Antineoplastic Agents. 511. Direct Phosphorylation of Phenpanstatin and Pancratistatin[†]

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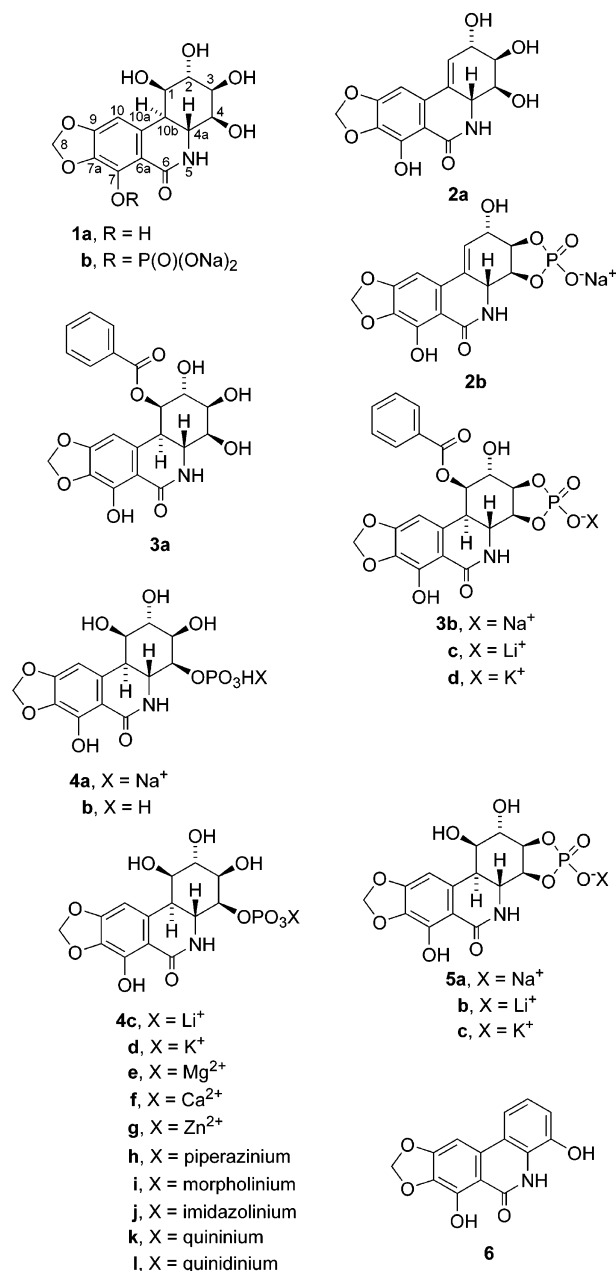
Selective phosphorylation of phenpanstatin (**3a**) with tetrabutylammonium dihydrogen phosphate and dicyclohexylcarbodiimide in pyridine followed by cation-exchange chromatographic procedures was found to provide an efficient route to a new series (**3b**−**3d**) of promising 3,4-*O*-cyclic phosphate prodrugs designated phenpanstatin phosphates. Application of analogous reaction conditions to pancratistatin (**1a**) led to a mixture of monophosphate derivatives where sodium pancratistatin 4-*O*-phosphate (**4a**) was isolated and the structure confirmed by X-ray crystallography. Modification of the reaction conditions allowed direct phosphorylation of pancratistatin followed by cation-exchange chromatography to afford sodium pancratistatin 3,4-*O*-cyclic phosphate (**5a**), which was selected for preclinical development.

In 1984, we first succeeded in isolating and elucidating the structure (by X-ray employing the 7-methoxy derivative) of (+)-pancratistatin (**1a**),[2] the principal anticancer constituent of the Amaryllidaceae tropical spider lily *Pancratium littorale*, which was later reidentified as *Hymenocallis littoralis*.[3] Because of the early promise[2b] of **1a** as a new type of anticancer and antiviral (RNA viruses)[4] drug, various phases of preclinical development[5] have been underway for over 17 years. Meanwhile, we and others have been increasingly successful in developing the availability of pancratistatin (**1a**) by horticultural[3] and synthetic approaches[6] as well as further defining SAR requirements.[7] When the preclinical drug formulation of **1a** began to present another serious challenge owing to its sparing (53 µg/mL in H_2O) solubility, we began to investigate structural modifications[5] that were expected to greatly increase aqueous solubility while serving as a successful delivery-type prodrug.[5c] Those studies led to useful syntheses (four steps)[5] of sodium pancratistatin 7-*O*-phosphate (**1b**) with considerably improved aqueous solubility (20 mg/mL).[5b]

While the 7-*O*-phosphate salt **1b** proved to have attractive aqueous solubility properties, the yield-penalizing synthetic steps from **1a** required us to continue parallel efforts to directly, but selectively, phosphorylate **1a**. The necessity of discovering more efficient techniques for converting **1a** to very effective phosphate prodrugs has been accelerating with the recent realization that the long elusive key mechanism of action by **1a** against in vivo neoplastic disease is cancer antiangiogenesis/vascular targeting.[8] Furthermore, pancratistatin (**1a**) has also recently been found to display remarkable activity against microspirochesis,[9] another potentially lethal challenge for some cancer patients.

