Antineoplastic Agents. 527. Synthesis of 7-Deoxynarcistatin, 7-Deoxy-*trans*-dihydronarcistatin, and *trans*-Dihydronarcistatin 1[1]

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The synthesis of sodium narcistatin (**8**) was improved (88% overall yield) and the modified reaction sequence was utilized to synthesize three new 3,4-cyclic phosphate prodrugs, sodium 7-deoxynarcistatin (**5**), sodium 7-deoxy-*trans*-dihydronarcistatin (**6**), and sodium *trans*-dihydronarcistatin (**7**). The human cancer cell line inhibitory isocarbostyril precursors were isolated from the bulbs of *Hymenocallis littoralis* obtained by horticultural production or reduction of narciclasine (**1a** → **4**) from the same source.

Advances in the chemistry of isocarbostyrils were already being achieved in the 1909−1920 period by the well-known organic chemists Dieckmann,[2a] Robinson,[2b] Perkin,[2b] and Chichibabin.[2c] However, interest in the potential of naturally occurring isocarbostyrils as anticancer agents only began with the isolation of narciclasine (**1a**)[3−8] in 1967 and our cancer bioassay-directed discovery[9] of pancratistatin (**2**) as a constituent of *Pancratium littorale* (now *Hymenocallis littoralis*, Amaryllidaceae) accompanied by narciclasine (**1a**) and 7-deoxynarciclasine (**1b**, lycoricidine, margetine). Later, we isolated another new anticancer constituent from *H. littoralis* identified as 7-deoxy-*trans*-dihydronarciclasine (**3a**).[10a] These discoveries and the need for scale-up quantities of this series of important isocarbostyrils for preclinical development led us to use a biotechnological approach involving an initial tissue culture cloning of *H. littoralis* bulbs.[10,11a,b] Large numbers of *H. littoralis* bulbs[10] were then grown in Arizona specifically for production of (+)-pancratistatin (**2**) and narciclasine (**1a**), along with 7-deoxynarciclasine (**1b**) and 7-deoxy-*trans*-dihydronarciclasine (**3a**). The pancratistatin (**2**) and narciclasine (**1a**) constituents were used for subsequent structural modifications,[12] conversion to phosphate prodrugs,[13] and anticancer evaluations.[10−14] A 7-deoxynarciclasine (**1b**)/7-deoxy-*trans*-dihydronarciclasine (**3a**) fraction provided the starting material for analogous objectives that included the present study.

Because isocarbostyrils **1b**, **3a**, and **4**[4,9c] also exhibited strong cancer cell growth inhibition against a minipanel of human cancer cell lines (Table 1), they were next selected for conversion to 3,4-cyclic phosphate prodrugs[13a] for the purpose of improving aqueous solubility and transport to tumors for cancer antiangiogenesis/vascular targeting.[15,16]

Results and Discussion

The 7-deoxynarciclasine (**1b**) and 7-deoxy-*trans*-dihydronarciclasine (**3a**) mixture separated from *H. littoralis* was acetylated with acetic anhydride in pyridine and the peracetate mixture separated by silica gel column chromatography to afford isocarbostyrils **1c** (60% recovery) and **3b** (19% recovery). Both 2,3,4-triacetoxy-7-deoxynarciclasine (**1c**) and 2,3,4-triacetoxy-7-deoxy-*trans*-dihydronarciclasine (**3b**) were then deprotected with potassium carbonate in aqueous MeOH to afford the corresponding triols **1b** and **3a** in 72% yields. The 3,4-cyclic phosphates **5**, **6**, and **7**[4,9c] were synthesized employing an improvement in the procedure we developed for synthesis of sodium narcistatin (**8**)[13a] using tetrabutylammonium dihydrogen phosphate and an excess of dicyclocarbodiimide in dry pyridine under argon at 80 °C for 48 h.

In each case, ^{1}H NMR of the crude product showed the reaction to be only 50% complete following a 24 h period. Additional reagents were added at this stage, and the reaction was allowed to proceed to completion (a further 24 h). Increasing the amount of tetrabutylammonium dihydrogen phosphate from 0.65 equiv to 1 equiv in the first 24 h did not increase the reaction rate. Water was added to the reaction mixture to precipitate the dicyclohexylurea (DCU), and the pyridine/water filtrate was concentrated to remove the pyridine. An aqueous extract of the residue was passed through a Dowex 50WX8-400 ion-exchange column (sodium form). The UV-responsive fractions were combined and lyophilized to afford the new 3,4-cyclic phosphates designated sodium 7-deoxynarcistatin (**5**, 88% yield), sodium 7-deoxy-*trans*-dihydronarcistatin (**6**, 65% yield), and sodium *trans*-dihydronarcistatin (**7**, 94% yield). More recently,[17] we found that deletion of the *p*-toluenesulfonic acid component increased the yield of phosphate **8** to 88% versus the original[13a] 50%. That was one of the major improvements that allowed new phosphates **5**, **6**, and **7** to be obtained in very good yields.

