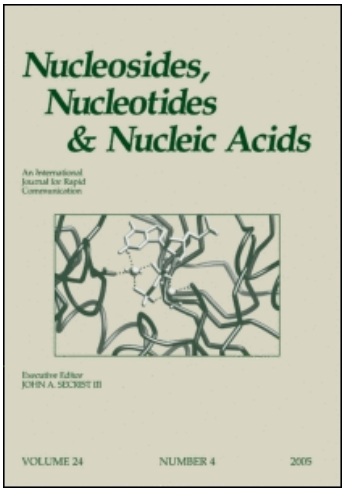

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

2',3'-Dideoxyadenosine Analogs of the Nucleoside Antibiotics Tubercidin, Toyocamycin and Sangivamycin

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To cite this Article Krawczyk, Steven H. and Townsend, Leroy B.(1989) '2',3'-Dideoxyadenosine Analogs of the Nucleoside Antibiotics Tubercidin, Toyocamycin and Sangivamycin', Nucleosides, Nucleotides and Nucleic Acids, 8: 1, 97 — 115
To link to this Article: DOI: 10.1080/07328318908054160
URL: http://dx.doi.org/10.1080/07328318908054160

NUCLEOSIDES & NUCLEOTIDES, 8(1), 97-115 (1989)

2',3'-DIDEOXYADENOSINE ANALOGS OF THE NUCLEOSIDE ANTIBIOTICS TUBERCIDIN, TOYOCAMYCIN AND SANGIVAMYCIN

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Abstract: Application of previously described methodologies, for the synthesis of 2',3'-dideoxy-2',3'-didehydro nucleosides from the parent ribonucleosides, to the antibiotics tubercidin (1), toyocamycin (6) and sangivamycin (10) has provided the corresponding 2',3'-unsaturated nucleosides 4, 9, and 13. A reduction of the 2',3'-unsaturated moiety has afforded the 2',3'-dideoxynucleoside antibiotics 5, 14, and 15.

The 2',3'-dideoxynucleoside class of compounds have been reported[1,2] to be some of the most active anti-HIV agents, with 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxycytidine (ddCyd) demonstrating the most potency.[3,4] Although both ddCyd and AZT are pyrimidine nucleosides, the 2',3'-dideoxyribofuranosyl purines have also demonstrated significant activity against HIV.[1,4] On the basis of these studies, vide supra, substitution of the 2',3'-dideoxyribose moiety by either a purine or a pyrimidine base can result in anti-HIV activity.

To date, while a variety of 2',3'-dideoxypyrimidine analogs have been synthesized and tested, only a few purine analogs of 2',3'-dideoxyadenosine,[5] have been synthesized and tested.[1-4]

This has prompted us to initiate research designed to synthesize and evaluate various heterocyclic analogs of 2',3'-dideoxyadenosine. Our interest in pursuing modifications of the purine moiety instead of the carbohydrate moiety stemmed from the

fact that minor modifications of the sugar moiety have been reported to reduce or in some cases completely eliminate activity. Although the 2',3'-dideoxyribosyl moiety appears to be the optimal carbohydrate moiety for a variety of heterocyclic substituents, there appears to be more latitude allowed in modifications involving the heterocycle.[4]