Toward the objective of efficiently phosphorylating pancratistatin (**1a**), the more abundant but closely related Amaryllidaceae biosynthetic product narciclasine (**2a**),[10] which also required conversion to a phosphate prodrug for anticancer preclinical development, served as a model for most of the exploratory phosphorylation[11] experiments. Eventually reaction conditions were found for efficient

1a, R = H
  b, R = P(O)(ONa)₂

2a

2b

3a

3b, X = Na⁺
c, X = Li⁺
d, X = K⁺

4a, X = Na⁺
 b, X = H

4c, X = Li⁺
d, X = K⁺
e, X = Mg²⁺
f, X = Ca²⁺
g, X = Zn²⁺
h, X = piperazinium
i, X = morpholinium
j, X = imidazolinium
k, X = quininium
l, X = quinidilum

5a, X = Na⁺
b, X = Li⁺
c, X = K⁺

6

phosphorylation of **2a** with tetrabutylammonium dihydrogen phosphate using dicyclohexylcarbodiimide (DCCI) in pyridine containing *p*-toluenesulfonic acid to yield the very useful 3,4-*O*-cyclic phosphate **2b** as a pyridinium salt that separated from the reaction mixture.[12] By application of ion-exchange methods, the pyridinium salt was easily converted to a series of metal and ammonium cation derivatives.[12]

Results and Discussion

When the preceding direct phosphorylation reaction was applied to pancratistatin-1-benzoate,[6a] renamed phenpanstatin (**3a**, another of the pancratistatin series we have in preclinical development that required a suitable phosphate derivative), the main product was again the 3,4-*O*-cyclic phosphate. The crude tetrabutylammonium salt was converted by an ion-exchange and LH-20 Sephadex chromatographic separation series to the corresponding sodium salt designated sodium phenpanstatin (**3b**) and subsequently to the Li$^+$ and K$^+$ cation derivatives **3c** and **3d**. The 3,4-*O*-cyclic phosphate structure (**3b**) was well supported by the NMR spectra, with the ^{31}P NMR shift downfield at 13.0 ppm close to that of 3,4-*O*-cyclic phosphate **2b** (δ 20.3), whose structure had been confirmed by X-ray crystal structure determination,[12] as well as by other evidence that now follows.

Detailed analysis of the ^{1}H, ^{13}C, COSY, HMQC, and D$_2$O exchange experiments was carried out in an effort to assign the carbon and proton spectrum. A downfield shift of the ring protons H-3 and H-4 was observed when compared with the ^{1}H spectrum of the starting material **3a**. There were no OH-3 and OH-4 signals observed in the spectrum. The signal for the OH-2 proton was observed downfield at δ 6.13 as a broad singlet, which disappeared when a D$_2$O exchange experiment was performed.

Analysis of the COSY spectrum indicated the H-1 signal was downfield at δ 5.73 as expected, showing strong correlation peaks with the signals at δ 4.19 and 3.3 for H-2 and H-10b, respectively. The H-2 signal in turn correlated with the signal at δ 4.32 (H-3). The H-10b signal correlated with the signal at δ 4.41 (H-4a). The signal at δ 4.25 was assigned to H-4. The OH-2 signal at δ 6.13 showed a correlation peak with the signal assigned to H-2 at δ 4.19. The D$_2$O exchange experiment resulted in a downfield shift of the H$_2$O peak, which allowed one to see the signal for H-10b at δ 3.27 (D$_2$O/DMSO-d_6) as a broad doublet. The ^{13}C spectrum was examined using HMQC. There was a noticeable downfield shift of the C-3 and C-4 signals from δ 68.8 in the ^{13}C spectrum of **3a** to 74.8 (C-3) and 74.9 (C-4) in **3b**, further proof that the cyclic phosphate had been prepared.

The lithium and potassium salts (**3b** and **3c**) were prepared by passing the sodium salt through an ion-exchange column of the respective cation.

When the tetrabutylammonium dihydrogen phosphate reaction was applied to phosphorylation of pancratistatin (**1a**) using the procedure that provided 3,4-*O*-cyclic phosphates **2b** and **3b**, the result was quite different, presumably owing to the unprotected 1-hydroxy group. The reaction was performed at 80 °C over 5 days and appeared complete by 300 MHz NMR analysis at that point. The mixture of pancratistatin phosphate salts was converted to the sodium salts for ease of separation using an ion-exchange (Dowex 50WX8-200, Na$^+$ form) and Sephadex G-10 sequence. The phosphates were retained in preference to other components on the G-10 Sephadex column, and further separation by recrystallization of the phosphate

mixture from H$_2$O−MeOH yielded (3.7%) pancratistatin 4-*O*-phosphate (**4a**, ^{31}P NMR δ 4.73). The second and third crops of crystals and the mother liquor residue were found to be mixtures (^{31}P δ 6.81, 6.00, 4.90, and 3.26) of phosphates.

