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THE PREPARATION AND BIOLOGICAL ACTIVITY OF NOVEL AMINO ACID ANALOGS OF BUTIROSIN*

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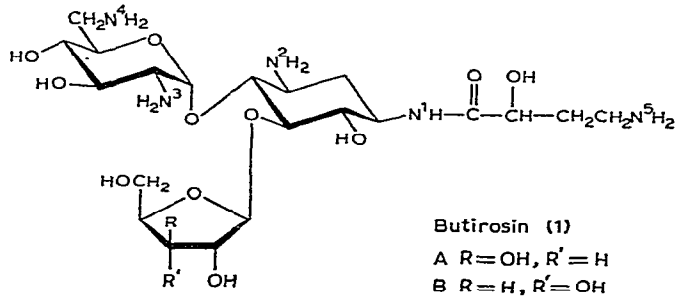
ABSTRACT

The aminoglycoside antibiotic butirosin (**1**) has been chemically modified in its amino acid side-chain. The (*S*)-(−)-4-amino-2-hydroxybutyryl side-chain was removed by alkaline hydrolysis of the tetrakis(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl) derivative. The resulting deacylated, trisubstituted derivative **3** was reacylated with a wide variety of mono and polyfunctional amino acids. The cyclohexenyl protecting groups were then removed by chlorine gas and the new analogs isolated chromatographically. Structure–activity relationships were determined with five microorganisms, including *Pseudomonas aeruginosa*. The specific side-chain structures that exhibited maximum potency against *Pseudomonas* are identified.

Butirosin[1], a new aminoglycoside antibiotic complex produced by mucoid strains of *Bacillus circulans* NRRL B-3312 and B-3313, is the first example of the aminocyclitol class of compounds to contain an amino acid in its chemical structure. Complete structural assignments for butirosin A and B have been described[2] and are shown in formula **1**. The amino acid, which is connected by an amide linkage to N^1 of the deoxystreptamine moiety, was found to be the unique (*S*)-(−)-4-amino-2-hydroxybutyric acid[2]. The butirosins have marked activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa*, both *in vitro* and *in vivo*, as well as low toxicity in mammalian species[3,4]. They were therefore likely candidates for structural-modification studies.

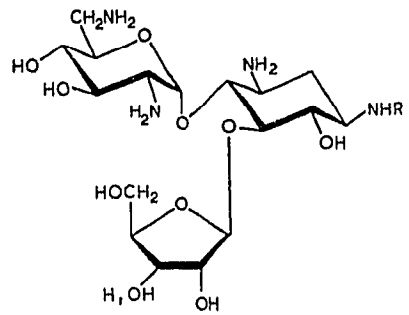


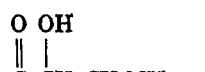
Butirosin (1)
A R=OH, R'=H
B R=H, R'=OH

*Dedicated to Dr. Louis Long, Jr., in honor of his 70th birthday.

TABLE I

BUTIROSIN ANALOGS



Compd. No.	*R*	*Configuration*[a]	*Formula*	*Analyses*[b]					
				Carbon		*Hydrogen*		*Nitrogen*	
				Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
23		Synthetic *S*	$C_{21}H_{41}N_5O_{12}\cdot H_2CO_3\cdot 2H_2O$	40.43	40.98	7.25	7.61	10.71	10.87
24		Synthetic *R*	$C_{21}H_{41}N_5O_{12}\cdot H_2CO_3\cdot 2H_2O$	40.43	40.67	7.25	7.60	10.71	11.07
25		*S*	$C_{20}H_{39}N_5O_{12}\cdot 0.2H_2CO_3\cdot 2H_2O$	41.12	41.60	7.04	7.04	11.87	11.72
26		*R*	$C_{20}H_{39}N_5O_{12}\cdot 0.5H_2CO_3\cdot 1.5H_2O$	41.06	41.22	7.23	7.13	11.68	12.13
27		*SR*	$C_{20}H_{39}N_5O_{12}\cdot 0.5H_2CO_3\cdot 2.5H_2O$	39.87	39.47	7.34	6.97	11.34	11.01

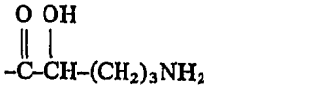
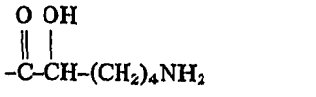
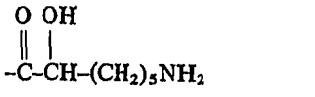
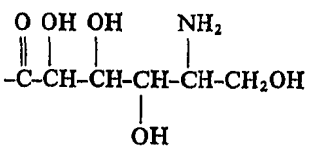
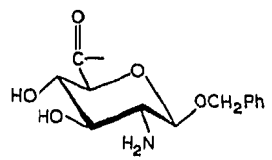
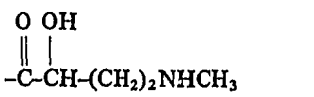
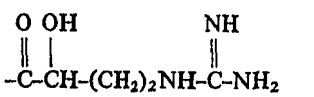
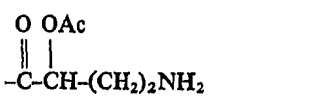
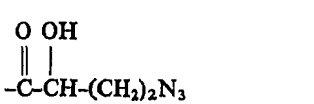
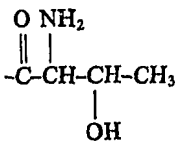
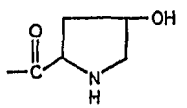
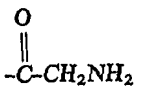
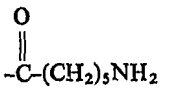
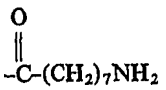
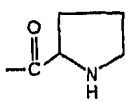
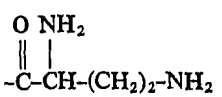
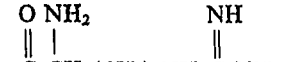
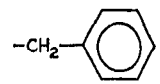
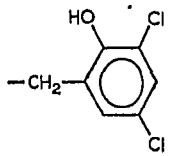
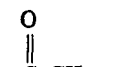
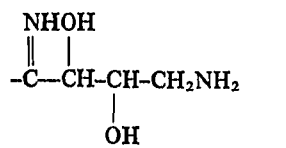
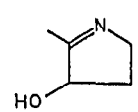
28		*S*	$C_{22}H_{43}N_5O_{12}\cdot 3H_2CO_3\cdot 2H_2O$	37.92	37.92	6.75	6.42	8.84	9.22
29		*S*	$C_{23}H_{45}N_5O_{12}\cdot 2H_2CO_3$	42.42	41.80	6.98	6.78	9.90	10.53
30		*SR*	$C_{24}H_{47}N_5O_{12}\cdot 2H_2CO_3\cdot H_2O$	42.21	42.44	7.21	7.26	9.47	9.86
31		*S*	$C_{23}H_{45}N_5O_{15}\cdot 2H_2CO_3$	39.75	39.60	6.53	6.30	9.27	10.17
32		D-*gluco*	$C_{30}H_{49}N_5O_{15}\cdot 2H_2CO_3\cdot H_2O$	44.60	44.70	6.43	6.35	8.12	8.17
33		*SR*	$C_{22}H_{43}N_5O_{12}\cdot 0.4H_2CO_3\cdot 2.5H_2O$	42.37	42.52	7.68	7.53	11.03	10.91
34		*S*	$C_{22}H_{43}N_5O_{12}\cdot 2H_2SO_4\cdot 6H_2O$	29.30	29.68	6.59	6.39	10.87	9.99
35		*S*	$C_{23}H_{43}N_5O_{13}\cdot 2H_2SO_4$	28.90	28.99	6.85	6.63	7.33	8.01
36		*SR*	$C_{21}H_{39}N_7O_{12}\cdot 0.75H_2CO_3\cdot 3H_2O$	38.29	38.46	6.87	6.72	14.38	14.11