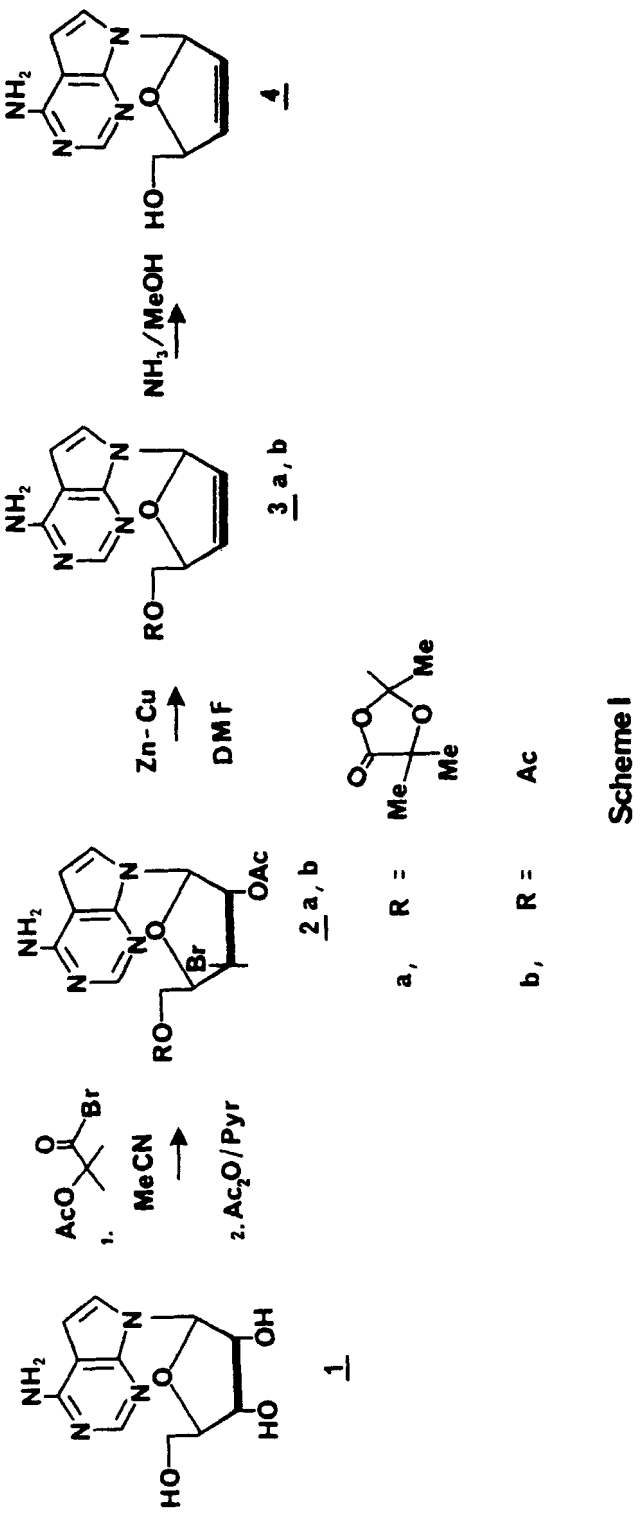
The chronic nature of the HIV infection necessitates an excellent toxicological profile in an agent designed for its treatment. Therefore, the design phase of our research involved the selection of compounds which would potentially have low cytotoxicity, especially at high concentrations of the drug. We noted that 2',3'-dideoxycytidine was only ten fold more active than 2',3'-dideoxyadenosine and yet 2',3'-dideoxyadenosine possessed one of the highest therapeutic indices of any 2',3'-dideoxynucleoside tested.[4] A priori, it would appear that heterocyclic analogs of 2',3'-dideoxyadenosine may possess a notable lack of cytotoxicity, while retaining the anti-HIV activity characteristic of nucleosides bearing the 2',3'-dideoxyribofuranosyl substituent.

We examined previous reports involving various chemical modifications of the 6-aminopurine (adenine) moiety, which have resulted in a retention or increase of biological activity. Substitution of the 7-nitrogen with carbon, or a carbon bearing a substituent, was one of the most attractive choices since several nucleosides of pyrrolo[2,3-d]pyrimidines have exhibited exceptional biological and chemotherapeutic activities.[6] Because of our own interest in sugar modified analogs of the pyrrolo[2,3-d]pyrimidine nucleoside antibiotics,[10,11] and the fact that some arabino, and xylo analogs of pyrrolo[2,3-d]pyrimidine nucleosides have been recently shown to possess antiviral activity,[7-9] we initiated a study designed to synthesize the 2',3'-dideoxy- and 2',3'-dideoxy-2',3'-didehydro analogs of the nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin.

Previously reported methodology[12] for the conversion of adenosine to 2',3'-dideoxy-2',3'-didehydroadenosine was applied to

the nucleoside tubercidin (Scheme I). This procedure furnished the corresponding unsaturated nucleoside 4-amino-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]-pyrimidine[14,16] (2',3'-dideoxy-2',3'-didehydrotubercidin, 4) in good yield, without the Dowex column chromatography which was reported[12] to be necessary for the isolation of the adenosine analog. However, application of this methodology to toyocamycin failed to furnish a pure product, without resorting to preparative reverse-phase chromatography. This was presumably due to the tendency of the 5-cyano moiety to add methanol during the extended reaction time required for a removal of the bulky 5'-O-dioxolanyl-moiety in methanolic ammonia. However, a slight modification, in which the 5'-dioxolane moiety is replaced by an acetyl group during the first reaction of the sequence, resulted in the isolation of a pure final product by direct crystallization.

Treatment of toyocamycin (6) with 2-methyl-2-acetoxy-propionyl bromide in acetonitrile afforded a mixture of compounds which appeared[12] as two bands on TLC (chloroform/methanol, 9:1, Rf = 0.6, 0.4). The lower band was presumed to be 4-amino-5-cyano-7-(2-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)pyrrolo[2,3-d]-pyrimidine while the upper band was presumed to be the corresponding 5'-trimethyldioxolanone derivative. The addition of methanol at this point effected a complete removal of the 5'-trimethyldioxolanone moiety to afford the 5'-hydroxy derivative. Subsequent acetylation afforded 4-amino-5-cyano-7-(2,5-di-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)pyrrolo[2,3-d]pyrimidine (7) which could then be crystallized and separated from the by-products of the deblocking step. This material was then treated with a zinc-copper couple[15] in DMF to afford the 5'-acetate of the desired unsaturated product, 4-amino-5-cyano-7-(5-O-acetyl-2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]pyrimidine (8). It was found that a careful purification of the reaction mixture from the acetylation step in fact hindered the elimination step. Thus, 7, which had been purified by crystallization, had to be heated to ca. 140° before the elimination proceeded. Compound 7