Detailed analyses of the ^{1}H, ^{13}C, COSY, HMQC, HMBC, and D_2O exchange spectra were conducted in an effort to assign the carbon and proton spectra of each of the cyclic phosphates and confirm the structure assignments. The ^{31}P NMR (DMSO-d_6 with neat H_3PO_4 as external standard) spectra corresponding to phosphates **5**, **6**, and **7** showed downfield signals for the phosphorus atom at δ 15.23, 13.45, and 13.36, respectively, which are consistent with the data previously observed for cyclic phosphate **8**.[13a] In addition, sodium narcistatin (**8**), prepared by the procedure described herein, was found to be identical (mp, ^{1}H NMR, ^{31}P NMR) with an authentic sample.[13a]

Downfield shifts for the H-3 and H-4 resonances displayed by 3,4-cyclic phosphates **5**−**8** were observed in all cases when compared with the spectra of each of the starting isocarbostyrils. The ^{1}H NMR spectrum of 7-deoxynarciclasine (**1b**) revealed the H-3 and H-4 resonances at δ 3.69 and 3.76, respectively, whereas the derived phosphate (**5**) showed H-3 and H-4 downfield as a multiplet at δ 3.93−3.79. In *trans*-dihydro derivative **3a**, the multiplet

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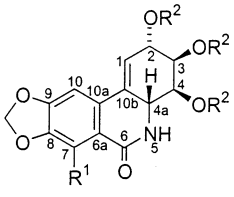
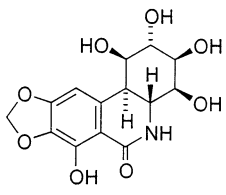
Table 1. Aqueous Solubility, Human Cancer Cell Line, and Murine P-388 Lymphocytic Inhibitory Activities

		ED_{50} (μg/mL)	GI_{50} (μg/mL)					
isocarbostyril	aqueous solubility 25 °C (mg/mL)	leukemia P388	pancreas-a BXPC-3	breast MCF-7	CNS SF268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145
1b	<1	0.019	0.070	0.046	0.120	0.053	0.084	0.051
3a	<1	0.029	0.046	0.034	0.059	0.043	0.051	0.040
4	<1	0.0024	0.012	0.0053	0.020	0.0092	0.015	0.0066
5	>190	1.7	5.3	4.0	6.3	4.7	5.6	3.9
5a	>10	0.42	>1	>1	>1	>1	>1	>1
5b	>10	1.4	>1	>1	>1	>1	>1	>1
6	>10	1.6	>10	7.2	>10	>10	>10	>10
6a	>5	0.39	>1	>1	>1	>1	>1	>1
6b	>5	1.7	>1	>1	>1	>1	>1	>1
7	>10	0.88	5.6	4.6	8.8	7.8	9.6	5.2
7a	>1	0.26	>1	>1	>1	>1	>1	>1
7b	>1	0.35	>1	0.64	>1	>1	>1	0.54



1a, R^1 = OH, R^2 = H
b, R^1 = R^2 = H
c, R^1 = H, R^2 = $COCH_3$



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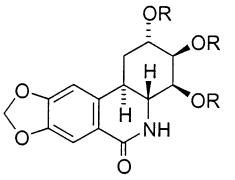
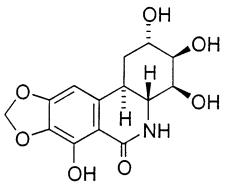


3a, R = H
b, R = $COCH_3$



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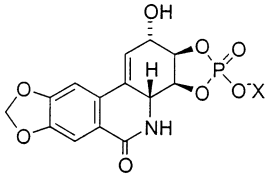
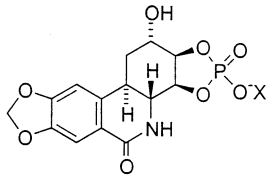