TABLE I (*Continued*)

Compd. No.	*R*	*Configuration*[a]	*Formula*	*Analyses*[b]					
				Carbon		*Hydrogen*		*Nitrogen*	
				Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
37		L-*threo*	$C_{21}H_{41}N_5O_{12}\cdot H_2CO_3\cdot H_2O$	41.57	41.73	7.14	7.19	11.01	11.36
38		L-*allo*	$C_{22}H_{41}N_5O_{12}\cdot 2H_2CO_3$	41.68	41.48	6.56	6.39	10.13	10.68
39			$C_{19}H_{37}N_5O_{11}\cdot H_2CO_3$	41.88	41.69	6.85	6.42	12.21	11.84
40			$C_{23}H_{45}N_5O_{11}\cdot H_2CO_3\cdot 1.5H_2O$	43.90	43.70	7.60	7.85	10.67	10.40
41			$C_{25}H_{49}N_5O_{11}\cdot 3H_2CO_3\cdot 2H_2O$	41.12	41.14	7.27	7.14	8.56	9.07
42		L	$C_{22}H_{41}N_5O_{11}\cdot 2H_2CO_3\cdot 0.5H_2O$	42.10	42.17	6.77	6.87	10.23	10.30
43		L	$C_{21}H_{42}N_5O_{11}\cdot H_2CO_3\cdot 1.5H_2O$	41.06	41.07	7.36	6.96	13.06	12.84

44		L	$C_{23}H_{46}N_6O_{11}\cdot 2H_2CO_3\cdot H_2O$	41.43	41.56	7.23	7.02	11.60	11.36
45		L	$C_{23}H_{46}N_8O_{11}\cdot 5.5HCl\cdot H_2O$[c]	33.31	33.53	6.50	6.27	13.51	13.54
46	$-CH_2CH_2CH_3$		$C_{20}H_{40}N_4O_{10}\cdot H_2CO_3\cdot 2H_2O$	42.41	43.50	7.79	7.38	9.42	8.90
47			$C_{24}H_{40}N_4O_{10}\cdot H_2CO_3$	49.50	49.30	6.98	7.38	9.24	9.54
48			$C_{24}H_{38}Cl_2N_4O_{11}\cdot 0.5H_2CO_3$	44.50	45.00	5.95	6.41	8.48	8.61
49			$C_{19}H_{36}N_4O_{11}\cdot H_2CO_3\cdot H_2O$	41.66	41.50	6.99	7.02	9.71	9.40
50		*SR*	$C_{21}H_{42}N_6O_{11}\cdot 2.5H_2SO_4\cdot 5H_2O$	28.34	28.09	6.46	6.49	9.44	8.70
51		*S threo*	$C_{21}H_{42}N_6O_{12}\cdot 2H_2SO_4\cdot 4H_2O$	30.07	29.72	6.49	6.50	10.02	9.54
52		*SR*	$C_{21}H_{39}N_5O_{11}\cdot 2H_2SO_4\cdot H_2O$[d]	33.55	33.12	6.03	5.82	9.31	9.20

[a]Configuration of α-carbon. [b]Representative examples were analyzed semi-quantitavely for water and carbon dioxide. In all cases, i.r. spectra indicated carbon dioxide either as hydrogen carbonate or carbonate salts. [c]Cl: Calc., 23.51; Found, 23.52. [d]S: Calc., 8.52; Found, 8.68.