Scheme I

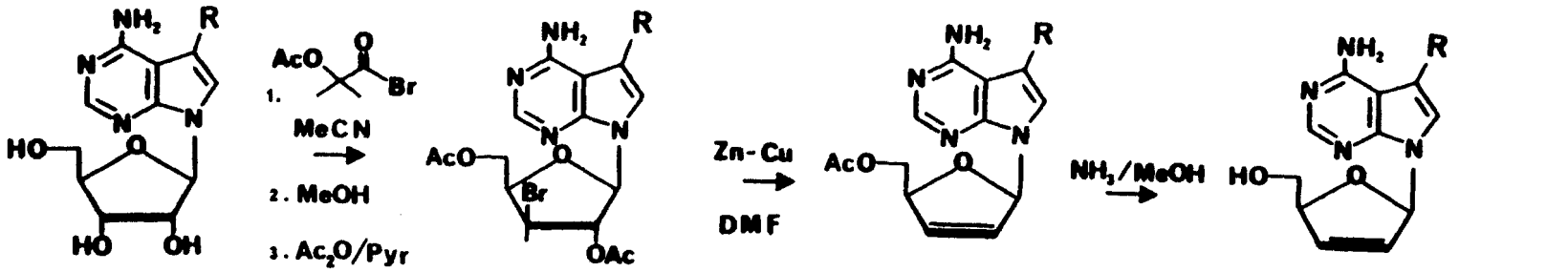
which had been purified only by an extraction of the ethyl acetate solution with water and evaporation to a foam was found to react at ca. 80°. The key factor was subsequently traced to the presence of acetic acid in the foam. Thus, the addition of ca. 10 mole % acetic acid allowed the elimination to proceed at room temperature. The 5'-acetate derivative 8 was then deblocked with methanolic ammonia to afford the desired compound 4-amino-5-cyano-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pentofuranosyl)pyrrolo-[2,3-d]pyrimidine (9) in an overall yield of 20%.

Synthesis of the sangivamycin derivatives, 4-amino-7-(2,5-di-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)pyrrolo[2,3-d]-pyrimidine-5-carboxamide (11), 4-amino-7-(5-O-acetyl-2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamide (12), and 4-amino-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamide (13), were accomplished using sangivamycin as our starting material and a procedure analogous to the one described for the synthesis of the toyocamycin derivatives 7, 8, and 9.

Hydrogenation of compounds 4, 9, and 13 with palladium on charcoal afforded compounds 4-amino-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]pyrimidine[13] (5), 4-amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]pyrimidine (14), and 4-amino-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine-5-carboxamide (15) without any major complications.

EXPERIMENTAL SECTION

General Methods. Melting points were determined on a Thomas Hoover unimelt apparatus and are uncorrected. Silica gel used for chromatography was silica gel 60 (E. Merck, Darmstadt, West Germany; 230-400 mesh) or 10 micron TLC grade silica without binder (Analtech Corp. Newark, DE). Thin layer chromatography (TLC) was performed on prescored SilicAR 7GF, (Analtech Corp. Newark, DE) silica gel plates (0.25 mm thick). Compounds were visualized on the eluted plates by observation under UV light (254 nm) and by spraying the plates with 20% methanolic sulfuric



6 R = CN

10 R = $CONH_2$

7 R = CN

11 R = $CONH_2$

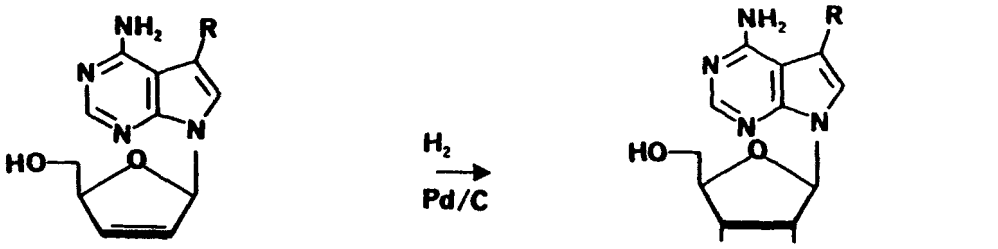
8 R = CN

12 R = $CONH_2$

9 R = CN

13 R = $CONH_2$

Scheme II



4	R =	H	5	R =	H
9	R =	CN	14	R =	CN
13	R =	$CONH_2$	15	R =	$CONH_2$

Scheme III

acid containing 2% p-methoxybenzaldehyde, followed by heating on a hot plate to develop the characteristic color of particular components. The solvents used were as follows: Solvent A, chloroform/methanol (9:1, v:v). All evaporations were conducted at water bath temperature (35-45°) unless otherwise noted. Ultraviolet spectra were obtained on a Hewlet-Packard 8450-A UV/VIS spectrophotometer. IR spectra were obtained using a Perkin Elmer 281 spectrophotometer. ^{1}H NMR spectra were obtained using a IBM WP 270-SY or a Bruker WM 360 instrument operating in the FT mode at 270 MHz and 360 MHz, respectively. Where necessary, deuterium exchange, and decoupling experiments were performed to confirm proton assignments.