5, X = Na^+
5a, X = Li^+
5b, X = K^+



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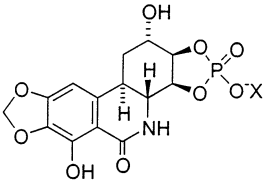
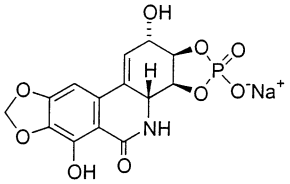
6a, X = Li^+
6b, X = K^+



7, X = Na^+
7a, X = Li^+
7b, X = K^+



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at δ 3.69 was assigned to H-3 and H-4 and in cyclic phosphate **6** they were at δ 4.11 (H-3) and 4.03 (H-4). Similarly, the downfield signals for protons H-3 and H-4 appeared as part of a multiplet at δ 4.14−4.03 for phosphate **7**. Furthermore, the usual signals for OH-3 and OH-4 were absent from the ^{1}H NMR spectra of each of the 3,4-cyclic phosphates. The OH-2 signals for 3,4-cyclic phosphates **5**−**7** were found downfield at δ 5.71, 5.22, and 5.26, respectively. By way of verification, these signals were absent in the spectrum of each when a D_2O exchange ^{1}H NMR experiment was performed. The D_2O exchange experiment also allowed assignment of the N−H resonances for 3,4-cyclic phosphate **5** at δ 7.65, **6** at δ 7.53, and **7** at δ 8.40.

The ^{13}C spectra were examined using HMQC and HMBC. With 7-deoxynarciclasine (**1b**) → 3,4-cyclic phosphate **5**, there was a downfield shift of the C-3 and C-4 signals from δ 69.9 to 75.6 and 74.8 (C-3, C-4). The corresponding downfield shifts for **3a** → **6** were δ 70.4 and 72.3 for C-3 and C-4, respectively, to δ 75.7 (C-3) and 75.9 (C-4), and for phosphate **7** C-3 and C-4 appeared at δ 74.9.

Analysis of the COSY spectrum for phosphate **5** indicated the H-1 signal was downfield at δ 6.35 and showed strong correlation peaks with the OH signal at δ 5.71 and the H-2 signal at δ 4.24. The H-4a signal was assigned to the multiplet at δ 4.24. The COSY spectrum for phosphate **6** showed H-1_{ax} at δ 1.47 ppm and H-1_{eq} at δ 2.27 exhibiting strong correlation peaks with the multiplet at δ 2.84 (H-10b) and the H-2 resonance at δ 4.03. The H-10b proton showed cross-peaks with the proton signal at δ 3.54, which was assigned to H-4a. Cross-peaks were also observed between H-4a and the H-4 signal in the multiplet at δ 4.03. The H-3 resonance was therefore assigned to the narrow multiplet at δ 4.11. An HMBC spectrum of phosphate **6** was used to assign the protons H-7 and H-10. The H-7 resonance (δ 7.27) showed cross-peaks with the C-6 carbonyl signal at δ 164.7, whereas the H-10 (δ 6.93) showed cross-peaks with the C-10a signal at δ 124.2 and the C-10b at δ 32.7. The proton spectrum from phosphate **7** was analyzed using COSY in a similar manner. The resonances for H-1_{ax} and H-1_{eq} were assigned at δ 1.50 and 2.26, respectively, as expected. These protons showed strong correlation peaks with the H-2 resonance in the multiplet between δ 4.07 and 4.03 and the H-10b signal at δ 2.84. Consequently, the H-4a signal was easily identified as a doublet of doublets at δ 3.59. The narrow multiplet at δ 4.14 was assigned to H-3, as a strong correlation peak was observed between the H-4a signal and the H-4 signal in the multiplet at δ 4.07−4.03. These 2D NMR spectral assignments for 3,4-cyclic phosphates **5**, **6**, and **7** were consistent with those already reported for sodium narciclasine, where the structure was unequivocally established by X-ray crystallography.[13a]

The cyclic phosphate prodrugs **5**, **6**, and **7** along with the parent compounds **1b**, **3a**, and **4** were evaluated against a minipanel of human cancer cell lines and murine P388 lymphocytic leukemia (Table 1). Results of the cancer cell line evaluations reconfirmed the strong cancer cell growth inhibitory activity of 7-deoxy-*trans*-dihydronarciclasine (**3a**) and *trans*-dihydronarciclasine (**4**). As expected, the corre-

sponding 3,5-cyclic phosphates owing to comparatively slow release of the parent active compound were less inhibitory under the experimental conditions employed.[13a] However, cleavage of the phosphate groups is expected to be very effective in vivo,[16] and such anticancer evaluations are now underway as part of the further preclinical development of these new anticancer drug candidates.

