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Glycocinnamoylspermidines, a New Class of Antibiotics. 3. The Structures of LL-BM123β, γ1, and γ2

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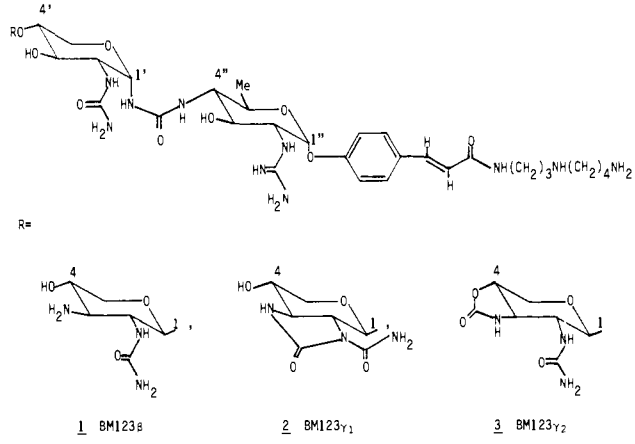
Abstract: On the basis of hydrolytic experiments in conjunction with ^1H NMR, ^{13}C NMR, and x-ray analysis, the structures of three novel broad-spectrum antibiotics called LL-BM123β, γ1, and γ2 have been determined.

LL-BM123β, γ1, and γ2 are three new broad-spectrum antibiotics which were recently isolated in these laboratories from fermentations of an unidentified species of *Nocardia*.[1,2] The γ1 and γ2 components are of special interest because of their broad-spectrum activity against gram-negative organisms and their protective effects against infections produced in mice.[2]

Initial attempts at structural characterization of these antibiotics centered on a possible x-ray solution. Although considerable effort was made to prepare a crystalline heavy-atom derivative, this approach was not successful. However, from the results of hydrolytic experiments and identification of the degradation products by spectral and, where possible, single-crystal x-ray analysis, we propose structures **1, 2,** and **3** for LL-BM123β, γ1, and γ2, respectively. These antibiotics contain several unusual structural features including the glycosylurea linkage and substituted 2,3-dideoxy-2,3-diaminoxylopyranoside moieties. The 2,4,6-trideoxy-2,4-diaminohexopyranoside has been observed only once before in nature as the 4-*N*-acetamide from mild acid hydrolysis of a polysaccharide from *Bacillus lichenformis*.[3] Also of interest is the 1′-*N*-carbamoylimidazolidone unit in **2** reminiscent of the 1′-*N*-carboxylimidazolidone portion of the biotin derivative postulated to be involved in biotin-dependent carboxylase systems.[4]

All three antibiotics are amorphous and strongly basic compounds. They are positive to ninhydrin and Sakaguchi tests and also to Ehrlich's reagent[5] for ureido groupings. Although elemental and mass spectral (including field desorption) analyses were of little value in obtaining molecular formulas,



^{13}C NMR experiments[6] indicate clearly the presence of 37 carbons in **2** and **3**, and 36 in **1**. These spectra also suggest a close relationship between the three antibiotics. Indeed, mild basic hydrolysis of **2** and **3** provided **1**, the loss of one carbonyl grouping being the only significant change as indicated by the ^{13}C NMR data. The *trans*-*p*-coumaroyl moiety is common to all three antibiotics as indicated by the UV maximum at 286 nm and the very characteristic ^1H NMR (220 MHz in D_2O) signals at δ 6.2 and 7.0 (1 H d's, J = 15.5 Hz) and 6.8 and 7.2 (1 H d's, J = 8.0 Hz). Other prominent ^1H NMR signals common to all three metabolites are those assigned to the secondary *C*-methyl at δ 1.4 (J = 6.0 Hz), the spermidine

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Table I. ^{13}C Chemical Shifts of the Carbohydrate Moieties (ppm) Downfield from Me_4Si[a,b]

	1·H_2SO_4	2·H_2SO_4	3·H_2SO_4	4·H_2SO_4	14 (Me_2SO)	2·H_2SO_4 (Me_2SO)	4·H_2SO_4 (Me_2SO)	13·HCl	NH_2 free 18·base	19·HCl	24·HCl	27 ($CDCl_3$)
C_1	101.8	98.2 (d)	103.2		100.6	96.8		103.4	102.5	102.0	101.7 (d)	99.7 (d)
2	53.2	56.1 (d)*	56.0*		55.3*	55.3		53.0	57.3*	55.7*	53.4 (d)*	47.6 (d)*
3	57.0†	60.8 (d)	60.7		59.8	59.1		57.4	57.2*	60.5	57.4 (d)	48.9 (d)*
4	66.9*	70.0 (d)	77.0		68.8	68.8		66.9*	70.8	77.0	67.0 (d)	68.0 (d)
5	66.7*	66.9 (t)	64.6		66.2	66.2		66.4*	67.1	64.6	66.6 (t)	58.9 (t)
					MeO 55.1*			MeO 58.1				
$C_{1'}$	82.2	82.4 (d)	82.3			80.2			82.3	82.3	55.1 (d)	169.5 (s)
2′	55.4	55.8 (d)*	55.6*			52.0			55.3	55.5*	53.8 (d)*	94.5 (s)
3′	73.2	73.4 (d)	73.2			69.8			73.2	73.2	70.3 (d)	37.5 (t)
4′	78.0	75.3 (d)	78.1			73.4			77.9	78.0	80.6 (d)	78.6 (d)
5′	64.6	64.6 (t)	64.6			59.7			64.5	64.6	61.5 (t)	74.7 (t)
$C_{1''}$	96.0	96.4 (d)	96.2	96.0		96.1	96.1		96.0	96.1	14.8 (q) Me's	
2″	58.2	58.3 (d)*	58.2	58.3*		57.3*	57.4*		58.3	58.3	14.6 (q)	
3″	69.2	69.2 (d)	69.2	69.3		68.2	68.2		69.3	69.2	26.5 (t) CH_2's	
4″	57.1†	57.3 (d)*	57.2	57.2*		56.0*	56.0*		57.2*	57.2	26.0 (t)	Acetyl 20.9 (q)
5″	70.8	71.0 (d)	70.9	71.0		69.8	70.1		70.8	70.8		Me's 22.9 (q)
6″	17.5	17.7 (q)	17.6	17.5		17.7	17.9		17.8	17.8		23.8 (q)
												26.1 (q)
						Carbonyl Signals						
$C_{2''}$	158.3*	158.3 (s)*	158.3*	158.3*		157.1*	157.2*		158.3	158.3		Acetyl 172.3 (s)
4″	157.9*	158.0 (s)*	157.9*	157.9*		157.5	157.7*		159.6	159.6		C=O's 171.9 (s)
2′	159.7	159.7 (s)	159.7			156.8			160.5	160.6	161.7 (s)(2)	169.9 (s)(2)
2	161.7	156.5 (s)	160.5		153.2	154.1		161.5	161.9	161.9		Urea 153.4 (s)
		160.8 (s)			159.5	158.8						C=O's 153.3 (s)
4			163.0							162.8		

[a] Off-resonance patterns are in parentheses. [b] Signals marked † or * may be interchanged in the column where they appear.

