.4 Hz, 1H), 7.52 (s, 1H), 7.37−7.40 (m, 3H), 6.99 (s, 1H), 6.4 (s, 1H), 6.13−6.01 (m, 3H), 5.10 (m, 4H), 4.25 (m, 2H), 3.92 (m, 5H), 3.6−3.2 (m, 6H), 2.42 (m, 1H), 2.2−2.12 (m, 1H), 1.91−1.84 (m, 1H), 1.60 (m, 2H), 1.47−1.38 (m, 1H).

Quininium narcistatin (3l): cream-colored solid; 55 mg, mp 195 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.72 (s, 1H), 8.70 (bs, 2H), 7.93 (d, J = 8.4 Hz, 1H), 7.57 (bs, 1H), 7.45−7.39 (m, 3H), 6.99 (s, 1H), 6.41 (s, 1H), 6.05 (m, 3H), 5.80−5.73 (m, 2H), 5.07−4.93 (m, 2H), 4.25 (bs, 2H), 4.03−3.85 (m, 5H), 3.38 (m, 6H), 1.91 (m, 4H), 1.71 (m, 1H), 1.47 (m, 1H).

Imidazolium narcistatin (3m): off-white solid, 39 mg, mp 210 °C; ^{1}H NMR (DMSO-d_6 300 MHz) δ 13.73 (s, 1H), 13.4 (s, 1H), 8.71 (s, 1H), 8.06 (bs, 1H), 7.21 (bm, 2H), 6.98 (s, 1H), 6.41 (s, 1H), 6.11 (bs, 1H), 6.04 (m, 2H), 4.25 (m, 2H), 3.99 (m, 1H), 3.84 (m, 1H).

Morpholinium narcistatin (3n): off-white solid, 20 mg, mp 230 °C; ^{1}H NMR (DMSO-d_6 300 MHz) δ 13.73 (s, 1H), 8.68 (s, 1H), 6.99 (s, 1H), 6.41 (s, 1H), 6.04 (d, J = 2.7 Hz, 2H), 5.76 (bs, 1H), 4.25 (bm, 2H), 3.97 (m, 1H), 3.92−3.71 (m, 5H), 3.03 (m, 4H), 1.22 (s, 1H).

Piperazinium narcistatin (3o): off-white solid, 21 mg, mp 270 °C; ^{1}H NMR (DMSO-d_6 300 MHz) δ 13.74 (s, 1H), 8.66 (s, 1H), 6.98 (s, 1H), 6.40 (s, 1H), 6.04 (d, J = 1.8 Hz, 2H), 5.74 (bs, 1H), 4.24 (bm, 2H), 3.93 (m, 1H), 3.81 (m, 1H), 3.14 (s, 2H), 2.83 (s, 9H).

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Supporting Information Available: X-ray data for pyridinium narcistatin hydrate (**3a**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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