Experimental Section

General Experimental Procedures. Reagents were purchased from Acros Chemical Company unless otherwise noted and used as received. Solvents were distilled prior to use, and pyridine was dried over KOH pellets and distilled. The 7-deoxynarciclasine, 7-deoxy-*trans*-dihydronarciclasine, and *trans*-dihydronarciclasine were isolated[10] from *Hymenocallis littoralis* grown by the ASU-CRI research group in Tempe, AZ. These isocarbostyrils were visible as blue fluorescent spots on TLC plates under long-wave ultraviolet light and developed with I_2 vapor (see also ref 13a). Dowex 50WX8-400 cation-exchange resin (H^+ form) was first eluted with MeOH, 1 N HCl, and deionized H_2O. The cation forms of the resin were prepared by eluting with a 1 N solution of the appropriate base followed by deionized H_2O.

Melting points were obtained using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Thin-layer chromatography was conducted using Analtech silica gel GHLF plates. High-resolution mass spectra were obtained by the Washington University Mass Spectrometry Laboratory (St. Louis, MO) and by 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 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 a Varian Inova 400 MHz instrument.

Separation of 7-Deoxynarciclasine (1b) and 7-Deoxy-*trans*-dihydronarciclasines (3a). The *H. littoralis* fractions[10] (8.6 g) containing isocarbostyrils **1b** and **3a** were acetylated by dissolving in pyridine (20 mL) and adding acetic anhydride (20 mL, 2.4 equiv). The mixture slowly became a solution with stirring overnight at rt, and TLC (CH_2Cl_2−CH_3OH, 2%) showed no starting material. Ice water (200 mL) was added to the reaction mixture with vigorous stirring. A cream-colored precipitate developed and was collected after stirring for 2 h to provide 13.7 g. The acetylation product (9 g) was separated on a silica gel column (37 cm × 7 cm) by elution using 7:3 toluene−ethyl acetate to yield 2,3,4-triacetoxy-7-deoxynarciclasine (**1c**) (5.45 g, 60.5%) followed by a mixture of both **1c** and **3b** acetates (1.08 g) and finally 2,3,4-triacetoxy-7-deoxy-*trans*-dihydronarciclasine (**3b**, 1.7 g, 19%). Recrystallization of peracetate **1c** from CH_2Cl_2−CH_3OH gave colorless needles: mp 238−240 °C; $[\alpha]^{23}_D$ +151° (*c* 1.04, CH_2Cl_2) [lit.[9c] 244−246 °C, $[\alpha]^{27}_D$ +219° (*c* 1.0, $CHCl_3$)]; ^{1}H NMR($CDCl_3$, 300 MHz) δ 7.50 (1H, s, H-7), 7.01 (1H, s, NH), 6.99 (1H, s, H-10), 6.10 (1H, m, H-1), 6.06−6.04 (2H, m, −OCH_2O-), 5.46 (1H, m, H-3), 5.34 (1H, m, H-2), 5.26−5.23 (1H, dd, J = 9.3, 2.1 Hz, H-4) 4.66 (1H, bd, J = 9.3 Hz, H-4a) 2.15 (3H, s, −$OCOCH_3$), 2.1 (3H, s, −$OCOCH_3$), 2.09 (3H, s, −$OCOCH_3$); ^{13}CNMR-($CDCl_3$, 300 MHz) δ 169.9 (C, *O*COCH3), 169.2 (2C, *O*COCH3), 163.8 (C, C-6), 151.3 (C), 148.7 (C), 133.6 (C), 129.8 (C), 121.8 (C), 116.7 (CH, C-1), 107.0 (CH, C-7), 102.9 (CH, C-10), 101.6 (CH_2, −OCH_2O−), 70.7 (CH, C-4), 68.0 (CH, C-2), 67.7 (CH, C-3), 49.7 (CH, C-4a), 20.5 (CH_3, −OCO*CH$_3$*), 20.4 (CH_3, −OCO*CH$_3$*), 20.2 (CH_3, −OCO*CH$_3$*); HRAPCI$^+$ *m/z* 418.1134 $[M + 1]^+$ (calcd for $C_{20}H_{20}NO_9$ 418.1138 $[M + 1]^+$).