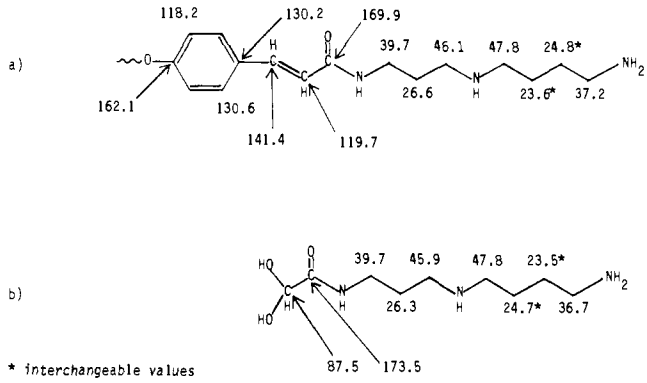


Figure 1.

methylene signals at δ 2.0 (4 H, m), 2.2 (2 H, t), and 3.2 (6 H, m), and an anomeric proton δ 5.4 (1 H d, J = 2.0 Hz). ^{13}C NMR chemical shift assignments for the p-coumaroylspermidine portion are essentially identical for all three antibiotics. The values for 2·H_2SO_4 are given in Figure 1a and are based on comparison studies with spermidine (free base and protonated forms) and p-methoxycinnamic acid. In Table I, the chemical shifts of carbons associated with the carbohydrate portions of **1, 2, 3,** and pertinent degradation products are compared. Assignments are based on these comparisons and in general on single-frequency off-resonance decoupling studies, general ^{13}C NMR data,[7] and the wealth of recent studies on aminoglycoside antibiotics.[8]

Hydrolytic Studies

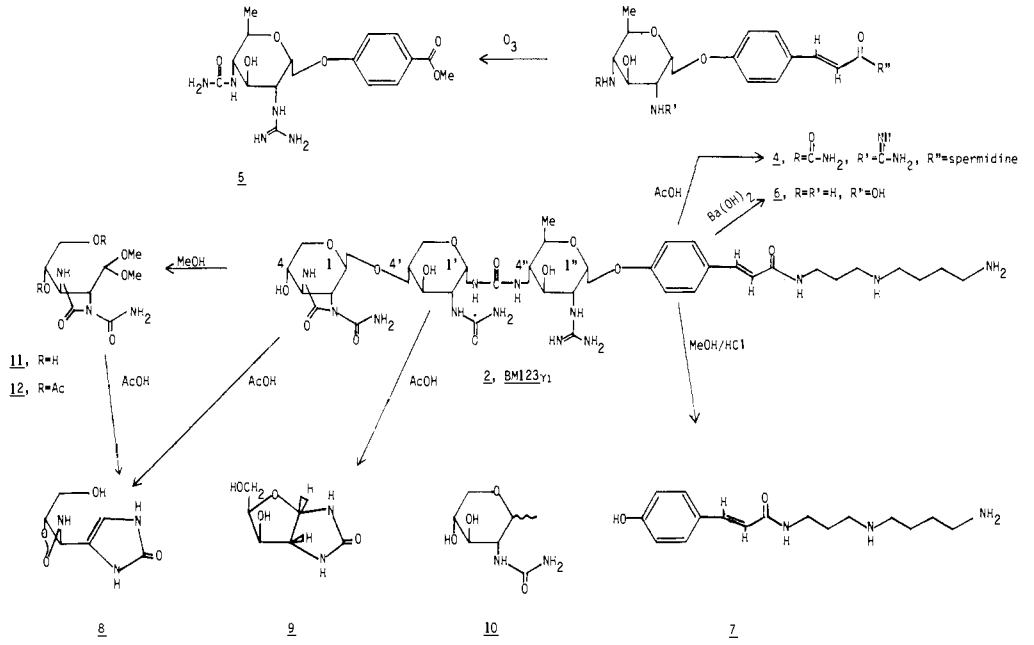
Before describing the hydrolytic studies carried out on these antibiotics, it should be pointed out that although **1** and **2** were obtained essentially pure (^1H and ^{13}C NMR data), no significant quantity of **3** completely free of **2** was isolated. Therefore, almost all the degradative chemistry, outlined in Schemes I and II, was done on **1** and **2.**

Mild acid hydrolysis of **2** (Scheme I) with 50% acetic acid at 37 °C for 7 days cleaved the molecule into the three fragments **4, 8,** and **9.** These degradation products were isolated by resolving the crude hydrolysate into acetone-soluble and -insoluble fractions. Cellulose chromatography of the insoluble fraction gave **4** while **8** and **9** were isolated from the acetone-soluble portion and will be discussed below. Compound **4,** $[\alpha]_D$ +96°, was converted by ozonolysis in methanol to the crystalline methyl benzoate **5**[9] and glyoxalylspermidine hydrochloride characterized by its ^1H and ^{13}C NMR (Figure 1b) spectra. X-ray analysis of **5** provided an unequivocal assignment. The D absolute configuration is assigned to the hexose on comparison of the specific rotation of the α-glycoside **6** (+152°) (obtained along with spermidine from barium hydroxide hydrolysis of **2**) with that of β-D-glucosyloxy-p-coumaric acid (−78°)[10] and coniferin (−67°)[11], the β-D-glucopyranoside of coniferyl alcohol.

The point of attachment of spermidine to coumaric acid was determined by ^1H NMR spin-decoupling studies on the coumaroylspermidine **7** (Scheme I). This degradation product was obtained by methanolysis of **2** with 14 N methanolic hydrogen chloride or by Dowex-50 hydrolysis of **1.** Thus, irradiation of the C_1 methylene proton signal at δ 3.37 (2 H triplet) collapsed the C_2 proton signal at δ 1.95 (2 H multiplet) to a triplet. Likewise, irradiation of the δ 1.95 signal collapsed the δ 3.37 triplet to a singlet. The 4 H multiplet at δ 1.79, assigned to the C_5 and C_6 methylene protons, was undisturbed. These experiments indicate clearly the point of attachment of spermidine as shown.

Comparison ^{13}C NMR spectral studies of **4** with **1, 2,** and **3** (Table I) showed no significant differences in the chemical shifts for the relevant carbons indicating that **4** occurs intact in all three antibiotics thus accounting for 24 carbons, the UV chromophore (286 nm), and the strongly basic nature of these

Scheme I

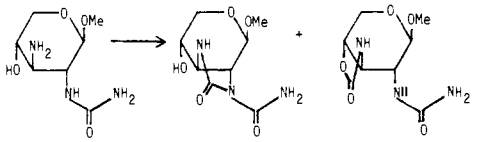


compounds. Mild acetic acid hydrolysis of **1** and **3** also gave **4** as indicated by spectral and TLC comparisons.

The remaining 13 carbons in **2** were accounted for by examination of the acetone-soluble fraction from the above acetic acid hydrolysis of **2**. Celite partition chromatography of the crude residue from this fraction gave two crystalline pentose derivatives: **8**, $C_7H_9N_3O_4$, and **9**, $C_6H_{10}N_2O_4$. Their structures were determined by single-crystal x-ray analysis and the data obtained from compound **9** were sufficient to indicate the D absolute configuration. Although ^{13}C and ^1H NMR spectral data for **2** indicate that both these fragments do not exist as such in the antibiotic, they do account for the remaining carbon atoms. The lack of a 61–63-ppm hydroxymethyl triplet in the ^{13}C NMR off-resonance experiment on **2** rules out the presence of an unlinked hydroxymethyl group[12] and, in conjunction with other hydrolytic evidence to be discussed below, suggests the pyranose structures. Thus **9** must have originated from the corresponding 2-deoxy-2-ureido-D-xylopyranoside **10**. Indeed there is excellent precedent for the formation of **9** from the postulated **10** in studies on 2-deoxy-2-ureidohexoses which are transformed to the corresponding furanoimidazol-2-ones on mild acid treatment.[13]

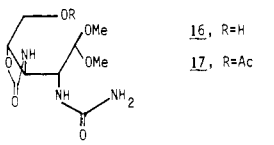
The origin of **8** was less obvious until mild methanolysis of **2** with Amberlyst-15 sulfonic acid resin overnight at room temperature yielded the crystalline dimethyl acetal **11**. An x-ray analysis of the diacetate **12** provided the structure. Treatment of **11** with hot 85% acetic acid gave **8** in good yield apparently by means of an acyl migration and subsequent cyclization at C_1 of the ureido grouping. Thus the formation of **11** on methanolysis of **2** in conjunction with the above-mentioned ^{13}C NMR considerations, points to the corresponding pyranoside of **11** as the precursor.