4-Amino-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine (4, 2',3'-Dideoxy-2',3'-didehydro-tubercidin)[14]

2-Methyl-2-acetoxypropionyl bromide (7.07 g, 33.9 mmole) was added, with vigorous stirring to a suspension of finely ground tubercidin (3.0 g, 11.3 mmole) in acetonitrile (100 mL, dried over

3 A° sieves) and the mixture then stirred at room temperature for 1.5 hr. The resulting solution was partitioned between ice-cold 50% saturated sodium bicarbonate (300 mL) and ethyl acetate (300 mL). The organic layer was washed with brine (100 mL), dried over sodium sulphate, filtered, and the filtrate was evaporated to afford a crisp white foam (5.8 g). This foam was dissolved in pyridine (100 mL), 4-dimethylamino-pyridine was added (40 mg, 0.4 mmole), and the solution was treated with acetic anhydride (4.0 mL, 43 mmole). The mixture was stirred at room temperature for 1 hr and then evaporated to a thick oil. This oil was partitioned between ethyl acetate (300 mL) and water (300 mL). The organic layer was then washed with brine (100 mL), dried over sodium sulphate, filtered, and the filtrate evaporated to a yellow foam which was kept in vacuo for 72 hr. This foam was dissolved in dimethyl-formamide (150 mL) and treated with zinc-copper couple (20 g). The mixture was stirred for 1.5 hr at 80°, then filtered through Celite. The Celite bed was washed with dimethylformamide (50 mL) and the combined filtrates were evaporated at 50-60° to afford a syrup which was partitioned between ethyl acetate (100 mL) and water (300 mL). The water layer was then extracted with ethyl acetate (2 x 100 mL) and the combined organic fractions were washed with brine (200 mL), dried over magnesium sulphate, filtered and the filtrate was evaporated to a thick oil. This oil was further evaporated in vacuo to afford a soft foam. This foam was dissolved in methanol (35 mL), methanolic ammonia (35 mL, saturated at 0°) was added, and the solution was sealed in a pressure bottle and stirred at room temperature for 12 hr. The solution was evaporated to a thick oil and then further evaporated in vacuo at 50-60° to afford a very thick oil. This oil was dissolved in chloroform (10 mL) and the solution was applied to a bed of silica (2 cm deep, 3 cm wide) slurry packed in a fritted disc funnel. This bed was eluted with solvent system A (300 mL). The first 100 mL of eluant was discarded, and the next 200 mL of eluant was collected and evaporated at 50-60° to afford a thick oil. This oil was triturated with ether (5 x 20 mL), chloroform

(15 mL) was added to the remaining residue and this mixture then heated at reflux. Ether was then slowly added (30 mL) and the mixture solidified upon cooling to room temperature. Additional ether was added (50 mL), the solid was collected by filtration and air dried to yield a tan solid (1.63 g). The mother liquor was combined with the ethereal extracts, the resulting solution was evaporated and then triturated with chloroform/ether (1:2, 30 mL) to afford an additional 0.4 g of solid. The solids were combined and suspended in boiling ethyl acetate (200 mL). Isopropanol (30 mL) was added, and the boiling mixture was stirred until all solid material had dissolved. Norit (200 mg) was added, and the mixture was filtered through Celite. The Celite bed was washed with ethyl acetate (20 mL). The combined filtrates were reduced in volume to approx. 50 mL by boiling. The solution was allowed to stand at room temperature for 4 hr. The solid was collected by filtration and air dried to afford 1.3 g of a tan solid. The material was further purified by dissolving 1.2 g in boiling ethanol (150 mL) and reducing the volume of the solution to 50 mL by boiling. The solution was allowed to cool to room temperature and then allowed to stand at 4° for 16 hr. The solid was collected by filtration to yield a solid (0.93 g), after drying at 80° under reduced pressure over phosphorous pentoxide. Some additional product (0.32 g) could be obtained by evaporating the ethyl acetate and ethanol mother liquors and crystallizing the resulting residue from ethanol (25 mL) for a total yield of 1.25 g (48%): mp 204-205°; ^{1}H NMR (DMSO-$\underline{d}_6$): δ 8.07 (s, 1H, H-2), 7.15 (d, 1H, H-6, $J_{(5,6)}$=3.71 Hz), 7.12 (bs, 1H, H-1'), 7.02 (bs, 2H, NH_2), 6.56 (d, 1H, H-5), 6.42 (ddd, 1H, H-3', $J_{(2',3')}$=6.01 Hz, $J_{(3',4')}$=$J_{(3',1')}$ =1.62 Hz), 6.02 (ddd, 1H, H-2'), 4.96 (t, 1H, 5'-OH), 4.79 (m, 1H, H-4'), 3.50 (m, 2H, H-5'a,b); UV: λ_{max} (nm) (log epsilon): MeOH, 271 (4.11); pH 1, 272 (4.04), 226 (4.36); pH 11, 271 (4.04), 224 (3.82); TLC: solvent A, R_f = 0.21; Anal. Calcd. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.20; N, 24.13. Found: C, 56.88; H, 5.22; N, 24.09.