Detailed NMR analysis was carried out in an attempt to establish the position of the phosphate (**4a**). The ring protons were assigned using a COSY spectrum where H-10b (δ 2.96) showed strong correlation signals with H-4a (δ 3.74) and H-1 (δ 4.24). In turn, H-4a exhibited strong correlation signals with H-4 (δ 4.29). The H-3 and H-2 resonances were assigned to the narrow multiplet at δ 3.95 integrating for two hydrogens. The correlation peaks associated with this multiplet were in accord with those predicted. A D$_2$O exchange NMR experiment led to elimination of the four signals at δ 13.34 (phenolic OH), 10.22 (NH), 4.74−4.72 (OH), and 3.47 (OH). The OH resonance at δ 4.73 gave a strong correlation peak in the COSY spectrum with the resonance assigned to H-1. Therefore, this signal was assigned to OH-1. A ^{31}P NMR spectrum displayed one signal at δ 4.73, implying one phosphorus atom per molecule. An X-ray crystal structure determination was required to confirm the structure of **4a**. Recrystallization of **4a** from H$_2$O−MeOH provided a crystal suitable for crystallography.

The cyclic phosphate of pancratistatin (**5a**) was eventually prepared following a series of exploratory experiments by decreasing the tetrabutylammonium dihydrogen phosphate from 8 equiv to 1.9 equiv and maintaining an excess of dicyclohexylcarbodiimide. The *p*-toluenesulfonic acid was also decreased from 3 to 1.3 equiv. The reaction goes to completion overnight at 80 °C to yield one product by ^{1}H NMR. This method was found to be low yielding, as the workup caused decomposition of the cyclic phosphate to a 2:1 ratio of **5a**:**4a**. Recrystallization from MeOH−CH$_2$Cl$_2$ yielded pure cyclic phosphate (28%); however, further purification of the mother liquor, which contains a mixture of **5a**, **4a**, and sodium tosylate, was not achieved. The cyclic phosphate (**5a**) was eventually prepared in high yield (48% following recrystallization from H$_2$O−MeOH−CH$_2$Cl$_2$) when the *p*-toluenesulfonic acid was excluded and the reaction was allowed to proceed for 48 h with additional amounts of dicyclohexylcarbodiimide and tetrabutylammonium dihydrogen phosphate being added after 24 h.

Detailed NMR analyses using ^{1}H, ^{13}C, COSY, HMQC, and D$_2$O exchange experiments were carried out to confirm the 3,4 position of the cyclic phosphate. Analysis of the product by ^{31}P NMR shows a phosphorus peak downfield at δ 13.22 (DMSO-d_6), which indicated the presence of a cyclic phosphate group. A D$_2$O exchange experiment showed exchangeable peaks to be at δ 13.25, 8.10, 5.42, and 4.69, OH-7, N-H, OH-2, and OH-1, respectively. The COSY spectrum revealed strong correlation peaks between the multiplets at δ 2.85 (H10b), 4.27 (H-1), and 4.09 (H-4a). The peak at δ 4.02 was assigned to H-2 due to the correlation peaks observed with H-1 and the OH peak at δ 5.42. The multiplet at 4.21 was assigned to H-3 due to cross-peaks observed with the H-2 peak at δ 4.02. The multiplet at δ 4.08−4.04 was assigned to the H-4 signal.

The ^{1}H NMR spectrum of **5a** was compared with the ^{1}H NMR spectrum of **1a**. Downfield shifts of the H-3, H-4, and H-4a signals by 0.38, 0.36, and 0.36, respectively, were observed. The downfield shifts observed for H-1 and H-2 were relatively minor, 0.01 and 0.08 ppm, respectively. This proves that the cyclic phosphate was in the 3,4 position.

The lithium and potassium salts (**5b** and **5c**) were prepared by passage through an ion-exchange column

Table 1. Human Cancer Cell Line and Murine P-388 Lymphocytic Inhibitory Activities of Compounds **1a,b**, **2a,b**, **3a,b**, **4a−l**, and **5a**

compound	ED$_{50}$ (μg/mL) leukemia P-388	GI$_{50}$ (μg/mL) pancreas-a BXPC-3	breast MCF-7	CNS SF268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145
1a[2a,b]	0.017	0.02	0.023	0.014	0.032	0.025	0.015
1b[5b]	0.24	0.20	0.20	0.079	0.19	0.17	0.026
2a[10]	0.013	0.0035	0.0032	0.0031	0.0084	0.0032	0.0032
2b[12]	0.012	0.069	0.059	0.047	0.058	0.06	0.031
3a[6a]	0.0016	0.0019	0.00031	0.00055	0.0001	0.00037	0.00021
3b	0.061	0.25	0.041	0.17	0.029	0.13	0.13
4a	0.018	0.18	0.18	0.12	0.38	0.24	0.94
4b	0.047	0.36	0.43	0.35	0.42	0.33	0.20
4c	0.036	0.30	0.39	0.28	0.35	0.25	0.20
4d	0.025	0.45	0.46	0.39	0.40	0.30	0.22
4e	0.020	0.40	0.44	0.36	0.42	0.36	0.22
4f	0.21	0.25	0.36	0.26	0.27	0.23	0.12
4g	0.17	0.27	0.47	0.38	0.42	0.30	0.17
4h	0.019	0.31	0.38	0.27	0.35	0.24	0.21
4i	0.27	0.29	0.40	0.38	0.46	0.37	0.18
4j	0.063	0.25	0.33	0.21	0.25	0.13	0.13
4k	0.066	0.23	0.35	0.28	0.31	0.22	0.15
4l	0.21	0.34	0.38	0.27	0.36	0.23	0.20
5a	3.33	3.3	2.9	2.9	3.8	3.7	2.3