Recrystallization of the precipitate (**3b**) from CH_2Cl_2−CH_3OH gave colorless needles: mp 208 °C, $[\alpha]^{23}_D$ +103° (*c* 0.53 CH_2Cl_2) [lit.[15b] mp 148−149 °C]; ^{1}HNMR ($CDCl_3$, 300 MHz) δ 7.55 (1H, s, H-7), 6.72 (1H, s, 1H), 6.18 (1H, s, NH), 6.03 (2H, s, −OCH_2O−), 5.43 (1H, t, J = 3 Hz, H-3), 5.22−5.18 (2H, m, H-3, H-4), 3.79 (1H, dd, J = 12.75, 10.95, H-4a), 3.18 (1H, m, H-10b), 2.5 (1H, m, H-1eq), 2.07 (3H, s, −$OCOCH_3$), 2.09 (3H, s, −$OCOCH_3$), 2.14 (3H, s, −$OCOCH_3$), 1.94 (m, 1H, H-1ax); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.8 (C, *O*COCH$_3$) 168.7 (C, *O*COCH$_3$), 168.6 (C, *O*COCH$_3$), 165.3 (C, C-6), 150.9 (C), 146.5 (C), 135.2 (C), 122.6 (C), 107.7 (CH, C-7), 103.4 (CH, C-10), 101.3 (CH_2, −OCH_2O-), 70.9 (CH, C-4), 68.1 (CH, C-2), 67.0 (CH, C-3), 52.3 (CH, C-4a), 34.2 (CH, C-10b), 26.2 (CH, C-1), 20.5 (CH_3, −$OCOCH_3$), 20.4 (CH_3, −$OCOCH_3$), 20.2 (CH_3, −$OCOCH_3$); HRAPCI$^+$ *m/z* 420.1282 $[M + H]^+$ (calcd for $C_{20}H_{21}NO_9$ 420.1294 $[M + H]^+$).

7-Deoxynarciclasine (1b). To a solution of 2,3,4-triacetoxy-7-deoxynarciclasine (**1c**, 4.36 g) in CH_3OH−(99 mL) H_2O−(1 mL) CH_2Cl_2 (30 mL) was added K_2CO_3 (0.124 g) and stirring continued for 16 h at rt while a white precipitate separated. The mixture was neutralized with acetic acid (2 mL), stirred for 15 min, and concentrated to minimum volume. The colorless product was collected (2.18 g, 72%), and recrystallization from acetic acid−MeOH afforded fine needles: mp 205−210 °C (dec), 230 °C melts; $[\alpha]^{23}_D$ +147° (*c* 0.52, DMSO) [lit.[9c] mp 251−252°C, $[\alpha]^{33}_D$ +157.3° (*c* 0.96, DMSO)]; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.29 (1H, s, H-7), 7.24 (1H, s, H-10), 7.17 (1H, s, NH), 6.10−6.08 (3H, m, H-1, −OCH_3O−), 5.15 (2H, m, OH), 4.96 (1H, m, OH), 4.16 (1H, d, J = 8.1 Hz, H-4a), 4.01 (1H, m, H-2), 3.76 (1H, m, H-4), 3.69 (1H, m, H-3); ^{13}C NMR (DMSO-d_6, 75 MHz) 163.8 (C, C-6), 151.7 (C), 148.5 (C), 132.4 (C), 130.7 (C), 124.3 (C), 122.6 (CH, C-1), 106.9 (CH, C-7), 103.9 (CH, C-10), 102.5 (CH_2, −OCH_2O−), 73.2 (CH, C-1), 69.9 (2CH, C-4, C-3), 53.4 (CH, C-4a); HRAPCI$^+$ *m/z* 292.0770 $(M + H)^+$ (calcd for $C_{14}H_{14}NO_6$, 292.0891 $[M + H]^+$).