Despite the fact that we were unable to obtain a methyl pyranoside corresponding to **11** from methanolysis experiments of **2**, we were successful in synthesizing one for ^{13}C NMR spectral comparisons. Treatment of β-methyl-2,3-dideoxy-2-ureido-3-aminoxylopyranoside (**13**) (from **1**; see below) with *p*-nitrophenoxycarbonyl chloride[14] gave in low yield the *N*-carbamoylimidazolidone **14** (1740, 1670, 1620, and 1590 cm^{-1}) and, as the major product, the oxazolidone **15** (1750, 1660, and 1550 cm^{-1}). These water-insoluble crystalline compounds were separated from the crude reaction mixture by Celite partition chromatography and characterized, in



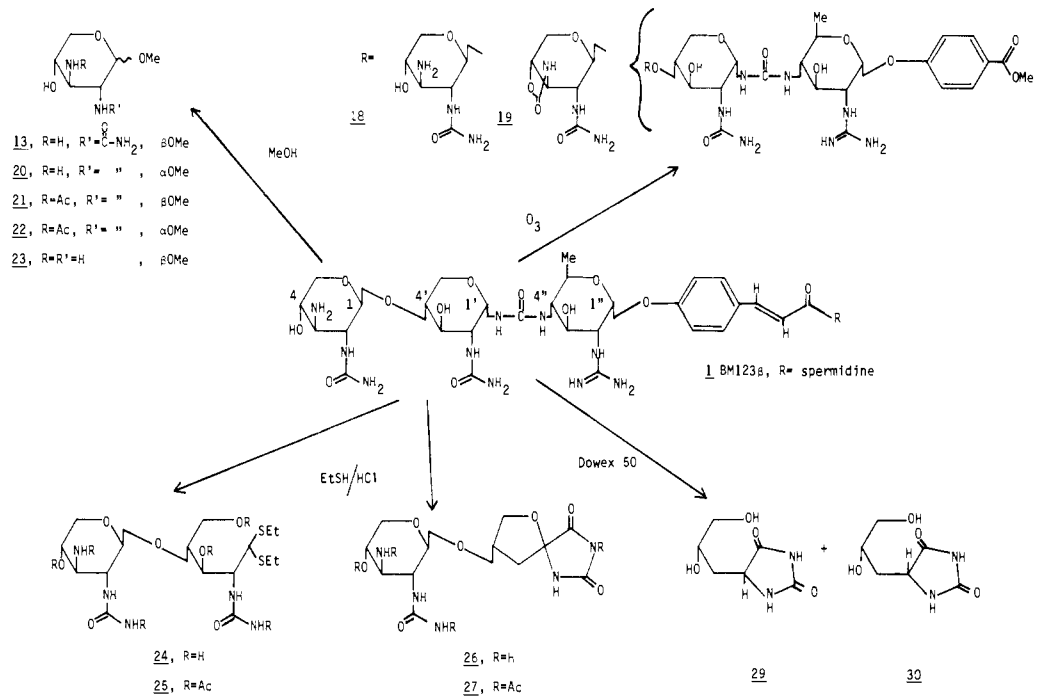
addition to the IR, by their very distinctive ^1H NMR spectra especially with regard to the NH signals.[15] For ^{13}C NMR studies, the spectrum of **14** was recorded in dimethyl sulfoxide. This, of course, necessitated rerunning **2** as well as **4** in dimethyl sulfoxide for comparison studies. The C_{2-5} and carbonyl signals of **14** correspond well with the relevant signals in **2** (Table I) thus indicating this *N*-carbamoylimidazolidone pentose as the terminal moiety in **2**. The 3.8-ppm variation in C_1 assignments between **2** and **14** is undoubtedly due to γ shielding effects at C_1 in **2** introduced by the branching at $C_{4'}$.[7,8] A possible explanation for the unexpectedly large variation in $C_{1'-5'}$ assignments (especially for $C_{3'}$ (Δ 3.6) and $C_{5'}$ (Δ 4.9)) between the spectrum of **2** in water and in dimethyl sulfoxide is solvent-induced changes in rotamer populations around the terminal glycosidic bond.

The oxazolidone structure for the terminal pentose in **3** was suggested by the formation of the amorphous oxazolidone **16** along with **11** from methanolysis (Amberlyst-15 resin) of a sample of **2** enriched in **3**. Acetylation of **16** gave the crystalline



acetate **17** (1760, 1745, and 1670 cm^{-1}; ^1H NMR Me H's 2.11, 3.45, 3.48). Further confirmation for the presence of the oxazolidone ring in **3** was obtained from ^{13}C NMR studies. In order to avoid having to compare the ^{13}C NMR spectrum of the water-insoluble **15** to that of **3** in dimethyl sulfoxide, a water-soluble model was sought.[16] Toward this end the amorphous but water-soluble **19** (1755 cm^{-1}) was obtained by *p*-nitrophenoxycarbonyl chloride treatment of **18** (from ozonolysis of **1**, Scheme II). As seen from Table I, the C_{1-5} and carbonyl ^{13}C NMR signals assigned to the ureido-oxazolidone

Scheme II



pentose unit in **19** are in close agreement with the corresponding signals in the spectrum of **3**. Weak signals attributable to a small amount of the *N*-carbamoylimidazolidone moiety, formed along with the oxazolidone, were also observed.

Evidence for the structure of the terminal pentose in **1** was likewise obtained by Amberlyst-15 catalyzed methanolysis (room temperature, 6 days) followed by elution of the resin with 1.5 N methanolic ammonia.[17] This gave the anomeric methyl glycosides **13** and **20**, $C_7H_{15}O_4N_3$. The β anomer (**13**), $[\alpha]_D$ −55°, was obtained by fractional crystallization from methanol. Treatment of the methanolic filtrate with acetic anhydride gave, after Celite partition chromatography, the corresponding *N*-acetyl derivatives **21**, $[\alpha]_D$ −130°, and **22**, $[\alpha]_D$ +36°. The structure of **13** was determined by x-ray analysis and the structures of **21** and **22** were determined by ^1H NMR spectral comparisons with **13**. The D form is indicated by the molecular rotation of −113° for **13** which corresponds well with the M_D of −106° of methyl β-D-xylopyranoside.[18] Further support for the D configuration was obtained from the M_D of −134° of **23** obtained from **13** by barium hydroxide hydrolysis. The ^{13}C NMR assignments of the C_{2-5} and ureido carbonyl signals of **13**·HCl agree well with the appropriate signals in **1** indicating the structure of the terminal pentose of **1** to be as shown (Table I). The 1.6-ppm difference in C_1 between **1** and **13** again reflects the branching at $C_{4'}$ in **1**.[7]

With the structures of the terminal pentoses elucidated, the previously mentioned conversion of **2** and **3** to **1**, with sodium carbonate can be readily explained in terms of hydrolysis of the strained heterocyclic rings in **2** and **3** with excision of the carbonyl group. This clearly accounts for the lack of imidazolidone (1745 cm^{-1}) and oxazolidone (1755 cm^{-1}) IR bands in the spectrum of **1** as well as the absence of the imidazolidone (156.5 ppm) and the oxazolidone (163.0 ppm) carbonyl signals in the ^{13}C NMR spectrum of **1**.

Since on the basis of ^{13}C NMR comparisons (to be discussed in detail below), no other differences exist between the three antibiotics other than in the substitution of the terminal pentoses, all the carbons are accounted for.

Position of Linkages

At this point it remained to show how the basic units delineated above are joined together in the antibiotics. As previously mentioned, the major degradation fragment **4** is common to all three antibiotics. Because of the constancy of the ^{13}C NMR signals assigned to **4** and the corresponding signals in **1, 2,** and **3** (no 8-10-ppm downfield shift due to alkylation), it follows that **4** is linked to the adjacent pentose through the $C_{4''}$ ureido grouping as opposed to the $C_{3''}$ hydroxy or the spermidine nitrogen atoms.

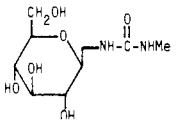
In order to obtain evidence for the linkage between the terminal pentoses and **10** in **1, 2,** and **3,** mercaptolysis experiments with ethyl mercaptan were carried out on **1**. this antibiotic was chosen rather than **2** because of the ease with which the terminal pentose in **2** is liberated under mild acid conditions. Accordingly, treatment of **1** with ethyl mercaptan[19] (Scheme II) in the presence of concentrated hydrochloric acid at 4 °C overnight yielded a mixture of products which was resolved by CM-Sephadex chromatography into **4,** the amorphous dithioacetal **24,** and most unexpectedly the hydantoin **26.** The dithioacetal **24** (mol wt 471 by field desorption mass spectrometry, FDMS) was characterized as its hexaacetate **25.** The accurate mass of the molecular ion of **25** was determined (FDMS) at 723.2455 by peak matching against hexakis-(2,2,2-trifluoroethoxy)cyclotriphosphazine in agreement with the formula $C_{28}H_{45}O_{13}N_5S_2$. The novel hydantoin **26** was further purified by acetylation with pyridine/acetic anhydride to give, after Celite partition chromatography, the corresponding acetate **27** (mol wt 513 by FDMS; ^1H NMR Me H's 2.08, 2.09, 2.24, and 2.56) as an amorphous powder. Methanolysis of both **24** and **26** yielded the methyl glycosides **13** and **20** which were characterized as their crystalline *N*-acetyl derivatives.