containing the respective ion. When the cyclic phosphate **5a** was passed through an ion-exchange column (H$^+$ form) in an effort to prepare the cyclic phosphoric acid, it decomposed to the 4-*O*-phosphoric acid (**4b**). This development led to the preparation of the 4-*O*-phosphate salts **4c−4l**.

The lithium salt (**4c**) was prepared by passage of **4b** through a column containing Dowex 50WX8-200 cation-exchange resin containing that cation. The potassium salt was then prepared from the lithium salt by dissolving **4c** in H$_2$O and passing it through a column containing Dowex 50WX8-200 cation-exchange resin (K$^+$ form).

The magnesium (**4d**), calcium (**4e**), and zinc (**4f**) salts were obtained by dissolving **4b** in MeOH and adding 1 equiv of the respective metal acetate. The resulting opaque solutions were stirred for several days as the salt precipitated from solution. The mixture was then concentrated, and the salts were washed with MeOH. A selection of ammonium salts were prepared by allowing **4b** to react with the respective amine at room temperature. The reaction mixture was concentrated and the solid washed with a suitable solvent to give the ammonium salts **4h−4l**.

The new pancratistatin phosphate prodrug series was evaluated against a minipanel of human cancer cell lines and the murine P388 lymphocytic leukemia cell line. Results of the cancer cell line evaluations compared to the parent pancratistatin (**1a**) and phenpanstatin (**3a**) appear in Table 1 and confirm retention of cancer cell line inhibitory activity comparable to the respective anticancer drug.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. TLC was performed on Analtech silica gel GHLF plates. HRMS was provided by the Washington University Mass Spectrometry Resource. All ^{1}H NMR spectra were initially 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 Inova 400 and Varian Unity 500 MHz instruments.

(+)-Pancratistatin (**1a**) was isolated from *Hymenocallis littoralis* as previously described.[3] Reagents were purchased from Acros Chemical Company unless otherwise noted and used as received. Solvents were distilled prior to use, and

pyridine preceding distillation was dried over potassium hydroxide pellets. The pancratistatin derivatives were visible as green-blue fluorescent spots on TLC plates under long-wave ultraviolet light. Dowex 50WX8-200 cation-exchange resin (H$^+$ form) was washed with MeOH, 1 N HCl, and deionized H$_2$O. The cation forms of the resin were obtained by washing with a 1 N solution of the appropriate base followed by deionized water.

Sodium Phenpanstatin 3,4-*O*-Cyclic Phosphate (3b). Phenpanstatin (**3a**, 0.20 g, 0.26 mmol) was dissolved in pyridine (10 mL) and the solution heated to 80 °C under argon before addition of tetrabutylammonium dihydrogen phosphate (1.25 g, 3.68 mmol, 8 equiv), dicyclohexylcarbodiimide (1.20 g, 5.8 mmol, 12.6 equiv), and *p*-toluenesulfonic acid (0.26 g, 1.36 mmol, 3 equiv). The reaction was monitored by ^{1}H NMR (DMSO-d_6), and after 24 h the NMR spectrum showed a 50:50 mixture of starting material to product. Dicyclohexylcarbodiimide (0.425 g) was added, and the reaction continued for a total of 48 h.

The mixture was cooled, and the dicyclohexylurea (DCU) precipitate was collected and washed with H$_2$O. Additional H$_2$O (100 mL) was added to the mother liquor and the DCU again collected. The mother liquor was extracted with butanol (2 × 40 mL), and the butanol fractions were combined and concentrated to a light brown oil. A solution of the oil in H$_2$O (minimum amount) was purified through a Dowex 50WX8-200 ion-exchange column (Na$^+$ form) (14 cm × 2 cm). The UV-active fractions were combined and freeze-dried to yield a white solid, which was further purified in methanol on a Sephadex LH-20 column (70 cm × 2 cm, eluent MeOH at 1.8 mL/min): 0.145 g, 60%, mp 268 °C dec, [α]$_D$ −51.8° (*c* 0.5, CH$_3$-OH), R_f = 0.69 (BuOH−MeOH−H$_2$O−NH$_4$OH, 4:3:2:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (s, 1H), 8.52 (s, 1H), 8.1 (d, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 2H), 6.30 (s, 1H), 6.13 (bs, 1H), 6.01 (s, 1H), 5.96 (s, 1H), 5.73 (s, 1H), 4.41 (dd, *J* = 8.4, 13.2 Hz, 1H), 4.32−4.21 (m, 2H), 4.11 (bs, 1H), 3.33 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 165.4, 152.1, 145.8, 133.4, 133.3, 132.3, 130.2 (2C), 129.2, 128.7, 107.4, 101.9, 95.8, 74.9, 74.8, 69.1, 66.3, 66.1, 51.3, 35.7 ppm; ^{31}P NMR (162 MHz, DMSO-d_6) δ 12.99; HRFAB *m/z* calcd for [C$_{21}$H$_{17}$NO$_{11}$P]$^-$ 490.0539Z, found 490.0540.