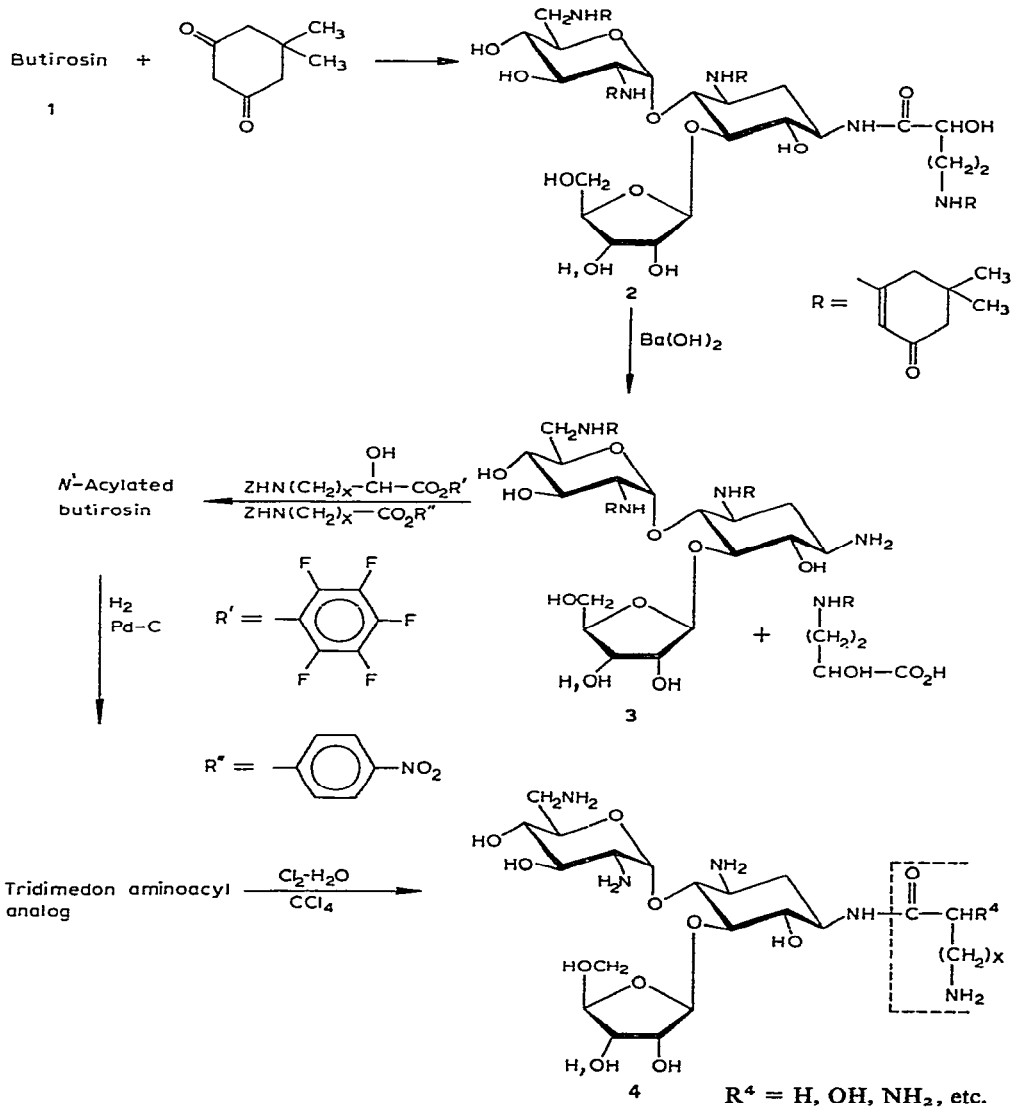
TABLE II

ANTIBACTERIAL ACTIVITY OF BUTIROSIN ANALOGS

	Minimum inhibitory concentration (μg/ml)[9]				
Compd. No.	S. aureus *UC-76*	Pseudomonas aeroginosa	E. coli	S. sonni	M. tuberculosis
1	1.0	10	5	2.5	25
23	1.0	10	1.5	1.5	5
24	5.0		5.0	10	
25	1.0	2.5	2.0	2.0	5
26	1.0	5.0	1.5	2.0	10
27	1.0	10	1.5	2.0	2.5
28	1.5		2.5	2.5	10
29	10		5	5	
30	20		20	25	
31	10		10	10	
32	←——inactive——→				
33	2.5		2.5	2.5	
34	1.5		2.5	2.5	10
35	20				
36	←——inactive——→				
37	←——inactive——→				
38	←——inactive——→				
39	←——inactive——→				
40	←——inactive——→				
41	←——inactive——→				
42	←——inactive——→				
43	←——inactive——→				
44	5.0		15	15	
45	2.0		15	15	
46	15		20	25	
47	←——inactive——→				
48	←——inactive——→				
49	←——inactive——→				
50	5.0	15	10	10	5
51	5.0		10	5	
52	5.0	20	2.5	2.5	

It was previously demonstrated[5] that the amino acid side-chain can be removed from butirosin by alkaline hydrolysis without affecting the structural integrity of the remainder of the molecule. The deacylated product thus obtained retained most of its inhibitory activity against *Staphylococci*, *Escherichia coli*, and *Shigella* but was relatively inert against *Pseudomonas aeruginosa*. The deacylated product from butirosin B has been isolated previously from culture filtrates of *Streptomyces ribosidificus* and is called Ribostamycin[6]. As the amino acid side-chain in the butirosins appeared to be a prerequisite for *Pseudomonas* activity, one of the primary objectives in our structural-modification studies was to replace the (*S*)-(−)-4-amino-2-hydroxybutyryl side-chain by other acyl moieties of various structures and functionality. Structure–activity relationships could then be observed against various strains of bacteria.

To achieve this objective, suitable protective substituents for the four primary amino groups in the intact antibiotic were sought. Such groups need to be stable enough in alkali to withstand conditions for complete hydrolysis of the amide bond and yet be ultimately removable under conditions mild enough to retain the newly formed amide linkage. The protecting group found the most suitable was the conjugated enamine formed by reaction of the amino groups with dimedone (5,5-dimethylcyclohexane-1,3-dione). Dimedone has been previously utilized as an alkali-stable blocking group in peptide synthesis[7] and with modification studies on kasugamycin[8].



Scheme 1

These vinylogous amides produced from aminoglycosides showed excellent solubility and chromatographic properties, and were conveniently monitored during isolation by their intense u.v. absorption. The tetrakis(dimedone) derivative (**2**) of butirosin (**1**) could be readily separated from partially reacted products by chromatography on Dowex-50 X1 followed by elution with aqueous pyridine. The amino acid side-chain was then readily removed by hot saturated barium hydroxide solution without affecting the cyclohexenylamino groups. The resulting tris(dimedone), deacylated butirosin (**3**), following chromatographic purification on Dowex-50, was then utilized for analog synthesis employing procedures used for peptides. In most instances, activated esters of *N*-[(benzyloxy)carbonyl]ated amino acids produced the desired derivatives in satisfactory yields. For 2-hydroxy amino acids, the pentafluorophenyl ester was used. The *p*-nitrophenyl ester was employed with all other amino acids. Following removal of protecting groups on the amino acid side-chain, the dimedone groups were conveniently removed with chlorine gas at 5°; the reaction sequence is shown in Scheme 1. The new antibiotic analogs (**4**) were usually isolated as free bases from columns of Amberlite IRC-50 resin by elution with aqueous ammonia. Each new compound was characterized by microanalysis, i.r. spectrum, and t.l.c. or paper chromatography. The structures are listed in Table I. Strong-base analogs, such as guanidino or amidino compounds, were isolated as acid salts from columns of Sephadex G-10.

The antibacterial activities of these analogs are listed in Table II. Inhibition assays were conducted by the Microbiology Section in these laboratories using the gradient-plate technique[9] against the microorganisms listed. The absence of a number in the tables indicates no significant growth-inhibition at concentrations of 25 μg/ml or above.