Although the MS, ^1H NMR, and ^{13}C NMR (Table I) spectra of **24** are consistent with the assigned structure, this data does not rule out the possibility of a C_1-$C_{3'}$ glycosidic linkage between the two pentose moieties. However, the spectral data described below for the hydantoin **26** and its tetraacetate **27** defines these structures unequivocally as in-

dicated with the intersugar aglyconic carbon at $C_{4'}$. Strong IR bands at 1770 and 1740 cm^{-1} in the spectrum of **26** in conjunction with the corresponding 1815 and 1760 cm^{-1} absorptions in the spectrum of **27** suggest the presence of the hydantoin moiety in these compounds.[20] Distinctive features of the ^{13}C NMR spectrum of **27** include signals at 37 and 94 ppm which are assigned to $C_{3'}$ and $C_{2'}$, respectively, on the basis of their chemical shifts and off-resonance patterns. Consistent with the $C_{3'}$ methylene grouping is the presence of an apparent 2 H doublet at δ 2.5 (J = 7.0 Hz) in the ^1H NMR spectrum. The $C_{5'}$ ^{13}C NMR signal of **27** is a triplet at 74.7 ppm in the off-resonance spectrum and the glycosylated $C_{4'}$ carbon shows a doublet at 78.6 ppm. As a ^{13}C NMR model for the acetylated hydantoin ring in **27**, the *N*-acetyl derivative (**28**) of 5,5-dimethylhydantoin was prepared (pyridine/acetic anhydride). The two ring carbonyl signals at 152.2 (C_3) and 168.9 ppm (C_1) for **28** agree well with the corresponding signals at 153.3 and 169.5 ppm in the spectrum of **27** while the hydantoin *N*-acetyl carbonyl signal varies ca. 3 ppm (175.4 ppm for **28** and 172.3 ppm for **27**). This acetyl methyl signal is, however, virtually identical in both **28** and **27** (26.2 vs. 26.1 ppm).

The origin of the spiro-3-deoxyhydantoin pentose unit in **26** seems best rationalized by an initial acid-catalyzed Lobry de Bruyn–Alberda van Ekenstein-type transformation[21] of the 2-deoxy-2-ureidoxylopyranoside moiety. Subsequent ring contraction and an oxidation step could yield this unusual structural moiety. In connection with this transformation, it was observed that Dowex-50 hydrolysis of **1** gave the neutral 3-deoxyhydantoin **29** (1755 and 1720 cm^{-1}) after purification by partition chromatography. The structure of **29** was secured by a single-crystal x-ray analysis which also indicated that carbons 2 and 4 have the *S* absolute configuration. A second hydantoin (1775 and 1730 cm^{-1}) was also obtained which is probably the C_2 epimer **30** on the basis of ^1H NMR spectral comparisons with **29**. These 3-deoxyhydantoin pentose derivatives undoubtedly originate from the same pentose which gave the hydantoin unit in **26**, i.e., **10** (Scheme I).[22]

Evidence that the pentose disaccharide moiety in **1, 2,** and **3** is linked to the $C_{4''}$ ureido grouping in **4** via the $C_{1'}$ of **10** was obtained by consideration of the anomeric carbon signals in the ^{13}C NMR spectra of the antibiotics. The 82-ppm signal



which occurs in all three metabolites is suggestive of a ureido glycosidic carbon and the model compound **31**[23] shows the C_1 signal at 81.9 ppm consistent with this assignment. The α linkage is indicated by the small $J_{1',2'}$ value (broad singlets) in the ^1H NMR spectra of the antibiotics (Table II).

Discussion of ^{13}C NMR Assignments of Trisaccharide Moieties

Chemical shift assignments of the trisaccharide carbons of **1, 2,** and **3** and the pertinent degradation products used for ^{13}C NMR comparison studies were in most cases straightforward. A few, however, must be regarded as tentative, especially some of the ureido and guanidino carbonyl signals (Table I).

Assignments for $C_{1''}$ (96.0 ppm) and $C_{6''}$ (17.5 ppm) in **4** are of course unambiguous. The 71.0 ppm signal is assigned to $C_{5''}$ and the upfield signal at 69.3 ppm to $C_{3''}$ on the basis that the latter carbon should experience shielding from both the flanking ureido and guanidino groupings. In addition, the ether linkage at $C_{5''}$ should shift that signal downfield compared with $C_{3''}$. The $C_{2''}$ (58.3 ppm) and $C_{4''}$ (57.2 ppm) assignments are interchangeable.

Table II. Chemical Shifts and J's of Anomeric Protons for **1, 2,** and **3**

		Me_2SO/D_2O (Me_4Si) at 100 MHz		D_2O (acetone standard) at 220 MHz[a]	
		δ	J, Hz	δ	J, Hz
1	C_1	4.54	6.5	4.55	7.7
	$C_{1'}$	4.77	bd s		
	$C_{1''}$	5.58	2.0	5.45	3.0
2	C_1	5.19	5.0	4.94	5.5
	$C_{1'}$	4.78	bd s		
	$C_{1''}$	5.60	s	5.46	3.0
3	C_1	4.56	6.5	4.52	6.5
	$C_{1'}$	4.78	bd s		
	$C_{1''}$	5.57	s	5.46	3.0

[a] $C_{1'}$ signals are obscured by the H_2O peak.

Unfortunately, we were unable to obtain a good ^{13}C NMR model for the middle sugar. However, subtracting out the signals of **13** and those of **4** from the spectrum of **1** permitted assignments of the middle sugar carbons in **1**. Except for $C_{4'}$ the $C_{1'}$, $C_{2'}$, $C_{3'}$, and $C_{5'}$ signals of this pentose are virtually identical in all three antibiotics and are consistent with the assigned structure.

Finally, the chemical shift differences in the assignments of the terminal pentose carbons as well as $C_{4'}$ in **1, 2,** and **3** are uniquely accounted for by the substitution patterns of these sugars. Thus the ca. 3 ppm variance in the glycosylated $C_{4'}$ signal is most likely due to the proximity of the *N*-carbamoyl grouping in **2** compared with **1** and **3** (**1,** 78.0; **2,** 75.3; **3,** 78.1). The C_1 carbon shifts also reflect this change (**1,** 101.8; **2,** 98.2; **3,** 103.2) as do the H_1 anomeric proton signals in the ^1H NMR spectra (220 MHz; δ 4.55, J = 7.7 Hz, in **1**; 4.94, J = 5.5 Hz, in **2**; 4.52, J = 6.5 Hz, in **3**) (see Table II). The shift difference in the terminal pentose carbons 2, 3, and 4 can be explained by the shielding effects of the C_3–NH_3^+ grouping[24] in **1** on C_2 and C_4 in contrast to the presence of the neutral imidazolidone and oxazolidone moieties in **2** and **3**. The significant change in assignments for C_4 in **2** (70.0 ppm) and **3** (77.0 ppm) is reasonably explained by the effects of acylation[25] and ring strain.[26] Also, acylation causes a ca. 2 ppm upfield change for C_5 in **3** (β shift) compared with **2**.[25]

The 156.5 ppm signal in the spectrum of **2** is tentatively assigned to the imidazolidone carbonyl by comparison with the corresponding assignment (156.0 ppm) in the spectrum of 1′-*N*-methoxycarbonyl-D-biotin methyl ester,[4b] although the latter was run in $CDCl_3$. The 156.2 ppm signal in the spectrum of **11** (in D_2O) is likewise tentatively assigned to the imidazolidone carbonyl grouping and the 159.4 ppm signal to the *N*-carbamoyl moiety.

The oxazolidone carbonyl signal in **3** is observed at 163.0 ppm and agrees well with the new carbonyl signal at 162.8 ppm in the spectrum of **19** formed from **18** by reaction of the latter with *p*-nitrophenoxycarbonyl chloride.