General Procedure for Preparation of the Phenpanstatin Phosphate Prodrugs (3c−3d). Sodium phenpanstatin 3,4-cyclic phosphate (6 mg) was dissolved in H$_2$O (0.5 mL) and eluted with H$_2$O through a column (1 cm × 5 cm) of Dowex 50WX8-200 ion-exchange resin (respective cation). The UV-active fractions were combined and freeze-dried to give the corresponding phenpanstatin phosphate prodrug.

Lithium Phenpanstatin 3,4-Cyclic Phosphate (3c): glassy solid, 4 mg, mp 280 °C (dec); ^{1}H NMR (300 MHz, CD$_3$-

OD) δ 8.05 (d, *J* = 7.2 Hz, 2H), 7.53 (t, *J* = 7.35 Hz, 1H), 7.42 (t, *J* = 7.35 Hz, 2H), 6.39 (s, 1H), 5.96 (s, 1H), 5.90 (s, 1H), 4.61−4.51 (m, 3H), 4.33 (bs, 1H), 3.44 (m, 1H).

Potassium Phenpanstatin 3,4-Cyclic Phosphate (3d): white solid, 3.9 mg, mp 245 °C (dec); ^{1}H NMR (300 MHz, CD₃-OD) δ 8.05 (d, *J* = 7.2 Hz, 2H), 7.53 (t, *J* = 7.35 Hz, 1H), 7.42 (t, *J* = 7.65 Hz, 2H), 6.39 (s, 1H), 5.96 (s, 1H), 5.90 (s, 2H), 4.62−4.51 (m, 3H), 4.33 (bs, 1H), 3.46−3.41 (m, 1H).

Sodium Pancratistatin 4-*O*-Phosphate (4a). Pancratistatin (**1a**, 0.2 g, 0.615 mmol) was added to pyridine (10 mL), and the solution was heated. Next, tetrabutylammonium dihydrogen phosphate (1.67 g, 4.92 mmol, 8 equiv), dicyclohexylcarbodiimide (1.01 g, 4.92 mmol, 8 equiv), and *p*-toluenesulfonic acid (0.35 g, 1.84 mmol, 3 equiv) were added. The resulting solution was stirred under argon and monitored by ^{1}H NMR at 80 °C for 2 days. As starting material was still present in the reaction mixture, additional DCCI (0.5 g) was added. The mixture was stirred and heated (80 °C) for a total of 5 days. After addition of H₂O (100 mL), dicyclohexylurea precipitated. The mixture was stirred for 1 h, and the solution filtered and concentrated to a brown oil. A solution of the oil in the minimum amount of H₂O was passed through an ion-exchange column (Dowex 50WX8-200, 12 cm × 3.5 cm, sodium form) and eluted with H₂O. The UV-active fractions were combined according to TLC mobility (n-BuOH−CH₃OH−H₂O−NH₄OH, 4:3:2:1) to give a crude white solid with considerable impurities (0.83 g). After further separation on a G-10 Sephadex column (60 cm × 2 cm), eluting with H₂O (at 10 mL/8 min), 254 fractions were collected and combined according to the TLC data (n-BuOH−CH₃OH−H₂O−NH₄OH, 4:3:2:1). Sodium pancratistatin 4-*O*-phosphate was found in fractions 41−88 and crystallized from a concentrated solution of these fractions in H₂O−MeOH to afford 4-*O*-phosphate (**4a**) as a colorless solid, insoluble in MeOH: 9.2 mg (3.7% yield), mp 280 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (s, 1H, Ph-OH), 10.22 (bs, 1H, NH), 6.45 (s, 1H, Ar-H), 6.03 (s, 1H), 5.99 (s, 1H), 4.74−4.72 (m, 1H, OH), 4.29−4.24 (m, 2H), 3.95 (narrow m, 2H), 3.74 (dd, *J* = 13.4 Hz, 10.2 Hz, Hz, H-4a), 3.47 (bm, 2H, OH), 2.96 (m, 1H, H-10b); [13]C (120 MHz, DMSO-d_6) δ 168.6, 151.6, 145.3, 135.6, 131.6, 107.9, 101.5, 97.6, 72.7, 72.1, 69.9, 68.5, 50.2, 39.8; [31]P (162 MHz, DMSO-d_6) δ 4.73. The second and third crops (21 mg) were found to be mixtures of several phosphates when analyzed by ^{31}P NMR, and the mother liquor was found to be a mixture (39 mg) of two, in a total of 79.6 mg (32%). The remaining material on the column visible by UV long-wave light was eluted using a gradient of MeOH−H₂O to MeOH and was identified by NMR as a narciprimine (**6**) derivative (27 mg) pointing to aromatization of the cyclitol ring during this phosphorylation reaction.