4-Amino-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]-pyrimidine (5, 2',3'-dideoxytubercidin)

A solution of compound 4 (0.5 g, 2.14 mmole) in absolute ethanol (75 mL), containing 10% palladium on charcoal (0.04 g) was hydrogenated for 7 hr on a Parr hydrogenation apparatus at a pressure of 50 psi. The catalyst was removed by filtration through Celite. The Celite bed was washed with ethanol (10 mL) and the filtrates were evaporated to afford an oil. This oil was dissolved in chloroform (30 mL) and the solution was evaporated to afford a foam. After being kept in vacuo for 16 hr, this foam was dissolved in chloroform/methanol (6:1, v:v, 3 mL) and the solution was applied to a silica column (300 mm x 22 mm). The column was eluted at a flow rate of 17 mL/min, with chloroform/methanol (6:1, v:v). The elution was followed by observing the absorption at 275 nm. The major fraction (eluting between 7.2 and 16.4 min) was evaporated to dryness and kept in vacuo for 48 hr to afford 380 mg of a crisp foam (75%): ^{1}H NMR (DMSO-d_6): δ 8.04 (s, 1H, H-2), 7.33 (d, 1H, H-6, $J_{(5,6)}$=3.6 Hz), 6.99 (bs, 2H, NH_2), 6.55 (d, 1H, H-5), 6.33 (dd, 1H, H-1', $J_{(1',2'a)}=J_{(1',2'b)}$=6.8 Hz), 4.99 (bs, 1H, 5'-OH), 4.03 (m, 1H, H-4'), 3.55 and 3.46 (2m, 2H, H-3'ab); UV λ_{max} (nm) (log epsilon): MeOH, 273 (3.99), 228 (4.35); pH 1, 273 (4.02), 277 (4.35); pH 11, 271 (4.07), 225 (3.95); TLC: solvent A, R_f = 0.24; Anal. Calcd. for $C_{11}H_{14}N_4O_2 \cdot (1/4\ H_2O)$: C, 55.34; H, 6.12; N, 23.47. Found: C, 55.25; H, 6.29; N, 23.27.

4-Amino-5-cyano-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pento-furanosyl)pyrrolo[2,3-d]pyrimidine (9, 2',3'-Dideoxy-2',3'-didehydrotoyocamycin)

A vigorously stirred suspension of finely ground toyocamycin (6, 5.8 g, 20 mmole) in acetonitrile (125 mL, dried over 3 A°) was chilled with an ice bath to ca. 5°. To this suspension was added 2-methyl-2-acetoxypropionyl bromide (11.7 g, 56 mmol) over a 5 min. period via syringe, with continuous stirring and cooling. After the addition was complete, the ice bath was removed and stirring was continued for an additional 1 h. After this time,

TLC analysis of the mixture revealed two bands (R_f = 0.60, 0.30; solvent system A; plate exposed to conc. ammonia vapor for ca. 1 sec before developing). The solution was then treated with methanol (5 mL) and stirring was continued for an additional 15 min. After this time, TLC analysis showed an absence of the faster running band. The solution was then evaporated to afford a sticky foam which was kept in vacuo for 0.5 hr and then dissolved in pyridine (150 mL). Acetic anhydride (12 mL, 127 mmole) was then added to this solution and stirring was continued for an additional 0.5 hr. At this time, TLC showed one major band (R_f = 0.6). The solution was then evaporated in vacuo to a thick oil which was partitioned between ethyl acetate (300 mL) and water (600 mL). The organic layer was washed with water (2 x 600 mL) and brine (300 mL), dried over magnesium sulphate, filtered, and evaporated to a foam which was treated with methanol (100 mL) and again evaporated to a foam which was then kept in vacuo for 0.5 hr. This foam was dissolved in hot methanol (125 mL) and the solution was allowed to cool to room temperature. The solution was allowed to stand at room temperature for 2 hr, then kept at ca. 5° for 1 hr. The crystalline product was collected by filtration, washed with cold methanol (30 mL) and dried at 80° under reduced pressure over phosphorous pentoxide for 16 hr to afford 4.2 g of a white solid. Additional material could be obtained by evaporating the mother liquor, triturating the residue with hot ligroin (3 x 75 mL), co-evaporating the residue with methanol (2 x 50 mL), and finally crystallizing the resulting foam from hot methanol (30 mL). Drying the solid as above, yielded 1.2 g of a hard glass which was identical by TLC (R_f = 0.6, solvent system A) to the first crop.