Experimental Section

The LL-BM123 antibiotics can be distinguished by cellulose TLC (0.1 mm thick, EM Laboratories, Inc., Elmsford, N.Y.) in the system 1-butanol/water/pyridine/acetic acid (15:12:10:1 by volume). The developed plates (overnight) were air dried for about 1 h before spraying with either ninhydrin or Sakaguchi spray reagents. The R_f values are as follows: compound **4**, 0.50; LL-BM123γ_1, 0.23; LL-BM123γ_2, 0.17; LL-BM123β, 0.08. Visualization of degradation products on TLC plates was by ninhydrin for amines, *tert*-butyl hypochlorite/potassium iodide/starch for both amines and amides, Sakaguchi for guanidines, and anisaldehyde/sulfuric acid for sugars.

Conversion of 2 and 3 to 1. A solution of 4 g of a mixture of **2** and **3** (mainly **2** by TLC) in 150 mL of water was treated with 1 g of sodium carbonate and 10 g of sodium bicarbonate. The solution (pH 9.2)

was allowed to stand at room temperature overnight. The reaction was then adjusted to pH 6.2 with 6 N hydrochloric acid and desalted over a 200-mL column of granular carbon. The carbon column was washed with water and then eluted with 500 mL of 65% aqueous acetone. The eluate was concentrated to 100 mL which was then chromatographed over a CM-Sephadex (NA^+) column ($\frac{3}{4} \times 16$ in.). Gradient elution (monitored at 286 nm) with 1–6% sodium chloride gave a band which was desalted as described above. Elution of the carbon column with 70% aqueous acetone followed by concentration to a small volume and lyophilization gave 558 mg of **1** as shown by TLC, ^1H NMR, and ^{13}C NMR comparisons.

Acetic Acid Hydrolysis of 2. A solution of 2 g of **2** in 40 mL of 50% acetic acid was warmed at 37 °C for 7 days and then precipitated by adding 2 L of acetone. The precipitate, consisting of the acetone-insoluble fraction, was filtered, washed well with acetone, and dried in a vacuum desiccator over anhydrous calcium sulfate to yield 1.62 g. The acetone filtrate and washes, comprising the acetone-soluble fraction, were evaporated to dryness to yield 390 mg.

Purification of Acetone-Soluble Fraction to Give 8 and 9. The above 390 mg residue was chromatographed over a 4 × 102.5 cm Celite partition column packed with the lower phase from heptane/1-butanol/water (10:100:25), and using the upper phase as the mobile phase.

5α-(Hydroxymethyl)-4β-(2-oxo-4-imidazolin-4-yl)-2-oxazolidinone (**8**) appeared from the column at 3.5 holdback volumes. After evaporation of the solvent the residue was taken up in water, clarified, and evaporated again to give 85 mg. The compound crystallized from water/acetone/ethyl acetate mixtures. High-resolution mass spectral analysis gave the empirical formula as $C_7H_9N_3O_4$ (molecular weight 199.0581); $[\alpha]_D$ + 65.0° (H_2O); IR 3365, 1730, and 1690 cm^{-1}; ^1H NMR (D_2O) δ 3.79 (1 H, dd, CH_2OH, J = 13.0 and 4.0 Hz), 3.92 (1 H, dd, CH_2OH, J = 13.0 and 2.5 Hz), 4.75 (2 H, m, $H_{3,4}$), and 6.58 (1 H, s, H_1).

(1*S*,2*R*,3*R*,4*R*)-1,2-Dideoxy-1,2-ureylene-D-xylofuranose (**9**) appeared from the column at 5.4 holdback volumes. The solvent was evaporated, the residue dissolved in water, the solution clarified and evaporated, the residue dissolved again in water, and the solution clarified and evaporated to yield 81 mg. This material was further purified by a repeat of the partition chromatography on a Celite column 1.7 × 97 cm in the above solvent system. Needles were obtained from moist ethyl acetate. High-resolution mass spectral analysis showed the empirical formula to be $C_6H_{10}N_2O_4$ (molecular weight 174.0644 found, 174.0641 calcd); $[\alpha]_D$ −67.2° (H_2O); IR 3390, 3335, 3205, 1690, and 1670 cm^{-1}; ^1H NMR (D_2O) δ 3.82 (1 H, q, CH_2OH, J = 11.5 and 7.0 Hz), 3.93 (1 H, q, CH_2 OH, J = 11.5 and 4.4 Hz), 4.14 (1 H, m, H_4, J = 7.0, 4.4, and 2.5 Hz), 4.24 (1 H, d, H_3, J = 2.5 Hz), 4.30 (1 H, d, H_2, J = 6.3 Hz), and 5.88 (1 H, d, H_1, $J_{1,2}$ = 6.3 Hz); ^1H NMR of NH signals in Me_2SO-d_6 6.65 and 7.20 (1 H each, s, NH of imidazolidone).

Spermidine *p*-(2,4,6-Trideoxy-2-guanidino-4-ureido-α-D-glucopyranosyloxy)cinnamoylamide Hydrochloride (4). The 1.62 g of acetone-insoluble fraction from acetic acid hydrolysis was dissolved in 50 mL of 50% acetic acid and 67 mL of *tert*-butyl alcohol and purified by cellulose column chromatography with 310 g of cellulose (Avicel) dry packed to form a column 4.3 × 52 cm. The column was developed with a 4:1:1 mixture of *tert*-butyl alcohol/acetic acid/water.

The main component **4** was eluted with 13.8 holdback volumes. The solvent was removed in vacuo and the residue dissolved in 15 mL of 50% acetic acid and clarified. The acetic acid solution was precipitated by addition to 900 mL of acetone. The precipitate was filtered and washed with acetone to yield 688 mg of **4** after drying under vacuum over calcium sulfate: λ_{max} (MeOH) 289 nm ($E^{1\%}_{1cm}$ = 345); IR 3280, 1655, 1595, and 1545 cm^{-1}; $[\alpha]_D$ +96° (H_2O); ^1H NMR (D_2O) δ 1.18 (3 H, d, Me, J = 6.0 Hz), 1.82 (4 H, m, $(CH_2)_2$), 3.15 (6 H, m, $(CH_2)_3N$), 3.42 (3 H, m), 3.90 (3 H, m, $H_{2'',3'',5''}$), 5.69 (1 H, d, $H_{1''}$, $J_{1'',2''}$ = 3.0 Hz), 6.54 and 7.48 (1 H each, d, trans vinyl H's, J = 16.0 Hz, 7.16 and 7.60 (2 H each, d, aromatic H's, J = 8.0 Hz).

Anal. Calcd for $C_{24}H_{40}N_8O_5 \cdot 3HCl$: C, 45.75; H, 6.88; N, 17.79, Cl^-, 16.88. Found: C, 44.19; H, 6.90; N, 16.57; Cl^- (15.49); loss on drying, 3.59; corrected for 3.59% H_2O: C, 45.84; H, 6.5; N, 17.19; Cl^-, 16.06.

Field desorption molecular weight: calcd for $C_{24}H_{40}N_8O_5$, 520; found, 521 (M + H).

Methyl *p*-(2,4,6-Trideoxy-2-guanidino-4-ureido-α-D-glucopyranosyloxy)benzoate Hydrochloride (5). A solution of **4** (371 mg) in 3.7 mL of water and 37 mL of methanol was treated with ozone in a methanol/dry ice bath until the chromophore of the cinnamoyl moiety was destroyed. Nitrogen was then bubbled through the cold reaction solution to remove ozone. Dimethyl sulfide (1.8 mL) was added and the solution allowed to stand at room temperature for 1.5 h. Concentration to dryness in vacuo gave a residue which was taken up in methanol and reevaporated. This was done several times to give 392 mg of a gummy residue which was chromatographed over a Celite partition column with the upper phase of the system heptane/1-butanol/water (25:100:25). The column was monitored by UV at 255 nm. Fractions comprising the first band were combined to give 107 mg of a gum after concentration to dryness in vacuo. Spectral analysis (IR, UV, and ^1H NMR) indicated this material to be the methyl benzoate **5.** Several fractional precipitations on crude **5** in mixtures of methanol/cyclohexane/ethyl acetate were carried out. The noncrystalline precipitates were discarded and the mother liquor was concentrated to dryness. The residue on standing in a small amount of methanol gave crystals of **5** with some of the largest suitable for x-ray analysis: IR (KBr) 1710, 1650, 1600, and 1555 cm^{-1} bd; UV (0.1 N HCl) 250 nm (ε 13 920); ^1H NMR (D_2O) δ 1.18 (3 H d, Me, J = 6 Hz), 3.91 (3 H, s, OMe), 5.82 (1 H, d, $H_{1''}$, $J_{1'',2''}$ = 3 Hz), 7.21 and 8.00 (2 H, d's, aromatic H's, J = 8.5 Hz).