X-ray Crystal Structure Determination. Sodium pancratistatin 4-*O*-phosphate (**4a**) hydrate: long, colorless, rod-shaped crystal (∼0.58 × 0.16 × 0.13 mm), grown from a MeOH−H₂O solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 123 ± 2 K on a Bruker SMART 6000 diffractometer. Final cell constants were calculated from a set of 8435 reflections from the actual data collection. Frames of data were collected in the θ range 5.59−69.67° (−8 ≤ *h* ≤ 8, −17 ≤ *k* ≤ 17, −18 ≤ *l* ≤ 19) using 0.396° steps in ω such that a comprehensive coverage of the sphere of reflections was performed. After data collection, an empirical absorption correction was applied with the program SADABS.[13] Subsequent statistical analysis of the complete reflection set using the XPREP[14] program indicated the space group was $P2_12_12_1$.

Crystal data: $C_{14}H_{15}NNaO_{11}P\cdot2H_2O$ (hydrate), *a* = 7.35630(10) Å, *b* = 14.6555(2) Å, *c* = 15.8310(2) Å, *V* = 1706.74(4) Å³, λ = (Cu Kα) = 1.54178 Å, ρ_c = 1.803 g cm⁻³ for *Z* = 4 and fw = 436.26, *F*(000) = 960. A total of 12 108 reflections were collected, of which 3026 were unique (R_{int} = 0.0366), and 2918 were considered observed ($I_o > 2\sigma(I_o)$). These were used in the subsequent structure solution and refinement with SHELXTL-V5.1.[14] All non-hydrogen atoms for **4a** were located using the default settings of that program. Hydrogen atoms were placed in calculated positions and assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso}
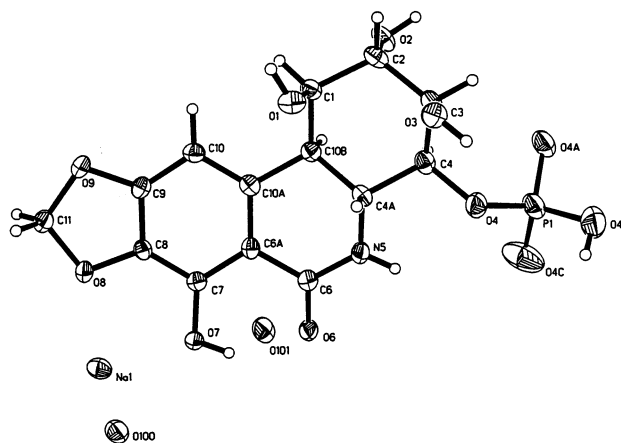

Figure 1. X-ray thermal ellipsoid plot (50% probability) of sodium pancratistatin 4-*O*-phosphate (**4a**) as the dihydrate.

value of the atom to which they were attached, then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. In addition to the parent sodium phosphate salt, two additional molecules of water solvent were also present in the asymmetric unit. The final standard residual R_1 value for the model shown in Figure 1 converged to 0.0438 (for observed data) and 0.0453 (for all data). The corresponding Sheldrick *R* values were wR_2 of 0.1205 and 0.1221, respectively, and the GOF = 1.062 for all data. The difference Fourier map showed residual electron density, the largest difference peak and hole being +0.682 and −0.404 e/Å³, respectively. The Flack absolute structure parameter for the model in Figure 1 refined to −0.04(4), indicating that the absolute structure for the enantiomer shown is correct. Final bond distances and angles were all within acceptable limits.

Pancratistatin 4-*O*-Phosphoric Acid (4b). Sodium pancratistatin 3,4-cyclic phosphate (**5a**, 0.0165 g, 0.04 mmol) was dissolved in H₂O (1 mL) and eluted through a column containing Amberlite IR 120 (H+) resin. The UV-active fractions were combined and freeze-dried, 0.011 g, 69%, mp 150 °C (dec). The material decomposed over several days. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.9 (s, 1H), 8.12 (s, 1H), 6.49 (s, 1H), 6.04−6.02 (m, 2H), 4.43 (m, 1H), 4.30 (m, 1H), 3.98−3.87 (m, 3H), 3.07−3.01 (m, 1H).

Lithium Pancratistatin 4-*O*-Phosphate (4c). Sodium pancratistatin 3,4-cyclic phosphate (**5a**, 0.144 g, 0.35 mmol) was dissolved in H₂O (1 mL) and eluted on an IR-120 (H+) ion-exchange resin to convert to the monophosphoric acid, and the active fractions were then directly eluted through a Dowex 50WX8-200 ion-exchange resin (Li⁺) form. The UV-active fractions were combined and freeze-dried to yield a white solid, which was washed with hot MeOH, and the solution was filtered to yield 0.083 g, 62% yield, mp 265 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.3 (s, 1H), 10.3 (s, 1H), 6.44 (s, 1H), 6.02 (s, 1H), 5.98 (s, 1H), 4.76 (m, 1H), 4.26−4.23 (m, 2H), 3.94 (m, 2H), 3.74 (m, 1H), 2.98−2.93 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.6, 151.6, 145.3, 135.5, 131.6, 107.9, 101.5, 97.6, 76.7, 72.1, 69.9, 68.5, 50.2, 48.6; ^{31}P NMR (DMSO-d_6, 400 MHz) δ 2.57; HRESI *m/z* calcd for [$C_{14}H_{15}NO_{11}P$]⁻ 404.0391, found *m/z* 404.0383.