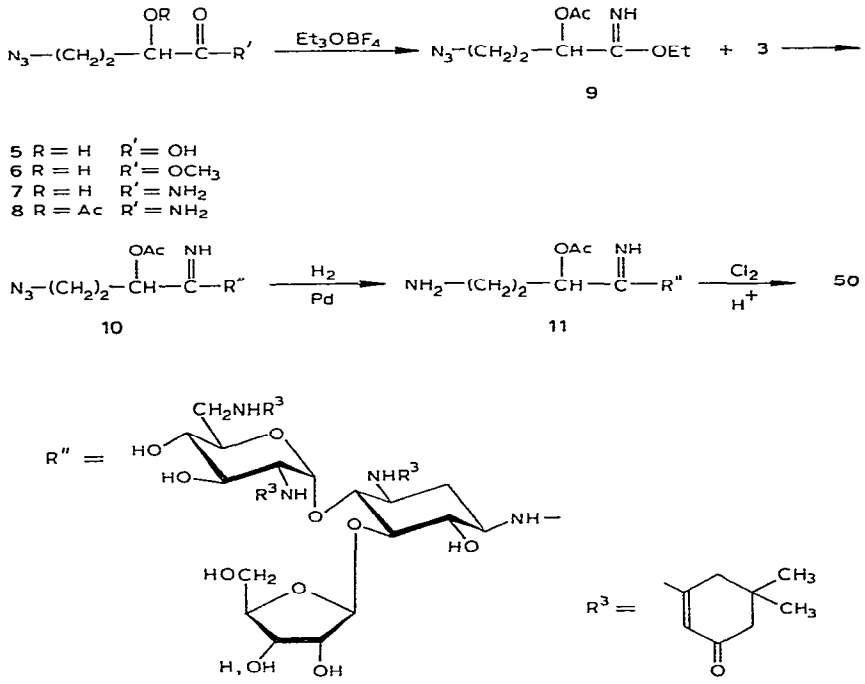
RESULTS AND DISCUSSION

In order to test the validity of the synthetic approach, the (*S*)-(−)-4-amino-2-hydroxybutyryl derivative of **3** was prepared from the optically pure amino acid (obtained from hydrolyzates of the antibiotic or by synthesis[10]). The *N*-[(benzyloxy)-carbonyl] pentafluorophenyl ester was used in the condensation. The synthetic product, after removal of protecting groups, was found to be identical with butirosin, chemically, physically, and biologically (within experimental error). The enantiomorphic (*R*)-derivative **24** was significantly less active than its optical antipode. Acetylation of the 2-hydroxyl group destroyed all but a trace of activity. The optimum activity observed with synthetic analogs occurred with 2-hydroxy-ω-aminoacyl side-chains. The optimum chain-length was four carbons or less, and the (*S*)-configuration was required for maximum activity. Increasing the number of hydroxyl groups in the chain (**31**, **32**) decreased the potency irrespective of configuration. Conversion of the ω-amino function into a stronger base, as by *N*-methylation or guanidination (**33**, **34**), destroyed the *Pseudomonas* activity only.

Replacement of the 2-hydroxyl group by amino or hydrogen completely

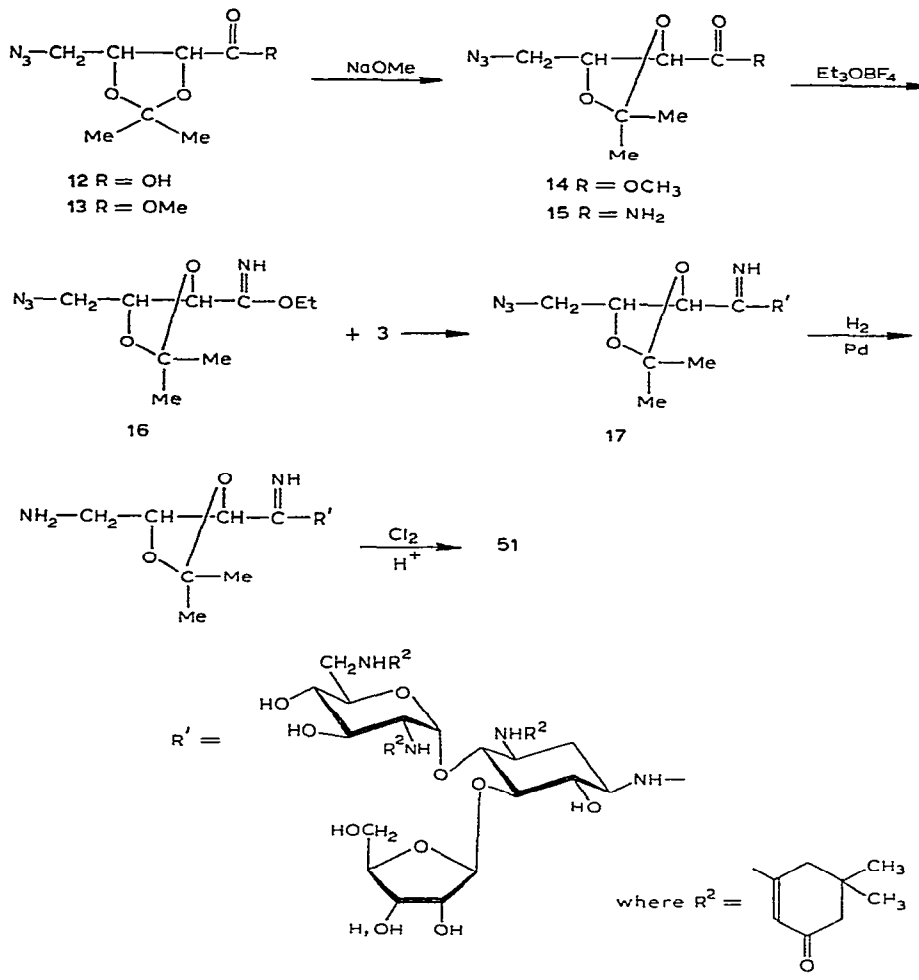
destroyed the *Pseudomonas* activity and markedly lowered the potency against the other microorganisms. Only with L-α,ω-diamino acids and L-arginine (**44, 45**) was any appreciable activity noted against the *Staphylococci*, *E. coli*, and *Shigella*. Alkylation or acylation (**46–49**) of the N^1-amino group of the deoxystreptamine moiety also lowered the potency. The specificity requirements for side-chain orientation of polar groups can be seen by the inactivity of the L-threonine and hydroxyproline analogs (**37, 38**).

Replacement of the carbonyl oxygen atom by nitrogen (that is, conversion of amide into amidine) resulted in retention of broad-spectrum activity (**50–52**). The preparation of these compounds is shown in Schemes 2, 3, and 4. Acyclic amidines (**50, 51**) were labile to base and had to be isolated as acid addition salts following desalting on columns of Sephadex G-10. The presence of the amidine linkage was confirmed by an i.r. absorption peak at 1695–1700 cm^{-1} in KBr. Interestingly, the cyclic hydroxyamidine (**52**) prepared from the butirosin side-chain (Scheme 4) retained a major portion of bioactivity. Therefore, an acyclic chain is not an absolute requirement for antibiotic activity.



Scheme 2

In summary, it can be stated that a highly specific arrangement and number of polar groups in the amino acid side-chain of butirosin are required for maximum antibacterial potency. The (*S*)-(−)-2-hydroxy and ω-amino functions are prerequisite for *Pseudomonas* activity as well as an optimum chain length of 3 to 4 carbon atoms.