Fractions from a second band from the above column were combined and concentrated to dryness in vacuo to give 49.7 mg of the corresponding aldehyde of **5** as indicated by IR, UV (λ_{max} 268 nm), and NMR. The column was then stripped with methanol and these washings were concentrated to dryness. The residue was taken up in water and lyophilized to give 158 mg of glyoxalylspermidine: IR (KBr) 1670 cm^{-1}; ^1H NMR (D_2O) δ 1.82 (4 H, m, 5 and 6 CH_2's of spermidine, see Scheme I), 1.98 (2 H, t, 2 CH_2), 3.12 (6 H, m, 3, 4, and 7 CH_2's, 3.40 (2 H, t, 1 CH_2), and 5.36 (1 H, s, HCO_2).

***p*-(2,4-Diamino-2,4,6-trideoxy-α-D-glucopyranosyloxy)cinnamic Acid (6).** To a solution of 3.0 g of **2** in 100 mL of water was added 23 g of barium hydroxide octahydrate. The mixture was heated at 80 °C for 48 h. On cooling the mixture was filtered through Celite and the filtrate sparged with carbon dioxide. The mixture was again filtered and the filtrate concentrated to dryness to a yellow, amorphous residue, which was chromatographed over a CM-Sephadex column (NH_4^+) (2.5 × 22 cm). The column was eluted with 500 mL of water, 400 mL of 0.05 N ammonium hydroxide, 250 mL of 0.2 N ammonium hydroxide, and finally 300 mL of 2 N ammonium hydroxide. The main band was concentrated in vacuo and the aqueous concentrate lyophilized to give 129 mg. This was combined with 31 mg of another preparation and triturated with methanol. The insoluble portion was taken up in water and lyophilized to give 84 mg of amorphous **6:** $[\alpha]_D$ +152° (H_2O); IR of hydrochloride (KBr) 1685, 1630, 1600, and 1520 cm^{-1}; UV (0.1 N HCl) 293 and 221 nm (ε 15 100 and 9700); UV (H_2O) 279 and 216 nm (ε 16 000 and 12 000); ^1H NMR (D_2O) + DCl) δ 1.40 (3 H, d, C_6Me, J = 6 Hz), 3.34 (1 H, t, H_4, $J_{3,4}$ = 10 Hz), 4.25 and 4.42 (m's, H_5 and H_3), 6.02 (1 H, d, H_1, $J_{1,2}$ = 4 Hz), 6.32 and 7.55 (1 H, d's, trans vinyl H's, J = 16 Hz), and 7.24 and 7.56 (2 H, d's, aromatic H's, J = 8 Hz).

Isolation of Spermidine. The above Sephadex column was then stripped with 0.2 N sulfuric acid. A yellow band was eluted which was neutralized with barium hydroxide and the barium sulfate was filtered off. The filtrate was sparged with carbon dioxide and the solution filtered again. Concentration of the filtrate to a small volume followed by lyophilization gave 384 mg of crude spermidine carbonate. This was purified by trituration with ethanol and the ethanol-insoluble portion slurried with methanol. The methanol-soluble portion was treated with acetone which precipitated out 193 mg of spermidine imines of acetone. The latter (10 mg) in 1 mL of water was treated with an excess of a saturated aqueous solution of picric acid. The resulting crystals (40 mg) were collected by filtration and dried, mp 211–213 °C; the picrate of authentic spermidine had mp 210–212 °C, mmp 211–213 °C.

4-(1,2-Dihydroxyethyl)-5-dimethoxymethyl)-2-oxo-1-imidazolidinecarboxamide (11). To a slurry of 4.0 g of **2** in 400 mL of methanol was added 75 mL of Amberlyst-15 sulfonic acid resin and the mixture stirred overnight at room temperature. The resin was removed by filtration and the filtrate adjusted to pH 6.5 with IR-45 (OH^-) resin. The resin was removed by filtration and the filtrate concentrated to 40 °C in vacuo to give 480 mg of a pale-yellow gummy residue. Trituration of this residue with methanol gave the analytical sample, mp 182–185 °C, $[\alpha]_D$ −33° (H_2O). High-resolution mass spectral analysis showed the empirical formula to be $C_9H_{17}N_3O_6$ (molecular

weight 263.1114 found; 263.1117 calcd); IR 3355, 3255, 1710, 1665, and 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.3 (2 H, bs, H_4), 3.39 (6 H, s, OMe's), 3.68 (1 H, m, H_3), 4.16 (1 H, dd, H_2, J = 3.5 and 2.3 Hz), 4.70 (1 H, d, H_1, $J_{1,2}$ = 2.3 Hz), 6.86 and 7.70 (1 H each, bs, NH_2's of *N*-carbamoyl), and 7.46 (1 H, bs, NH).

Diacetate of 11 (Compound 12). A slurry of 60 mg of **11** in 1 mL of pyridine and 0.5 mL of acetic anhydride was warmed on the steam bath for 1 min to effect solution and then allowed to remain at room temperature overnight. This was then concentrated in vacuo to a gummy residue which on crystallization with ethyl acetate/hexane gave 35 mg of diacetate **12:** mp 154–156 °C; $[\alpha]_D$ 0° (H_2O); CD 0.1076 mg/ml, 225 nm ($\Delta\epsilon$ -11.4×10^2); IR 3425, 3280, 1755, 1725, and 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 and 2.09 (3 H each, s, OAc's), 3.48 and 3.51 (3 H each, s, OMe's), 4.15 (4 H, m, $H_{2,3}$ and H_5), 4.81 (1 H, d, H_1, J = 2.3 Hz), 5.00 (1 H, q, H_4), 5.32 and 7.95 (1 H each, bs, NH_2's of *N*-carbamoyl), and 6.16 (1 H, s, NH).

The mother liquor from the above crystallization on standing overnight deposited long needles, suitable for x-ray analysis.

Isolation of 9 from Methanolysis Resin. The Amberlyst-15 resin from the above methanolysis reaction of **2** was placed in a column and eluted with 250 mL of 3 N hydrochloric acid. The eluate was immediately neutralized with 10 N sodium hydroxide and the neutralized solution passed through a 100-mL bed volume column of granular carbon (20–40 mesh). After the column was washed with water, it was eluted with 500 mL of 50% aqueous methanol which was subsequently concentrated in vacuo to an aqueous phase and lyophilized to give 230 mg of a colorless, amorphous solid. This was further purified by partition chromatography over a 20 g Celite column packed with the lower phase from heptane/1-butanol/water (10:100:25), and using the upper phase as the mobile phase. Compound **9** was eluted after 3.1 holdback volumes. Concentration in vacuo gave 193 mg of **9** as shown by IR and TLC comparison with an authentic sample prepared as previously described.

Conversion of 11 to 8. A solution of 50 mg of **11** in 1 mL of 85% aqueous acetic acid was heated on the steam bath for 6 h. The solution was diluted with water and lyophilized to give an amorphous solid which by ^1H NMR was composed of 90% **8** and 10% unreacted **11.** TLC analysis on silica gel plates in the system ethyl acetate/1-propanol/water (10:6:4) was consistent with the ^1H NMR result.

Conversion of 13 to the Imidazolidone 14 and the Oxazolidone 15. To a solution of **13** (150 mg) in 12 mL of water was added 3.7 mL of Dowex 1 × 2 (OH^-, 100–200 mesh) and the mixture cooled to 5 °C in an ice bath. A cold solution of *p*-nitrophenyl chloroformate (0.24 g) in 2.4 mL of acetone was added dropwise and the yellow mixture stirred at ice bath temperature for 10 min and then for 2 h at room temperature. The mixture was washed with ether and then filtered. The residual mass was washed with hot water and the filtrate and washings were made acidic (pH 2.5) with 6 N hydrochloric acid. The filtrate and washings were washed several times with ether and then neutralized with Dowex 1 × 2 (OH^-, 100–200 mesh) and lyophilized to give 170 mg of a hygroscopic residue. Celite partition chromatography (18-g column) with the system heptane/1-butanol/water (12:100:25) gave two compounds. Fractions from the first band were combined and concentrated to dryness. Trituration with ethanol gave 12 mg of crystals of **14:** mp 163–172 °C; IR (KBr) 1740, 1670, 1620, and 1570 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.30 (3 H, s, OMe), 4.82 (1 H, d, H_1, $J_{1,2}$ = 5.5 Hz), 5.40 (1 H, d, OH), 6.87 and 7.50 (1 H each, bs, NH_2's of *N*-carbamoyl), and 7.80 (1 H, bs, NH). The ^{13}C NMR was run in Me_2SO-d_6 and is given in Table I.