Potassium Pancratistatin 4-*O*-Phosphate (4d). Lithium pancratistatin 4-*O*-phosphate (0.08 mg) was eluted through an IR-120 (K⁺) ion-exchange resin, and the UV-active fractions were collected and freeze-dried, wt 0.006 g, mp 230 °C; ^{1}H NMR (DMSO-d_6/D₂O) δ 6.42 (s, 1H), 5.99−5.96 (m, 2H), 4.20 (m, 2H), 3.99 (m, 1H), 3.94 (m, 1H), 3.84 (m, 1H), 2.99−2.93 (m, 1H).

General Procedure for the Preparation of the Pancratistatin 4-*O*-Phosphate Divalent Cation Salts (4e−4g). The phosphoric acid (**4b**) (0.036 g, 0.086 mmol) was taken up in MeOH (3 mL). A 1 mL aliquot of this solution was added to a round bottom flask containing 1 equiv of the corresponding

metal acetate. The opaque solutions were allowed to stir for several days before concentrating to a residue, which was washed with MeOH and dried.

Magnesium Pancratistatin 4-*O*-Phosphate (4e): beige solid, 0.010 g, mp 255 °C (dec), insoluble in DMSO-d_6 and D$_2$O; HRESI *m/z* calcd for $[C_{14}H_{15}NO_{11}P]^-$ 404.0391, found *m/z* 404.0368.

Calcium Pancratistatin 4-*O*-Phosphate (4f): gray solid, 0.011 g, mp 290 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.2 (s, 1H), 9.65 (s, 1H), 6.45 (s, 1H), 6.02−5.99 (m, 2H), 4.96 (m, 1H), 4.34−4.26 (m, 2H), 3.96 (m, 2H), 3.85−3.73 (m, 1H), 3.02−2.97 (m, 1H).

Zinc Pancratistatin 4-*O*-Phosphate (4g): crystalline powder, 0.0085 g, mp 270 °C (dec), insoluble in DMSO-d_6 and D$_2$O; HRESI *m/z* calcd for $[C_{14}H_{15}NO_{11}P]^-$ 404.0391, found *m/z* 404.0372.

General Procedure for the Preparation of the Pancratistatin 4-*O*-Phosphate Ammonium Salts (4h−4l). Pancratistatin 4-*O*-phosphoric acid **4b** (0.012 g, 0.03 mmol) was dissolved in MeOH (1 mL), and the amine (1.2 equiv) was added with stirring at room temperature. The reaction was stirred for 4 days before concentrating to a residue, which was washed with MeOH, and the solid was filtered and dried.

Piperazinium Pancratistatin 4-*O*-Phosphate (4h): off-white solid, 0.011 g, mp 180 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.3 (s, 1H), 10.1 (s, 1H), 6.44 (s, 1H), 6.02−5.99 (m, 2H), 4.26−4.24 (m, 2H), 3.94 (m, 2H), 3.73 (m, 1H), 2.98−2.88 (m, 9H); HRESI *m/z* calcd for $C_{18}H_{27}O_{11}N_3P$ (M + H)$^+$ 492.1383, found 492.1383 (M + H)$^+$.

Morpholinium Pancratistatin 4-*O*-Phosphate (4i): hydroscopic solid, 0.01 g, mp 130 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.2 (bs, 1H), 9.93 (s, 1H), 6.45 (s, 1H), 6.03−5.99 (m, 2H), 5.19 (bm, 3H), 4.29−4.24 (m, 2H), 3.95 (nm, 2H), 3.75−3.63 (m, 5H), 2.99−2.86 (m, 5H).

Imidazolium Pancratistatin 4-*O*-Phosphate (4j): crystalline solid, 0.007 g, mp 125 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.2 (s, 1H), 9.5 (bs, 1H), 7.93 (s, 2H), 7.21 (bs, 4H), 6.45 (s, 1H), 6.03−5.99 (m, 2H), 4.57−4.25 (m, 2H), 3.95 (m, 2H), 3.8 (m, 1H), 3.01−2.97 (m, 1H).

Quinium Pancratistatin 4-*O*-Phosphate (4k): beige solid, 0.015 g, mp 173 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.19 (s, 1H), 9.9 (s, 1H), 8.71 (m, 1H), 7.92 (d, 1H), 7.58 (m, 1H), 7.48 (m, 1H), 7.39 (d, 1H), 6.45 (s, 1H), 6.02−5.99 (m, 2H), 5.82 (m, 2H), 5.6−4.92 (m, 3H), 4.29−4.24 (m, 2H), 3.95 (m, 4H), 3.79 (m, 2H), 3.4 (m, 2H), 3.15 (s, 1H), 3.01 (m, 4H), 2.5 (m, 1H), 1.9 (m, 2H), 1.71 (m, 1H), 1.44 (m, 1H).