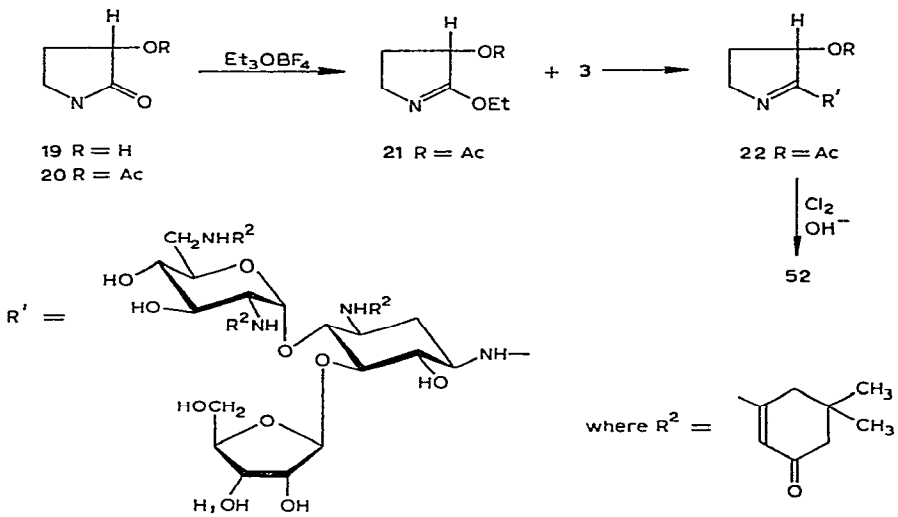
Scheme 3

Replacement of the carbonyl oxygen atoms by nitrogen afforded analogs with good biopotency.

In the work described herein, a mixture of butirosins A and B in an approximate ratio of 4:1 was used throughout. As no effort was made to separate these isomers, it is assumed that the analogs prepared consisted of a mixture of similar ratios.

EXPERIMENTAL

General methods. — Thin-layer chromatography was performed on Quanta-Gram Silica Gel Q1F plates, and products containing the 5,5-dimethyl-3-oxo-1-cyclohexen-1-yl group were detected by use of a 254 nm u.v. lamp or iodine vapor. Unsubstituted aminoglycosides were detected by ninhydrin or phosphomolybdate[14] sprays. Chromatography of final products utilized Quanta-Gram Q2F cellulose



Scheme 4

plates with ninhydrin as detector. Evaporations were performed *in vacuo* at 35°. Bacterial-inhibition assays were conducted by the Parke–Davis Microbiology Section using the gradient-plate technique[9]. Silica Gel-60 (E. Merck) was employed for column chromatography. Melting points were determined with a Thomas–Hoover capillary apparatus and are uncorrected. The i.r. and n.m.r. spectra of all new intermediates were consistent with the structures assigned.

Preparative procedures. — *A.* N-[*(Benzyloxy)carbonyl*]*aminohydroxy acids.* The preparation of *N*-[(benzyloxy)carbonyl]aminohydroxy acids was accomplished by reported procedures[11].

B. Preparation of activated esters. (*i*) *N*-[(Benzyloxy)carbonyl]aminohydroxy acids: Equimolar amounts of substituted amino acid, pentafluorophenol, and *N,N'*-dicyclohexylcarbodiimide were allowed to react in tetrahydrofuran (8 ml/mmol) for 16 h at 5°. The mixture was filtered to remove urea, which was rinsed with ether. After removal of the solvent in vacuum, the residue was used directly for the condensation with **3**.

(*ii*) *N*-[(Benzyloxy)carbonyl]amino acids: *p*-Nitrophenol was substituted for pentafluorophenol in the foregoing (*i*). Ethyl acetate, acetonitrile, and tetrahydrofuran were used as solvents and reactions were conducted at ambient temperature.

C. Condensation with **3**. A slight excess of activated ester was allowed to react with **3** in *N,N*-dimethylformamide for at least 24 h, at 5° in the case of hydroxyamino acids and room temperature for the *p*-nitrophenyl esters. Upon removal of solvent *in vacuo*, the crude product was obtained by triturating the residue with ether. The products were purified by chromatography on activated silica gel with chloroform–methanol mixtures. Fractions were analyzed by t.l.c. (10:6 $CHCl_3$–MeOH). The chromatographically pure fractions were combined and product was isolated by removal of solvent and trituration with ether.

D. Hydrogenolysis of N-(*benzyloxy*)*carbonyl groups.* Hydrogenolysis was accomplished in methanol–water mixtures with 20% Pd-on-carbon by passing hydrogen through a stirred suspension until evolution of carbon dioxide [$Ba(OH)_2$ solution] ceased.

E. Removal of dimedone groups and isolation of purified products. A stock solution of 3% *w/v* of chlorine in carbon tetrachloride was prepared and stored at 5°. The tri(dimedone)derivative (0.5 mmol) in 35 ml of water was rapidly stirred with an equal volume of chlorine stock solution for 15 min at 5°. After washing the aqueous layer with carbon tetrachloride and twice with chloroform, the pH was adjusted to 2.0 with ammonium hydroxide. An aqueous solution of sodium bisulfite was added to give a negative starch–iodide test (slight excess) and the pH was adjusted to 5–6.0 with ammonia. The solution was concentrated to 3.0–5.0 ml and passed through a column prepared from 1–2 g of Darco G-60 and an equal weight of Celite 545. The effluent tubes were checked for a chromophore absorbing at 290 nm. Tubes showing no u.v. absorption maxima were combined for final purification.

With mono-amino and hydroxyamino acid analogs, the final purification step was performed on Amberlite IRC-50 columns. A 10 to 15 ml column of resin was converted into its ammonium form and the carbon-column effluents from the foregoing step were cycled through, washed with water, and eluted with N ammonia solution. Concentration followed by lyophilization of the effluent afforded the analogs as free bases or partial carbonate or hydrogen carbonate salts.

Amino acid analogs containing guanidino, amidino, or alkali-labile groups were isolated as sulfate salts by desalting the carbon effluents on Sephadex G-10 columns, as described for **34**.

N^2,N^3,N^4,N^5-*Tetrakis*(*5,5-dimethyl-3-oxo-1-cyclohexen-1-yl*)*butirosin** (**2**). — Butirosin (**1**) free base (13.5 g) and dimedone (17 g) were refluxed in pyridine (200 ml) for 8 h and kept overnight at 25°. The solvent was removed under vacuum and replaced by methanol (50 ml). Water (50 ml) and dimedone (2 g) were added, and after refluxing the mixture for 6 h the solvent was removed and the product isolated by trituration with ether and filtration to afford 27.5 g of crude enamine, λ_{max}^{MeOH} 290 nm ($\varepsilon_{1cm}^{1\%}$ 940).

Colored impurities and excess dimedone were removed by passing the crude enamine in 1:1 methanol–water through a 500-ml column of anion-exchange resin (Amberlyst XN-1001, hydroxyl form). The crude product was then adsorbed from methanol–water (1:1) onto a Dowex 50 X1 (50–100 mesh) column (700 ml) which was eluted with 2% aqueous pyridine. Evaporation of the effluent and trituration with ether afforded the tetra(dimedone) derivative **2** (17.1 g); λ_{max}^{MeOH} 291 nm ($\varepsilon_{1cm}^{1\%}$ 1035); t.l.c. (in 10:6 chloroform–methanol) one zone R_F 0.7.

Anal. Calc. for $C_{53}H_{81}N_5O_{16}$: C, 60.96; H, 7.82; N, 6.71. Found: C, 60.55; H, 7.72; N, 6.51.

*The numbering system used for the nitrogen atoms in butirosin is given in formula **1**. Products lacking the original acyl group at N^1 are designated N^1H.