Fractions from the second band were combined and concentrated to dryness to give 12 mg of **15** from ethanol: mp 198–200 °C; IR (KBr) 1755, 1655, and 1565 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.32 (3 H, s, OMe), 4.32 (1 H, d, H_1, $J_{1,2}$ = 5.5 Hz), 5.50 (2 H, s, NH_2 of urea), 6.23 (1 H, d, J = 6.8 Hz, urea NH), and 7.91 (1 H, s, oxazolidone NH).

Anal. Calcd for $C_8H_{13}O_5N_3$: C, 41.56; H, 5.67; N, 18.18. Found: C, 40.90; H, 5.45; N, 17.74.

Field desorption molecular weight: calcd for $C_8H_{13}O_5N_3$, 231; found, 232 (M + 1).

Formation of the Oxazolidone Dimethyl Acetals 16 and 17. A 7.5-g sample of **2** enriched in **3** (60:40 by high-pressure liquid chromatography) was methanolyzed as described above for **2.** Evaporation of the methanolic filtrate and trituration of the residue with methanol gave 530 mg of **11.** The residue (417 mg) from the mother liquor was chromatographed over a 50-g Celite partition column with the upper phase of the solvent system heptane/1-butanol/water (10:100:25). Another 103 mg of crystalline **11** was obtained from a band eluted at 0.65 holdback volume. Compound **16** appeared after 2.3 holdback volumes. Evaporation of the solvent gave 67 mg of an amorphous residue which was acetylated with pyridine/acetic anhydride. The solution was concentrated to dryness and the residue crystallized from ethyl acetate/hexane to give 18 mg of **17:** mp 172–173 °C; $[\alpha]_D$ +0.63° (MeOH); IR (KBr) 1760, 1745, and 1670 cm^{-1}; ^1H NMR ($CDCl_3$ + 2 drops Me_2SO-d_6) δ 2.11 (3 H, s, Ac), 3.45 and 3.48 (3 H, s, OMe's), 4.00 (2 H, m, H_2, H_3), 4.25 (2 H, m, H_5), 4.33 (1 H, d, H_1, $J_{1,2}$ = 3 Hz), 4.60 (1 H, q, H_4), 5.28 (2 H, s, urea NH_2), 6.12 (1 H, s, NH of oxazolidone), 6.38 (1 H, d, J = 8 Hz, urea NH). Field desorption molecular weight: calcd for $C_{11}H_{19}O_7N_3$, 305; found, 306 (M + 1).

Conversion of 18 to 19. A solution of **18** (0.5 g) in 5 mL of water was treated with 3.4 mL of Dowex 1 × 2 (OH^-, 100–200 mesh) and the mixture cooled in an ice bath. To this stirred mixture was added dropwise 0.36 g of *p*-nitrophenyl chloroformate in 3.5 mL of acetone. The yellow mixture was stirred at ~5°C for 10 min and then at room temperature for 1 h. The reaction mixture was then washed with ether and filtered. The residual mass was washed with hot water and the filtrate and combined washings were acidified to pH 2.5 and these were washed with ether. The aqueous solution was neutralized to pH 6.5 with Dowex 1 × 2 (OH^-; 50–100 mesh) and lyophilized to give 0.44 g of **19** as an amorphous powder with a prominent IR band at 1755 cm^{-1}.

Methanolysis of 1. To a slurry of 5.0 g of **1** in 250 mL of methanol was added 100 mL of Amberlyst-15 sulfonic acid resin and the reaction mixture stirred for 3 days at room temperature. The mixture was decanted several times to remove a fine precipitate. After filtration and washing with methanol to remove any neutral substances, the resin was placed in a column and eluted with 500 mL of 2 N methanolic ammonium hydroxide. The eluate was concentrated at 40 °C in vacuo to give 1.35 g of a yellow-brown gum. Trituration with methanol gave 59 mg of β-methyl-2,3-dideoxy-2-ureido-3-aminoxylopyranoside (**13**): mp 211–213 °C; $[\alpha]_D$ −55° (H_2O); IR 3345, 3205, 2840, 1665, 1624, and 1550 cm^{-1}; ^1H NMR (D_2O) δ 2.72 (1 H, dd, H_3, $J_{2,3}$ = 9, $J_{3,4}$ = 10 Hz), 3.35 (1 H, t, H_2, $J_{1,2}$ = 8, $J_{2,3}$ = 9 Hz), 4.02 (1 H, q, H_5 eq, $J_{4a,5eq}$ = 4, $J_{5,5}$ = 10 Hz), 4.38 (1 H, d, H_1, $J_{1,2}$ = 8 Hz); ^1H NMR of urea NH signals in Me_2SO-d_6 δ 5.46 (2 H, s, NH_2 of urea) and 5.94 (1 H, d, J = 8.5 Hz, NH of urea).

Anal. Calcd for $C_7H_{15}O_4N_3$: C, 40.97; H, 7.37; N, 20.48. Found: C, 41.12; H, 7.31; N, 19.94.

The mother liquor from the above crystallization (20 mL) was treated with 2 mL of acetic anhydride and allowed to remain at room temperature overnight. Concentration in vacuo gave a gummy product which was purified by partition chromatography over a 100-g Celite column packed with the lower phase from heptane/butanol/water (12:100:25) and using the upper phase as the mobile phase. The α-methyl *N*-acetate, **22**, appeared from the column at 2.1 holdback volumes. Concentration in vacuo gave 169 mg of crude crystalline material which on recrystallization from ethanol gave 54 mg of **22:** mp 300 °C; $[\alpha]_D$ +36° (H_2O); IR 3335, 2925, 1665, 1580, and 1550 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.80 (3 H, s, NHAc), 3.30 (3 H, s, OMe), 4.49 (1 H, d, H_1, $J_{1,2}$ = 3.0 Hz), 4.90 (1 H, bd, OH), 5.53 (1 H, d, J = 8.5 Hz, NH of urea), 5.61 (2 H, s, NH_2 of urea), and 7.60 (1 H, d, J = 8.5 Hz, N*H*Ac).

Anal. Calcd for $C_9H_{17}O_5N_3$: C, 43.72; H, 6.93; N, 17.00. Found: C, 43.63; H, 6.91; N, 16.62.

The β-methyl *N*-acetate, **21,** was eluted from the above partition column at 3.2 holdback volumes. Concentration in vacuo gave 262 mg of crude crystalline material which on recrystallization from methanol gave 126 mg of **21:** mp 281–283 °C; $[\alpha]_D$ −130° (H_2O); IR 3390, 3280, 3095, 2935, 2840, 1655, 1605, and 1565 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.81 (3 H, s, NHAc), 3.32 (3 H, s, OMe), 3.63 (1 H, q, J = 8.5 and 8.5 Hz), 3.82 (1 H, q, H_{5e}, J = 10.7 and 3.5 Hz), 4.21 (1 H, d, H_1, $J_{1,2}$ = 6.5 Hz), 5.08 (1 H, d, OH), 5.49 (2 H, s, NH_2 of urea), 5.81 (1 H, d, NH of urea), and 7.57 (1 H, d, J = 8.0 Hz, N*H*Ac).

Anal. Calcd for $C_9H_{17}O_5N_3$: C, 43.72; H, 6.93; N, 17.00. Found: C, 43.58; H, 6.93; N, 16.94.