Quinidium Pancratistatin 4-*O*-Phosphate (4l): off-white solid, 0.017 g, mp 180 °C (dec); ^{1}H NMR (DMSO-d_6 300 MHz) δ 13.22 (s, 1H), 9.9 (s, 1H), 8.71−8.69 (m, 1H), 7.94−7.91 (d, 1H), 7.57 (m, 1H), 7.40−7.37 (m, 2H), 6.45 (s, 1H), 6.02−5.99 (m, 3H), 5.73 (s, 1H), 5.16−5.13 (m, 3H), 4.25 (m, 2H), 3.94 (m, 4H), 3.77 (m, 4H), 3.35 (m, 4H), 3.01−2.97 (m, 1H), 2.19 (m, 1H), 1.83 (m, 1H), 1.67 (m, 2H), 1.13 (m, 1H); HRESI *m/z* calcd for $C_{34}H_{41}N_3O_{13}P$ (M + H)$^+$ 730.2377, found 730.2378 (M + H)$^+$.

Sodium Pancratistatin 3,4-Cyclic Phosphate (5a). Pancratistatin (**1a**) (0.2 g, 0.615 mmol) was dissolved in pyridine (8 mL) and heated to 80 °C under argon before adding tetrabutylammonium dihydrogen phosphate (0.3 g, 1.47 equiv) and dicyclohexylcarbodiimide (0.92 g, 4.44 mmol, 7.25 equiv). The reaction was stirred at 80 °C for 24 h. ^{1}H NMR of a crude sample of the reaction mixture showed a 50:50 mixture of **5a** and starting material. Tetrabutylammonium dihydrogen phosphate (0.15 g) and dicyclohexylcarbodiimide (0.5 g) were added, and the reaction continued for a further 24 h. The reaction mixture was cooled and H$_2$O (100 mL) was added. The dicyclohexylurea (DCU) precipitate was filtered off and the mother liquor concentrated to a white residue. Water was added to effect solution, and the material was eluted on a Dowex 50WX8-200 (Na$^+$ form) ion-exchange resin. The UV-active fractions were combined and concentrated to a beige crystalline solid, 0.244 g, recrystallized from H$_2$O−MeOH−DCM to yield **5a**, 120 mg, 48%, mp >300 °C, [α]$_D^{25}$ −3.3° (*c* 0.54 H$_2$), R_f = 0.63 (n-BuOH−MeOH−H$_2$O−NH$_4$OH, 4:3:2:1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 13.25 (s, 1H), 8.11 (s, 1H),

6.48 (s, 1H), 6.03−6.02 (m, 2H), 5.42 (bs, 1H), 4.69 (d, *J* = 5 Hz, 1H), 4.28 (m, 1H), 4.22 (m, 1H), 4.09−4.03 (m, 3H), 2.85 (m, 1H); ^{13}C NMR (DMSO-d_6 125 MHz) δ 169.2, 152.1, 145.4, 135.2, 131.7, 107.4, 101.7, 97.6, 75.7, 75.3, 69.4, 67.7, 50.5, 37.0; ^{31}P NMR δ 13.22; HRFAB *m/z* found 386.0267 (M − Na)$^-$, $C_{14}H_{13}O_{10}NP$ requires 386.02771 (M − Na)$^-$.

General Procedure for the Preparation of Pancratistatin-3,4-Cyclic Phosphate Prodrugs (5b,5c). Sodium pancratistatin 3,4-cyclic phosphate (**5a**, 20 mg) was dissolved in H$_2$O and the solution passed through a column (1 × 20 cm) of Dowex 50WX8-200 bearing the respective cation. The UV-active fractions were combined and freeze-dried to give the corresponding pancratistatin 3,4-cyclic phosphate salt as a solid.

Lithium Pancratistatin 3,4-Cyclic Phosphate (5b): 23.9 mg, mp 240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.25 (s, 1H), 8.09 (s, 1H), 6.48 (s, 1H), 6.03−6.01 (m, 2H), 5.40 (bs, 1H), 4.69 (d, *J* = 5.1 Hz, 1H), 4.27−4.22 (m, 2H), 4.11−4.03 (m, 3H), 2.87−2.83 (m, 1H).

Potassium Pancratistatin 3,4-Cyclic Phosphate (5c): 17.8 mg, mp 238−248 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.25 (s, 1H), 8.11 (s, 1H), 6.48 (s, 1H), 6.03−6.01 (m, 2H), 5.41 (bs, 1H), 4.27−4.20 (m, 2H), 4.08−4.02 (m, 3H), 2.87−2.83 (m, 1H).

Supporting Information Available: Tables of X-ray crystallographic data for compound **4a** are available free of charge via the Internet at http://pubs.acs.org.

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