N^2,N^3,N^4-*Tris(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl)*-N^1*H-butirosin* (**3**). — Compound **2** (17.4 g) was heated in saturated aqueous barium hydroxide (655 ml) for 3 h on a steam bath. The cooled solution was neutralized to pH 5 with 2M sulfuric acid and filtered through Celite. The filtrate was absorbed on a Dowex-50 XI (H^+ form) column (600 ml) and the column was rinsed with water. Following elution of the dimedone–amino acid complex with 2% aqueous pyridine (2 liters) the title compound was eluted with 2N ammonium hydroxide. Compound **3** (12.7 g) was obtained after removal of solvent and lyophilization; λ_{max}^{MeOH} 291 nm ($\varepsilon_{1cm}^{1\%}$ 970); t.l.c. (in 20:1 methanol–2N ammonia) R_F 0.6 major zone.

Anal. Calc. for $C_{41}H_{64}N_4O_{13}\cdot H_2O$: C, 58.69; H, 7.93; N, 6.68. Found: C, 58.21; H, 7.82; N, 6.93.

(S)-*4-[(Benzyloxy)carbonyl]amino-2-hydroxybutyric acid.* — (*SR*)-4-Amino-2-hydroxybutyric acid[9] was used to prepare[11] (*SR*)-4-[(benzyloxy)carbonyl]amino-2-hydroxybutyric acid, m.p. 95–96°.

Anal. Calc. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.99; H, 6.08; N, 5.34.

A mixture of benzyloxycarbonyl derivative (16.4 g) and L-tyrosine hydrazide (12.7 g) in absolute ethanol (500 ml) was heated for 30 min on a steam bath[12]. The solution became cloudy after 15 min and crystallization began after 20 min. The slurry was filtered while hot and washed with ethanol, affording the salt (13.4 g), which was dissolved in hot ethanol (750 ml) and the solution was concentrated to half its volume, giving the crystalline salt (11.7 g) upon cooling to 25°. A solution of the salt (11.7 g) in 4N hydrochloric acid (44 ml) was extracted with four portions of ethyl acetate. The combined extracts were washed with N hydrochloric acid and brine, and dried (magnesium sulfate) to afford the crystalline (*S*)-4-[(benzyloxy)-carbonyl]amino-2-hydroxybutyric acid (6.4 g); m.p. 77–78°, $[\alpha]_D^{25}$ +5.7°, at 365 nm +25.6° (*c* 1, $CHCl_3$); reported* m.p. 77.5–76.5° and $[\alpha]_D^{25}$ +5.63°, 365 nm +26.2° (*c* 1, $CHCl_3$).

Anal. Calc. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.85; H, 6.15; N, 5.66.

(R)-*4-[(Benzyloxy)carbonyl]amino-2-hydroxybutyric acid.* — From another resolution starting with the racemate (3.9 g), three crops of mostly (*S*)-amino acid salt were separated before the residues from the first and third filtrates were combined and treated with warm methanol (100 ml). The slurry was filtered, and the filtrate on cooling to 20° deposited the salt (0.85 g). Liberation of the free acid as before afforded the (*R*)-enantiomorph (0.396 g) as crystals from ethyl acetate–pentane; m.p. 76.5–78°, $[\alpha]^{25}$ 589 nm −5°, 365 nm −31° (*c* 1, $CHCl_3$).

Anal. Calc. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.87; H, 6.15; N, 5.43.

(SR)-*2-Hydroxy-4-(methylamino)butyric acid.* — (*SR*)-4-Amino-2-hydroxybutyric acid (5.95 g) was converted[13] into its *N*-benzyl analog with benzaldehyde, M sodium

*We are indebted to Dr. P. W. K. Woo of Parke–Davis for these values (personal communication).

hydroxide, and hydrogen over 5% Pd–C. The product (5.6 g) obtained from Dowex-50 (H^+) by elution with dilute ammonia was crystallized from acetone–water; m.p. 200–202°.

Anal. Calc. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 62.66; H, 7.25; N, 6.72.

The foregoing *N*-benzyl derivative (4.89 g) was reductively methylated with formaldehyde–formic acid[13] to afford the *N*-benzylmethylamino derivative (3.95 g); m.p. 180–185°

Anal. Calc. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.23; H, 7.49; N, 6.15.

A solution of (*SR*)-4-(benzylmethylamino)-2-hydroxybutyric acid (3.8 g) in glacial acetic acid (90 ml) and water (10 ml) was hydrogenated with 20% palladium-on-carbon (1 g) for 6 h at 25° and 50 lb. in^{-2}. Filtration, evaporation of the filtrate, and crystallization from methanol–acetone, gave the title compound (2.2 g); m.p. 193–195°.

Anal. Calc. for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52. Found: C, 44.97; H, 8.05; N, 10.49.

The *N*-(benzyloxy)carbonyl derivative was an oil.

N-[(*Benzyloxy*)*carbonyl*]-(S)-*isoserine*. — *N*-[(Benzyloxy)carbonyl]-(*SR*)-isoserine, m.p. 114–116° (*Anal.* calc. for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.85. Found: C, 55.48; H, 5.48; N, 5.92) was resolved as the brucine salt by a method previously published for *N*-benzoyl-(*SR*)-isoserine[15]. The title compound, m.p. 129–131°, had $[\alpha]^{25}$ 189 nm +4.2°, 365 nm +25.5° (*c* 0.96, methanol) (Found: C, 55.17; H, 5.26; N, 5.60). Hydrogenation afforded (*S*)-isoserine, $[\alpha]^{25}$ 589 nm −35.0°, 365 nm −100° (*c* 1, water); reported[15] $[\alpha]^{20}$ 589 nm −32.6° (*c* 10, water).

The enantiomorphic (*R*)-*N*-(benzyloxy)carbonyl derivative was isolated from the mother liquors; m.p. 128–130°, $[\alpha]^{25}_{589}$ −3.3°, 365 nm −24.6° (*c* 1, water) (Found: C, 55.30; H, 5.65; N, 5.73).

N^1-[(S)-*4-Guanidino-2-hydroxybutyryl*]-N^1*H-butirosin* (**34**). — Compound **3** was condensed with the pentafluorophenyl ester of (*S*)-4-[(benzyloxy)carbonyl]amino-2-hydroxybutyric acid and hydrogenated to form the (*S*)-4-amino-2-hydroxybutyryl–tridimedone complex by the general methods described.

Anal. Calc. for $C_{45}H_{71}N_5O_{15}\cdot H_2CO_3\cdot 4H_2O$: C, 52.31; H, 7.73; N, 6.63. Found: C, 52.04; H, 7.60; N, 6.97.