To a solution of the material obtained as described above (7.5 g, 17 mmole), in dimethylformamide (25 mL), was added acetic acid (0.25 mL, 2.6 mmole) and zinc-copper couple (7.5 g). After stirring for 10 min., the mixture became warm and TLC analysis (solvent system A) showed the absence of starting material (R_f = 0.63, chars yellow) and the presence of a new spot (R_f = 0.57,

chars blue). The mixture was treated with water (10 mL) and filtered through Celite. The Celite bed was washed with dimethylformamide (20 mL) and the combined filtrates diluted to a volume of 200 mL with water. This solution was extracted with ethyl acetate (5 x 40 mL) and the combined extracts were washed with water (40 mL) and then brine (3 x 40 mL). The organic layer was then dried over magnesium sulphate, filtered, and evaporated to afford a sticky foam which was co-evaporated with methanol (2 x 40 mL). This foam was then dissolved in hot methanol (20 mL), filtered, and allowed to crystallize for 2 h at room temperature. The product was collected by filtration, and dried as described above to afford 2.7 g (52%) of the 5'-O-acetate of the title compound: mp 148-149°; ^{1}H NMR (DMSO-d_6): δ 8.25 (s, 1H, H-2), 8.09 (s, 1H, H-6), 7.13 (bs, H, H-1'), 6.88 (bs, 2H, NH_2), 6.51 (d, 1H, H-3', $J_{(2',3')}$ =6 Hz), 6.17 (d, 1H, H-2'), 5.06 (bs, 1H, H-4'), 4.16 (m, 2H, H-5'ab), 2.01 (s, 3H, 5'-CH_3CO).

A suspension of the 5'-O-acetate (2.0 g, 6.3 mmole) in methanolic ammonia (75 mL, saturated at 0°) was sealed in a pressure bottle and stirred at room temperature for 6 hr. The resulting yellow solution was reduced in volume by boiling on a steam bath to approx. 20 mL. The solution was then allowed to crystallize at room temperature. The solid was collected by filtration, washed with methanol (5 mL) and air dried to afford 1.2 g of a white solid (22 % overall from toyocamycin). A sample (1.0 g) of this material was recrystallized by dissolving it in methanol (40 mL), diluting the solution with water to a final volume of 100 mL, and allowing the solution to stand overnight at room temperature. The solid was collected by filtration and washed with water (10 mL) to yield 0.56 g of material after air drying. A sample of this material (0.2 g) was dissolved in solvent system A (10 mL) and the solution was chromatographed on a silica column (22 mm x 300 mm) using solvent system A at a flow rate of 17 mL/min. The elution was followed by observing the absorption at 280 nm. The desired fraction (eluting between 4.2 and 10.0 min.) was immediately evaporated under water aspirator

pressure at room temperature. The residue was suspended in methanol (5 mL) and filtered. The product cake was washed with methanol (5 mL) and ether (5 mL) to afford 0.12 g of a white powder, after air drying: mp 201-202°; ^{1}H NMR (DMSO-d_6): δ 8.25 (s, 1H, H-2), 8.22 (s, 1H, H-6), 7.13 (bs, 1H, H-1'), 6.87 (bs, 2H, NH_2), 6.48 (d, 1H, H-3', $J_{(2',3')}$=6.05 Hz), 6.07 (d, 1H, H-2'), 5.00 (t, 1H, 5'-OH), 4.87 (m, 1H, H-4'), 3.58 (m, 2H, H-5'ab); UV λ_{max} (nm) (log epsilon): MeOH, 279 (4.31), 229 (4.17); pH 1, 273 (4.26), 233 (4.39); pH 11, 278 (4.33), 231 (4.20); TLC: Solvent system A, R_f = 0.38; IR(KBr) 2220 cm^{-1} (cyano); Anal. Calcd. for $C_{12}H_{11}N_5O_2$: C, 56.03; H, 4.31; N, 27.23. Found: C, 56.08; H, 4.36; N, 27.16.