Sodium 7-Deoxynarcistatin (5). A solution of 7-deoxynarciclasine (**1b**, 0.2 g, 0.69 mmol) in pyridine (8 mL) was heated to 80 °C, and tetrabutylammonium dihydrogen phosphate (0.15 g, 0.45 mmol, 0.65 equiv) followed by dicyclohexylcarbodiimide (0.8 g, 5.6 equiv) were added. The reaction was allowed to proceed at 80 °C for 24 h. An ^{1}H NMR analysis of the reaction mixture composition indicated a 50:50 mixture of starting material to product. Tetrabutylammonium dihydrogen phosphate (0.15 g) was added followed by DCCI (0.8 g), and the reaction continued for a further 24 h. At this point, ^{1}H NMR analysis of a sample from the reaction mixture showed the reaction was complete. The reaction mixture was cooled, and H_2O (100 mL) was added. The precipitated dicyclohexylurea (DCU) was collected, and the pyridine−H_2O mother liquor was concentrated to minimum volume. The aqueous fraction was then passed through an ion-exchange column (DOWEX 50W8-400) in the sodium form. The UV-responsive fractions were combined and lypholized to yield phosphate **5** as a colorless solid: 227 mg (88%); mp 255°C (dec); $[\alpha]^{24}_D$ −7.2 (*c* 0.5, CH_3OH); ^{1}HNMR (DMSO-d_6, 400 MHz,) δ 7.65 (1H, s, NH), 7.35 (s, 1H, H-7), 7.31(1H, s, H-10), 6.35 (1H, m, H-1), 6.06−6.05 (2H, m, −OCH_2O−), 5.71 (1H, d, J = 6.5 Hz, OH), 4.26−4.22 (2H, m, H-4a, H-2), 3.93−3.79 (2H, m, H-3, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) 179.5 (C, C-6) 161.4 (C), 151.2 (C), 147.7 (C), 128.9 (C), 126.2 (CH, C-1), 120.7 (C), 106.1-(C), 101.8 (CH_2, CH, −OCH_2O−, C-7), 75.6 (CH, C-4), 74.8 (CH, C-3), 70.3 (CH, C-2), 54.04 (CH, C-4a); ^{31}P (DMSO-d_6, 162 MHz) δ 5.23; HRESI *m/z* 352.0202 $[M - Na]^-$ (calcd for $C_{14}H_{11}NOP^-$ 352.0222 $[M - Na]^-$).

General Procedure for the Preparation of 7-Deoxynarcistatin Prodrugs 5a and 5b. Sodium 7-deoxynarcistatin (**5**, 52 mg) was dissolved in H_2O (1 mL) and the solution passed through a column of Dowex 50WX8-400, bearing the respective cation. The UV-active fractions were combined and freeze-dried to give the corresponding narcistatin salt as a white solid.

Lithium 7-deoxynarcistatin (5a): 34 mg, mp 250 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.65 (1H, s, NH), 7.37 (1H, s, H-7), 7.32 (1H, s, H-10), 6.38 (1H, m, H-1), 6.08−6.07 (2H, m, −OCH_2O−), 5.72 (1H, d, J = 6 Hz, OH), 4.30−4.25 (2H, m, H-4a, H-2), 3.91−3.78 (2H, m, H-3, H-4).

Potassium 7-deoxynarcistatin (5b): 43 mg, mp 230−235 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.67 (1H, s, NH), 7.37 (1H, s, H-7), 7.32 (1H, s, H-10), 6.37 (1H, m, H-1), 6.08−6.07 (2H, m, −OCH_2O−), 5.70 (1H, m, OH), 4.24 (2H, bm, H-4a, H-2), 3.91−3.78 (2H, m, H-3, H-4).

7-Deoxy-*trans*-dihydronarciclasine (3a). The saponification of 2,3,4-triacetoxy-7-deoxy-*trans*-dihydronarciclasine (**3b**, 0.14 g) was performed in 9:1 aqueous MeOH−K_2CO_3 (0.003 g) and was conducted as described above for obtaining alcohol **1b** to yield triol **3a** as a colorless solid: 89 mg (91% yield); mp >300 °C (dec) [lit.[10a] mp 303−304 °C]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (1H, s, NH), 6.91 (2H, m, H-7, H-10), 6.05 (2H, m, −OCH_2O−), 4.95−4.90 (2H, m, OH), 4.78 (1H, s, OH), 3.86 (1H, m, H-4a), 3.69 (2H, m, H-3, H-4), 3.3 (1H, m, H-2), 2.86 (1H, m, H-10b), 2.12 (1H, m, H-1eq), 1.64 (1H, m, H-1ax); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.9 (C-6), 151.3 (C), 138.7 (2C) 123.9 (C), 107.6 (CH, C-7), 104.9 (CH, C-10), 102.2 (CH_2, −OCH_2O-), 72.3 (CH, C-4), 70.4 (CH, C-3), 69.3 (CH, C-2), 55.8 (CH, C-4a), 34.9 (CH, C-10b), 29.9 (CH, C-1); HRFAB$^+$ *m/z* 294.0967 [M + H]$^+$ (calcd for $C_{14}H_{15}NO_6$ 294.0978 [M + H]$^+$).