The foregoing compound (0.461 g) and 3,5-dimethylpyrazole-1-carboxamidine nitrate (0.1 g) in water (6 ml) was heated for 3 h on a steam bath. The cooled solution was washed with pentane and evaporated to dryness. The residue was precipitated from methanol–ether to give the crude guanidinated product (0.5 g). The dimedone protecting-groups were removed as already described, and the solution (6 ml) obtained from the carbon column was passed through a column of Sephadex G-10 (50 g) and developed with water. The salt-free fractions were combined and lyophilized to afford **34** (0.077 g).

N^1-[(SR)-*4-Amino-2-acetoxybutyryl*]-N^1*H-butirosin*. (**35**). — A solution of (*SR*)-

4-[(benzyloxy)carbonyl]amino-2-hydroxybutyric acid (1.0 g) and acetic anhydride (2.0 g) in pyridine (5 ml) was kept for 24 h at 5°. After 6 h at 25° the solvent was removed, and the residue was dissolved in benzene and washed with M sulfuric acid, and brine, and dried (magnesium sulfate) to afford the acetate (1.34 g) as an oil. This product was condensed with **3** as previously described, affording the tri(dimedone) acetic ester; ν_{max}^{KBr} 1667, 1703, and 1730 cm^{-1}.

Anal. Calc. for $C_{55}H_{79}N_5O_{19}\cdot 3H_2O$: C, 56.54; H, 7.33; N, 5.99. Found: C, 56.66; H, 7.50; N, 5.85.

Dimedone protecting-groups were removed as before and the product purified on a Sephadex G-10 column as described for **34**.

N^1-[(SR)-*4-Azido-2-hydroxybutyryl*]-N^1-*H-butirosin* (**36**). — (*SR*)-4-Azido-2-hydroxybutyric acid (**5**) was converted into its activated ester (pentafluorophenyl), condensed with **3**, and deprotected as already described to afford **36**, ν_{max}^{KBr} 2110 cm^{-1} (N_3).

(S)-*5-Amino-2-hydroxypentanoic acid.* — Diazotization of L-ornithine[16] gave the title compound; m.p. 190–192°, $[\alpha]_D^{25}$ −16° (*c* 10.25, H_2O) (reported[17] $[\alpha]_D^{25}$ −17°); *N*-(benzyloxy)carbonyl derivative, m.p. 107–108.5°.

Anal. Calc. for $C_{12}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.21. Found: C, 58.72; H, 6.50; N, 5.25.

(S)-*6-Amino-2-hydroxyhexanoic acid.* — L-Lysine hemisulfate was diazotized as previously described[16], m.p. 221–223° (reported for racemate[18], m.p. 222–224°), $[\alpha]_D^{25}$ −14° (*c* 1, H_2O); *N*-(benzyloxy)carbonyl analog, m.p. 79–81° (ethyl acetate–ligroin), $[\alpha]_{589}$ +2.7°, 365 nm +21.4° (*c* 1, $CHCl_3$).

(SR)-*7-Amino-2-hydroxyheptanoic acid.* — 7-Benzamidoheptanoic acid[19] was converted into the 2-chloro analog with sulfuryl chloride and chlorine[20]. Hydroxylation was accomplished with sodium hydroxide[18], affording the title compound, m.p. 207–211°; *N*-(benzyloxy)carbonyl analog, m.p. 71–74°.

Anal. Calc. for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.03; H, 6.97; N, 4.77.

Compound **31**. — Benzyl 2-[(benzyloxy)carbonyl]amino-2-deoxy-α-D-glucosiduronic acid[21] was converted into its pentafluorophenyl ester by the normal procedure. The solid obtained was homogeneous by t.l.c. (R_F 0.7 in ethyl acetate), λ_{max}^{MeOH} 257 nm ($E_1^1 = 11.1$), ν_{max}^{KBr} 1800, 1695, 1640, 1540, and 1460 cm^{-1}, and was condensed with **3** without further purification. The (benzyloxy)carbonyl and *O*-benzyl groups were removed by hydrogenolysis with 20% Pd-on-carbon at atmospheric pressure for 20 h in the presence of one equivalent of sulfuric acid. The hydrogenation was monitored by t.l.c. (20:1 methanol–2M ammonia) and continued until the complete disappearance of the nonreducing zone (R_F 0.7) and emergence of a homogeneous reducing zone (R_F 0.25). The product was reduced with sodium borohydride at 5° and isolated from Dowex-50 XI by M ammonia elution. Removal of the dimedone groups in the normal manner afforded **31**.

Compound **32**. — Removal of the *N*-(benzyloxy)carbonyl group from the intermediate described in the preceding example was accomplished by hydrogenolysis

for 1.5 h as already described, whereby no reducing zone was observed by t.l.c. Processing as already described afforded **32**.

Compound **45**. — N^G,N^G,N^2-*tris*[(*benzyloxy*)*carbonyl*]-L-*arginine*[22] was converted into its *p*-nitrophenyl ester*, m.p. 129–131° (Calc. for $C_{36}H_{35}N_5O_{10}$: C, 61.97; H, 5.06; N, 10.04. Found: C, 62.10; H, 5.11; N, 10.19), which was condensed with **3** and the protecting groups removed. The arginyl analog (**45**) was obtained by elution from a column of Amberlite IRC-50 with dilute hydrochloric acid followed by desalting on Sephadex G-10.

N^1-*Aryl*-N^1*H-butirosin: compounds* **46**, **47**, *and* **48**. — The corresponding aldehyde (1 ml) and tris(dimedone)-N^1H-butirosin (**3**) (0.6 mmol) in methanol (5 ml) was refluxed for 3 h. Sodium borohydride (0.25 g) was added at 5°, and the solution was stirred for 24 h at 25°. Solvent removal was followed by acidification with aqueous acetic acid. The aqueous layer was extracted twice with 1-butanol, which was removed under vacuum and the residue triturated with ether. Purification was accomplished by silica gel chromatography with chloroform and methanol. Removal of dimedone and final purification was accomplished in the usual manner.

Amidino analogs: compound **50** (*Scheme 2*), DL-*4-azido-2-hydroxybutyric acid* (**5**) *and its methyl ester* (**6**). — DL-2-hydroxybutyrolactone[23] (5.1 g) was heated with sodium azide (3.5 g) in *N*,*N*-dimethylformamide (25 ml) for 20 h at 100°. The solvent was removed, the residue was dissolved in water, which was then acidified to pH 2 with Dowex 50. The filtered solution was evaporated to dryness and the residue distilled to give **5** (1.3 g); b.p. 120–144° (0.1 nm); $\nu_{max}^{liq\ film}$ 2110 and 1740 cm^{-1}.

The acid **5** (5.4 g) was dissolved in ether and ethereal diazomethane was added until the solution became neutral. The methyl ester (**6**) (5.3 g) was obtained on distillation; b.p. 80° (1 mm); $\nu_{max}^{liq\ film}$ 2080 and 1738 cm^{-1}.