4-Amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine (14, 2',3'-Dideoxytoyocamycin)

A solution of compound 9 (0.4 g, 1.7 mmole) in ethanol/water (90:10, v:v, 30 mL) containing 5% palladium on charcoal (0.05 g) was hydrogenated on a Parr apparatus for 1 hr at a pressure of 50 psi. An additional portion (0.1 g) of the catalyst was then added, and the hydrogenation was continued for an additional 2 hr. The catalyst was removed by filtration through Celite, and the Celite bed was then washed with ethanol (20 mL). The combined filtrates were evaporated under aspirator pressure at 45-50°, and the residue was co-evaporated with ethanol (2 x 20 mL). The residue was crystallized from isopropanol (20 mL), and washed with ether (20 mL) to yield 0.23 g (57%) of a white solid, after air drying. A sample (0.1 g) of this material was dissolved in DMSO (2 mL) and the solution was chromatographed in two portions on a C-18 column (22 mm x 250 mm, 50 mm guard column) using water/methanol (70:30, v:v) at a flowrate of 12 mL/min. The elution was monitored by observing the absorption at 280 nm. The desired fraction eluted between 23 and 42 min. This fraction was combined with the corresponding fraction from a duplicate run and evaporated under water aspirator pressure at 35° to a volume of 8 mL. The resulting suspension was heated to dissolve the solids

and the solution was then allowed to stand at room temperature for 3 hr. The resulting solid was collected by filtration, washed with water (10 mL) and air dried to afford 0.053 g of a white solid: mp 197-197.5°; ^{1}H NMR (DMSO-d_6): δ 8.44 (s, 1H, H-2), 8.20 (s, 1H, H-6), 6.83 (bs, 2H, NH_2), 6.36 (dd, 1H, H-1',J=3.13, 6.84 Hz), 5.02 (t, 1H, 5'-OH), 4.08 (m, 1H, H-4'), 3.62 and 3.54 (2m, 2H, H-5'ab), 2.41 and 2.22 (2m, 2H, H-2'ab), 1.97 (m, 2H, H-3'ab); UV λ_{max} (nm) (log epsilon): MeOH, 280 (4.05), 231 (3.93); pH 1, 274 (3.92), 235 (4.08); pH 11, 279 (4.03), 234 (3.89); TLC: solvent system A, R_f = 0.40, chars yellow; IR(KBr) 2220 cm^{-1} (cyano); Anal. Calcd. for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05, N, 27.02. Found: C, 55.68; H, 5.08; N, 26.91.

4-Amino-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine-5-carboxamide (13, 2',3'-Dideoxy-2',3'-didehydrosangivamycin)

A rapidly stirred suspension of sangivamycin[17] (10, 6.18 g, 20 mmole) in acetonitrile (125 mL, dried over 3 A° sieves) was treated with 2-methyl-2-acetoxypropionyl bromide (10.4 g, 50 mmole) and the stirring was continued for 1.5 hr. An additional portion of the bromo compound was added (1 g, 5 mmole) and stirring was continued for another 5 min. Methanol was then added (5 mL) and the mixture was stirred for 10 min. The solvent was removed by evaporation. Pyridine (100 mL) and acetic anhydride (10 mL, 0.1 mmole) were added to the residue and the mixture was shaken vigorously for 5 min in order to cause dissolution of the gummy residue. After an additional 15 min of stirring, TLC analysis of the mixture showed one major product (R_f = 0.51, solvent system A). The solution was evaporated, and the resulting gummy residue was treated with ethanol/water (1:1, v:v, 100 mL) and the solution was once again evaporated to afford a gummy residue. This gum was treated with isopropanol (200 mL) and the mixture was heated to reflux temperature. The mixture was allowed to cool to room temperature, the solid was collected by filtration (6.8 g) and combined with the solid obtained (1.2 g) by

concentration of the mother liquor and trituration of the residue with isopropanol (100 mL). A sample (7.5 g) of this material was dissolved in hot isopropanol/methanol (4:2, v:v, 600 mL) and the volume of the solution was reduced to 300 mL by boiling on a hot plate. The solid, which had precipitated on cooling to room temperature, was collected by filtration, washed with isopropanol (20 mL) and ether (50 mL) to afford 5.2 g of a white powder. This powder (4.0 g) was dissolved in dimethylformamide (70 mL) and the solution was treated with zinc-copper couple (1.5 g). After stirring for 2 hr, TLC (solvent system A) showed the absence of starting material (R_f = 0.51) and the presence of product (R_f = 0.31). The spent zinc-copper couple was removed by filtration through Celite, and the Celite bed was washed with dimethylformamide (3 x 15 mL). The combined filtrates were evaporated to afford an oil which solidified upon treatment with ethanol (50 mL). The solid was collected by filtration and washed with ethanol (10 mL) to afford 3.1 g of the crude acetate of the title compound.