Sodium 7-Deoxy-*trans*-dihydronarcistatin (6). The conversion of 7-deoxy-*trans*-dihydronarciclasine (**3a**, 0.15 g, 0.51 mmol) to narcistatin **6** in pyridine (6 mL) with tetrabutylammonium dihydrogen phosphate (0.21 g, 0.62 mmol, 1.2 equiv) and dicyclohexylcarbodiimide (0.54 g, 2.62 mmol, 5.13 equiv) was conducted and the phosphate isolated as summarized for synthesis of narcistatin (**5**) including the additional tetrabutylammonium dihydrogen phosphate (0.21 g, 1.2 equiv) and DCCI (0.54 g) (cf. above). The aqueous extract of product was subjected to a Dowex 50WX8-400 ion-exchange column (sodium form), and the UV fluorescing fractions were combined and lyophilized as noted above (cf. **5**). A solution of the sodium salt was prepared in MeOH (15 mL with heating), the insoluble material was collected, and the filtrate was concentrated to yield 0.124 g, 65%; mp 297 °C (dec); $[\alpha]^{23}_D$ −19° (*c* 0.35, H_2O); ^{1}H NMR (DMSO-d_6, 400 MHz$_6$) δ 7.53 (1H, s, NH), 7.27 (1H, s, H-7), 6.93 (1H, s, H-10), 6.04−6.03 (2H, m, −OCH_2O−), 5.22 (1H, bs, OH), 4.12−4.09 (1H, m, H-3)), 4.07−3.98 (2H, m, H-2, H-4), 3.54 (1H, dd, J = 13, 8.6 Hz, H-4a), 2.84 (1H, m, H-10b), 2.27 (1H, m, H-1eq), 1.47 (1H, m, H-1ax); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.7 (C, C-6), 151.2 (C), 146.7 (C), 139.7 (C), 124.2 (C), 107.6 (CH, C-7), 105.2 (CH, C-10), 102.2 (CH_2, −OCH_2O−), 75.9 (CH, C-4), 75.7 (CH, C-3), 60.6 (CH, C-2), 56.9 (CH, C-4a), 32.7 (CH, C-10b), 29.5 (CH_2, C-1); ^{31}P NMR (162 MHz, DMSO-d_6) δ 13.45; HRESI *m/z* 354.5294 [M − Na]$^-$ (calcd for $C_{14}H_{13}NO_8P^-$ 354.0379 [M − Na]$^-$).

General Procedure for the Preparation of 7-Deoxy-*trans*-dihydronarcistatin Prodrugs 6a and 6b. Sodium 7-deoxy-*trans*-dihydronarcistatin (**6**, 30 mg) was dissolved in H_2O (1 mL) and the solution passed through a column of Dowex 50WX8-400, bearing the respective cation. The UV-active fractions were combined and freeze-dried to give the corresponding narcistatin salt as a white solid.

Lithium 7-deoxy-*trans*-dihydronarcistatin (6a): 25.4 mg, mp 253 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.57 (1H, s, NH), 7.28 (1H, s, H-7), 6.96 (1H, s, H-10), 6.06−6.05 (2H, m, −OCH_2O−), 5.19 (1H, m, OH), 4.10−4.00 (3H, m, H-4, H-3, H-2), 3.57 (1H, dd, J = 8.1, 12.9 Hz, H-4a), 2.86 (1H, m, H-10b), 2.28 (1H, m, H-1eq), 1.50 (1H, m, H-1ax).

Potassium 7-deoxy-*trans*-dihydronarcistatin (6b): 23.2 mg, mp 287 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.56 (1H, s, NH), 7.28 (1H, s, H-7), 6.96 (1H, s, H-10), 6.06 (2H, s, −OCH_2O−), 5.17 (1H, m, OH), 4.07−3.95 (3H, m, H-3, H-2, H-4), 3.59−3.52 (1H, m, H-4a), 2.84 (1H, m, H-10b), 2.28 (1H, m, H-1eq), 1.48 (1H, m, H-1ax).