DL-*4-Azido-2-hydroxybutyramide* (**7**). — The methyl ester **6** (1.0 g) was heated with methanolic ammonia (20 ml) in a pressure bottle for 2 h at 100°. Evaporation to dryness followed by crystallization from ethyl acetate afforded **7** (0.65 g), m.p. 100–102°; ν_{max}^{KBr} 2100, 2080, and 1640 cm^{-1}.

Anal. Calc. for $C_4H_8N_4O_2$: C, 33.34; H, 5.59; N, 38.87. Found: C, 33.63; H, 5.58; N, 39.12.

DL-*2-Acetoxy-4-azido-butyramide* (**8**). — The hydroxyamide **7** (1.0 g) was acetylated with acetic anhydride in pyridine for 3 days at 5°. Evaporation and crystallization from ether–ligroin afforded **8** (1.04 g); m.p. 48–50°; ν_{max}^{KBr} 2100, 2150, 1740, and 1660 cm^{-1}.

Anal. Calc. for $C_6H_{10}N_4O_3$: C, 38.70; H, 5.41; N, 30.09. Found: C, 38.79; H, 5.51; N, 29.95.

Compound **10**. — The acetoxy amide **8** (0.2 g) was treated with Meerwein reagent[24] (0.3 g) in dichloromethane (10 ml) overnight at 20°. The supernatant was decanted from the precipitate, which was washed several times with solvent. The organic layer was shaken with 2.5M potassium carbonate solution (4 ml) and the

*The authors are indebted to Dr. E. D. Nicolaides for this sample.

solvent layer was separated and dried over sodium sulfate. Filtration and evaporation gave the crude imido ester **9**, ν_{max}^{KBr} 2090, 1750, and 1700 cm^{-1}, which was condensed with **3** (0.41 g) as described for **22** to yield **10** (0.57 g); λ_{max}^{MeOH} 290 nm ($\varepsilon_{1cm}^{1\%}$ 674); R_F 0.7 (10:1 methanol–2M ammonia); ν_{max}^{KBr} 2100, 1740, and 1695 cm^{-1}.

Compound **50**. — Compound **10** (0.56 g) was hydrogenated over 20% Pd-on-carbon in aqueous methanol until no azide remained (i.r.). Filtration and evaporation gave **11**, which was treated with chlorine in the usual manner. The solution, after treatment with sodium bisulfite, was kept at pH 2 for 24 h at 5°. Conventional processing over carbon and Sephadex G-10 columns afforded **50** (0.5 g); ν_{max}^{KBr} 1695 and 1620 cm^{-1}.

Compound **51** (*Scheme 3*). — 4-Azido-4-deoxy-2,3-*O*-isopropylidene-D-erythronic acid[25] (**12**) was converted by ethereal diazomethane into its methyl ester **13**; b.p. 65–66° (0.02 mmHg), $[\alpha]_D^{25}$ +89° (*c* 1, methanol); n.m.r. ($CDCl_3$) δ 1.62 and 1.40 (2 s, CMe_2). Compound **13** was converted into the D-threonate **14** by epimerization[26] for 21 h in refluxing methanol–sodium methoxide. Analysis by g.l.c.* showed an 85% conversion into the diastereoisomer; b.p. 65–66° (0.02 mmHg), $[\alpha]_D^{25}$ +83° (*c* 1, methanol); n.m.r. ($CDCl_3$) δ 1.51 and 1.44 (2 s, CMe_2).

Anal. Calc. for $C_8H_{13}N_3O_4$: C, 44.67; H, 6.05; N, 19.53. Found: C, 44.90; H, 5.94; N, 19.63.

The methyl ester (**14**) was converted into its amide **15** as described for **7**; $\nu_{max}^{liq\ film}$ 2080, 1680, and 1580 cm^{-1}.

Compound **18**. — The amide **15** (1.0 g) and triethyloxonium tetrafluoroborate (1.3 g) in dichloromethane (10 ml) were allowed to react as described for **10** to give **16** as a syrup; $\nu_{max}^{liq\ film}$ 2080 and 1667 cm^{-1}. This was condensed directly with **3** to yield **17**; λ_{max}^{MeOH} 291 nm ($\varepsilon_{1cm}^{1\%}$ 500); ν_{max}^{KBr} 2100, 1650, and 1690 (shoulder) cm^{-1}; homogeneous by t.l.c. R_F 0.8 (10:1 methanol–2M ammonia). Reduction of the azide afforded **18** showing no azide absorption by i.r.

Compound **51**. — Compound **18** (0.51 g) was treated with chlorine in the usual manner. After chromatography on carbon the u.v.-transparent fractions were heated for 15 min at 60° at pH 2 (sulfuric acid). The pH was adjusted to 6 with alkali and compound **51** (33 mg) was obtained following desalting on Sephadex G-10; ν_{max}^{KBr} 1695 cm^{-1}.

Compound **52** (*Scheme 4*): (SR)-*3-acetoxy-2-pyrrolidinone* (**20**). — (*SR*)-3-Hydroxy-2-pyrrolidinone[2] (**19**, 1.0 g) was acetylated in pyridine (10 ml) and acetic anhydride (2 ml) for 5 h at 25°. The usual processing afforded **20** (1.0 g); m.p. 87–88° (ether); ν_{max}^{KBr} 1700 and 1753 cm^{-1}.

Anal. Calc. for $C_6H_9NO_3$: C, 50.35; H, 6.29; N, 9.79. Found: C, 50.49; H, 6.27; N, 9.64.

(SR)-*3-Acetoxy-2-ethoxy-1-pyrroline* (**21**). — (*SR*)-3-Acetoxy-2-pyrrolidinone (**20**, 0.434 g) in dichloromethane (5 ml) was treated with triethyloxonium tetrafluoro-

*F & M 810 10% Carbowax on Anakrom AB with a 4-foot glass column isothermal at 200°; *T* for *erythro* derivative 6.35 min, *T* for *threo* derivative 5.72 min.

borate (0.63 g) in the usual manner affording **21** as a crude oil; $\nu_{max}^{liq\ film}$ 1669 and 1750 cm^{-1}, very little NH absorption at 3450 cm^{-1} in carbon tetrachloride.

Compound **22**. — The lactim ether (**21**, 0.22 g) and compound **3** (0.45 g) in ethanol (20 ml) containing 1 drop of glacial acetic acid were refluxed for 3 h, at which time t.l.c. (10:3 methanol–2M ammonia) showed no starting material. The residue after removal of solvent upon trituration with ethyl acetate gave crude **22** (0.57 g), which was purified by chromatography (10:3 methanol–2M ammonia) to homogeneity; λ_{max}^{MeOH} 291 nm ($\varepsilon_{1cm}^{1\%}$ 780). No acetate peak at 1750 cm^{-1} was observed.

Compound **52**. — Dimedone groups were removed from **22** (0.28 g) in the normal manner and the product desalted over Sephadex G-10, affording **52** (76 mg) as the sulfate salt, ν_{max}^{KBr} 1700, 1640, and 1540 cm^{-1}.

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