A suspension of the acetate (2.0 g) in methanolic ammonia (40 mL, saturated at 0°) was stirred at room temperature in a pressure bottle for 24 hr and then kept at -20° for 24 h. The solid was removed by filtration and washed with methanol (5 mL) and ether (10 mL). The combined filtrates were evaporated to dryness and the residue was triturated with hot ethanol (30 mL). After cooling the suspension to room temperature, the solid material was collected by filtration, washed with ethanol (10 mL) and ether (10 mL) to afford 0.76 g of the crude title compound (29% overall from sangivamycin). A sample (0.5 g) of this material was dissolved in water (10 mL) and the solution was applied to a Sephadex LH-20 column (2.5 cm x 20 cm). The column was eluted with water while the elution was monitored by TLC (solvent system A). The first product containing fraction (R_f = 0.14, 10 mL) was discarded with the next 110 mL of eluent being collected and then evaporated to dryness. The residue was triturated with ethanol (20 mL), the solid was collected by

filtration, washed with ethanol (5 mL) and ether (10 mL) to afford 0.29 g of a white powder, after air drying. A sample (0.1 g) of this material was dissolved in chloroform/methanol/water (80:10:2, v:v:v, 3 mL) and the solution was chromatographed on a silica column (20 mm x 100 mm, 10 micron silica) using chloroform/methanol (8:1, v:v) at a flow rate of 15 mL/min. The elution was followed by observing the absorption at 280 nm and the fraction containing the product (eluting between 4 and 12 min.) was collected and evaporated. The residue was triturated with methanol (3 mL) and the solid was collected by filtration, washed with methanol (2 mL) and ether (5 mL) to afford 0.068 g of a white solid, after air drying: mp 240° dec.; ^{1}H NMR (DMSO-d_6): δ 8.10 (s, 1H, H-2), 8.00 and 7.32 (2bs, 2H, NH_2), 7.95 (s, 1H, H-6), 7.15 (bs, 1H, H-1'), 6.54 (d, 1H, H-3', $J_{(2',3')}$=5.98 Hz), 6.08 (d, 1H, H-2'), 4.92 (t, 1H, 5'-OH), 4.81 (bs, 1H, H-4'), 3.53 (m, 2H, H-5'ab); UV λ_{max} (nm) (log epsilon): MeOH, 281 (4.12), 230 (3.97); pH 1, 275 (4.02), 226 (4.10); pH 11, 280 (4.08), 233 (3.87); TLC: solvent system A, R_f = 0.14; Anal. Calcd. for $C_{12}H_{13}N_5O_3$: C, 52.36; H, 4.76; N, 25.44. Found: C, 52.16; H, 4.57; N, 25.28.

4-Amino-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-d]-pyrimidine-5-carboxamide (15, 2',3'-dideoxysangivamycin)

A suspension of compound 13 (0.15 g, 0.54 mmole) in methanol (50 mL) containing 5% palladium on charcoal (0.05 g) was hydrogenated on a Parr apparatus for 8 hr at 35 psi. The catalyst was removed by filtration through Celite and the Celite bed was washed with methanol (2 x 10 mL). The combined filtrates were evaporated to a thick oil which was then co-evaporated with ethanol (2 x 10 mL) and isopropanol (10 mL). The resulting foam was then treated with ether (30 mL), and the resulting solid was collected by filtration, washed with ether (10 mL) and air dried to afford 0.12 g of a white solid. A sample (0.09 g) was dissolved in chloroform/methanol (8:1, v:v, 5 mL) and the solution was chromatographed on a silica column (20 mm x 100 mm, 10 micron

silica) using chloroform/methanol (8:1, v:v) at a flowrate of 15 mL/min. The elution was monitored by observing the absorption at 280 nm and the fraction eluting between 5 min and 10 min was collected and evaporated. The residue was triturated with methanol (3 mL). The solid was collected by filtration, washed with methanol (3 mL) and ether (5 mL), then allowed to air dry to afford 0.071 g of a white solid (47%): mp 207-208°. ^{1}H NMR (DMSO-d_6): δ, 8.08 (s, 1H, H-2), 8.05 (s, 1H, H-6), 7.94, 7.32 (2bs, 2H, NH_2), 6.38 (q, 1H, H-1'), 4.86 (t, 1H, 5'-OH); 4.06 (q, 1H, H-4'), 3.54 (m, 2H, H-5'ab), 2.62 (m, 1H, H-2'ab), 2.20 (m, 1H, H-2'b), 2.05 (m, 2H, H-3'ab); UV λ_{max} (nm) (log epsilon): MeOH, 282 (4.08); pH 1, 232 (4.15), 276 (4.09); pH 11, 236 (3.93), 245 (3.89), 281 (4.13); TLC: Solvent system A R_f = 0.14; Anal. Calcd. for $C_{12}H_{15}N_5O_3 \cdot 1/4\ H_2O$: C, 51.15; H, 5.54; N, 24.86. Found: C, 51.43; H, 5.50; N, 24.67.

ACKNOWLEDGEMENT

The authors would like to thank the National Institutes of Health (Training grant #5-T32-GM-07767) and in part support from the Department of Health and Human Services under research contract number NO1-A1-42554. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We would also like to thank Mona Quesada, Constance Lopatin and Margaret Perrin for their assistance in the preparation of this manuscript.

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Received November 6, 1987.