Sodium *trans*-dihydronarcistatin (7). Synthesis of 3,4-cyclic phosphate **7** from *trans*-dihydronarciclasine[18,19] (**4**, 57 mg, 0.184 mmol) was accomplished in pyridine (2 mL) employing tetrabutylammonium dihydrogen phosphate (60 mg, 0.176 mmol, and 60 mg for the delayed addition) and dicyclohexylcarbodiimide (0.18 g, 0.87 mmol, and 0.18 g for the second addition) as described for the preparation of phosphate **5** (see above). The aqueous fraction eluted from the ion-exchange column (Dowex 50WX8-400, sodium form) provided sodium *trans*-dihydronarcistatin (**7**) as a colorless solid (86 mg, 94% yield): mp > 300 °C, $[\alpha]^{23}_D$ −32° (*c* 0.3, H_2O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.16 (1H, s, OH-7), 8.40 (1H, s, NH), 6.52 (1H, s, H-7), 6.03 (2H, m, −OCH_2O−), 5.26 (1H, bs, OH), 4.14−4.03 (3H, m, H-3,H-4, H-2), 3.59 (1H, dd, J = 13.6, 8.8 Hz, H-4a), 2.84 (1H, m, H-10b), 2.26 (1H, m, H-1eq), 1.50 (1H, m, H-1ax); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.5 (C-6), 163.9 (C), 152.1 (C), 145.5 (C), 137.6 (C), 106.9 (C), 101.8 (CH_2, −OCH_2O−), 96.7 (CH, C-10), 74.9 (3CH, C-2, C-3, C-4), 56.1 (CH, C-4a), 31.5 (CH, C-10b), 28.7 (CH_2,C-1); ^{31}P NMR (DMSO-d_6, 162 MHz) δ 13.36; HRESI *m/z* 370.0314 [M − Na]$^-$ (calcd for $C_{14}H_{13}NO_9P^-$ 370.0328 [M − Na]$^-$).

General Procedure for the Preparation of *trans*-Dihydronarcistatin Prodrugs 7a and 7b. Sodium *trans*-dihydronarcistatin (**7**, 0.010 g) was dissolved in water (1 mL) and the solution passed through a Dowex 50WX8-200 column, bearing the respective cation. The UV-active fractions were combined and freeze-dried to give the corresponding *trans*-dihydronarcistatin salt as a white solid.

Lithium *trans*-dihydronarcistatin (7a): 8 mg, mp 275 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.17 (1H, s, OH-7), 8.41 (1H, s, NH), 6.52 (1H, s, H-10), 6.04−6.02 (2H, m, −OCH_2O−), 5.23 (1H, s, OH), 4.14−4.02 (3H, m, H-2, 3, 4), 3.34−3.57 (1H, m, H-4a), 2.88−2.79 (1H, m, H-10b), 2.28−2.24 (1H, m, H-1eq), 1.52−1.45 (1H, m, H-1ax).

Potassium *trans*-dihydronarcistatin (7b): 7.1 mg, mp 230−235 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.16 (1H, s, OH-7), 8.41 (1H, s, NH), 6.51 (1H, s, H-10), 6.04−6.02 (2H, m, −OCH_2O−), 5.19 (1H, s, OH-2), 4.09−4.04 (3H, m, H-2, 3, 4), 3.57 (1H, m, H-4a), 2.82 (1H, m, H-10b), 2.28−2.22 (1H, m, H-1eq), 1.53−1.43 (1H, m, H-1ax).

Preparation of Sodium Narcistatin (8). Synthesis of 3,4-cyclic phosphate **8** from narciclasine (**1a**) (0.113 g, 0.368 mmol) was carried out in pyridine (4 mL) using tetrabutylammonium dihydrogen phosphate (0.075 g, 0.22 mmol) and dicyclohexylcarbodiimide (0.4 g, 1.94 mmol) with additional amounts of tetrabutylammonium dihydrogen phosphate (0.185 g) and dicyclohexylcarbodiimide (0.4 g) added after the first 24 h stirring at 80 °C. The reaction was stirred for 96 h, cooled, and filtered to remove precipitated DCU. Water (100 mL) was added and the mixture refiltered to remove any residual DCU. The mother liquor was concentrated to minimum volume. The aqueous fraction was eluted through an ion-exchange column (sodium form). The UV-active fractions were combined and lypholized to yield the phosphate **8** as a cream solid (88% yield). Analysis (^{1}H NMR, ^{13}C NMR, ^{31}P NMR) showed **8** to be identical with an authentic sample.[13a]

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