A mixture of 5 g of **1** in 650 mL of methanol was stirred at room temperature in the presence of 100 mL of Amberlyst-15 sulfonic acid resin for 5 days. The mixture was decanted several times to remove fines from the resin. The mixture was then filtered and the resin washed well with methanol. The resin was then slurried in methanol and placed in a column. After elution with methanolic ammonia (100

mL of concentrated ammonium hydroxide and 900 mL of methanol), the eluate was concentrated to dryness, and the residue was taken up in hot methanol, treated with carbon, and finally crystallized from methanol to give 143 mg of **13** with mp 216–218 °C identical by TLC and IR with the sample obtained previously. Crystals suitable for x-ray analysis were obtained by slow recrystallization from methanol.

β-Methyl-2,3-diamino-2,3-dideoxy-D-xylopyranoside (23). A solution of 34 mg of **13** in 15 mL of water was treated with 500 mg of barium hydroxide octahydrate and the mixture refluxed for 24 h. The mixture was filtered through a pad of Celite and the filtrate percolated through a 10-mL column of Dowex 1 × 2 (OH^-, 50–100 mesh) and the effluent evaporated to dryness in vacuo at 40 °C. Crystallization of the residue from absolute ethanol gave 8 mg of **23:** mp 205–210 °C; $[\alpha]_D$ −83° (H_2O). Field desorption molecular weight: calcd for $C_6H_{14}O_3N_2$, 162; found, 163 (M + 1).

Mercaptolysis of 1. To a solution of 10 g of **1** in 30 mL of concentrated hydrochloric acid was added 150 mL of ethyl mercaptan. The mixture was stirred in a dry ice/methanol bath for 3 h. The reaction was then transferred to the cold room (4 °C) and stirred for an additional 19–20 h. Neutralization to pH 5.5 was carried out with lead carbonate. The filtrate was subsequently adjusted to pH 7.4 with IR-45 (OH^-). The filtrate was then concentrated to 150 mL and chromatographed over a CM-Sephadex column (Na^+) with a gradient of 1–12% sodium chloride. Fractions were assayed with ninhydrin and on this basis homologous fractions were combined and desalted with granular carbon followed by elution with 50% aqueous acetone. Four bands were obtained but only the first and the last examined. TLC and spectral evidence showed that the material from the last band was **4.**

The 2.79 g from the first band was chromatographed over a Celite partition column (240 g) in the system *sec*-butyl alcohol/ethyl acetate/acetic acid/water (125:75:10:150). Homologous fractions were pooled, concentrated to dryness, taken up in water, and lyophilized. ^{13}C and ^1H NMR studies indicated that material from a first band was composed of the dithioacetal **24**, $[\alpha]_D$ −48° (MeOH). ^{13}C NMR and IR data indicated that material from a second band contained primarily the hydantoin **26.**

Acetylation of 24. The dithioacetal **24** (250 mg) was dissolved in 3 mL of pyridine and 1.2 mL of acetic anhydride. The solution was allowed to stand at room temperature for 20 h. Concentration to dryness gave an amorphous residue which was chromatographed over a Celite partition column in the upper phase of the system isobutyl alcohol/methanol/ethyl acetate/water (5:1.5:6:5) with 10-mL fractions. The main band was concentrated to dryness and lyophilized from *tert*-butyl alcohol to give 74 mg of **25.**

Acetylation of 26. A slurry of the hydantoin **26** (523 mg) in 2.5 mL of pyridine and 0.8 mL of acetic anhydride was warmed on the steam bath for 3 min to effect solution. After standing at room temperature overnight, the solution was concentrated to dryness in vacuo. The residue was chromatographed over a Celite partition column (50 g) with the solvent system heptane/1-butanol/water (100:100:15). Two main bands were concentrated to dryness. ^{13}C and ^1H NMR (Me's 2.08, 2.09, 2.24, and 2.56) studies on the first band (200 mg) indicated clearly that this material is the tetraacetate **27** while the second band (50 mg) consists of a closely related but as yet unidentified substance.

Methanolysis of 24. A methanolic solution (10 mL) of the dithioacetal **24** (100 mg) saturated with hydrogen chloride was heated at reflux for 2 h. The solution was concentrated to dryness, methanol was added, and the solution was concentrated again to dryness. The residue was dissolved in 10 mL of 10% methanol/water to which was added 0.5 mL of acetic anhydride and 20 mL of Dowex 1 × 4 (CO_3^{2-}). The mixture was stirred at 5 °C for 2 h. After standing at room temperature over the weekend, the mixture was filtered and the filtrate concentrated to dryness. Trituration with methanol gave crystalline **21** as shown by TLC and IR comparison with an authentic sample. Silica gel TLC (ethyl acetate/1-propanol/water, 10:6:4) on the mother liquor showed the presence of **22** in addition to **21** and two new materials presumably containing sulfur.

Methanolysis of 26. A solution of 185 mg of the hydantoin **26** in methanol saturated with hydrogen chloride was heated at reflux for 2 h. The solution was concentrated to dryness and taken up in methanol. The solution was reconcentrated to dryness. Finally the residue was dissolved in 10 mL of a 10% methanol/water mixture. To this was added 0.5 mL of acetic anhydride and 20 mL of Dowex 1 × 4 (CO_3^{2-}). The mixture was stirred at 5 °C for 2 h. After standing at room temperature over the weekend, the mixture was filtered and the filtrate concentrated to dryness. Trituration with methanol followed by filtration gave crystalline **21** as shown by TLC and IR comparisons with an authentic sample. Silica gel TLC (ethyl acetate/1-propanol/water, 10:6:4) of the mother liquor indicated the presence of **21** and **22.**

2-*N*-Acetyl-5,5-dimethylhydantoin (28). A solution of 495 mg of 5,5-dimethylhydantoin was treated with 0.5 mL of acetic anhydride and allowed to stand at room temperature for 3 days. The solution was evaporated to dryness in vacuo at 50 °C. Toluene was added to the residue and the solution reevaporated. Trituration with benzene gave 465 mg of white crystals of **28** with mp 193–195 °C with some prior softening; IR (KBr) 1805, 1760, and 1720 cm^{-1}; ^1H NMR ($CDCl_3$ + 1 drop Me_2SO) δ 1.46 (6 H, s, Me's), 2.58 (3 H, s, *N*-Ac), and 7.95 (1 H, s, NH).

Ozonolysis of 1. A solution of **1** (10 g) in 40 mL of water and 400 mL of methanol was cooled in a dry ice/methanol bath. Ozone was bubbled in for 12 min. The solution was allowed to come to room temperature and sparged with nitrogen until a negative starch iodide test was observed. Dimethyl sulfide was added and the solution allowed to stand overnight. The pH was adjusted from 3.62 to 5.88 with 1 N ammonium hydroxide and the solution concentrated in vacuo to dryness. Methanol was added and the solution evaporated once more. A 5-g portion was then purified on a Celatom partition column with the system methanol/1-butanol/water (0.5:10:4) and monitored by UV at 255 nm. Fractions in the main band were combined and concentrated to dryness in vacuo. The residue was dissolved in water and the solution filtered through Celite and lyophilized to give 1.82 g of an amorphous residue. The remaining 5 g was chromatographed in the same manner to give 2.40 g. The two preparations were combined to give 4.22 g of the methyl ester **18:** $[\alpha]_D$ +64° (H_2O); IR (KBr) 1705 sh, 1665, 1610 and 1555 cm^{-1} bd; UV (0.1 N HCl) 250 nm (ε 12 800); ^1H NMR (D_2O) δ 1.14 (3 H, d, Me, J = 6 Hz), 3.91 (3 H, s, OMe), 5.81 (1 H, d, $H_{1''}$, $J_{1'',2''}$ = 3 Hz), 7.20 and 7.96 (2 H, d's aromatic H's, J = 8.0 Hz).

[*S*-(*R,*R**)]-5-(2,3-Dihydroxypropyl)hydantoin (29).** To a solution of 5 g of **1** in 500 mL of water was added 100 mL of Dowex-50 (H^+, 50–100 mesh) and the mixture heated at reflux for 4 h. The mixture was filtered and the filtrate neutralized with IR-45 (OH^-). Concentration to dryness at 40 °C under reduced pressure gave 630 mg of a gummy residue. This was chromatographed over a 50 g Celite partition column (2.5 × 62 cm) with the system heptane/1-butanol/water (10:100:25). The first band was concentrated to dryness to give a residue which on crystallization from absolute alcohol gave 20 mg of **29:** mp 153–155 °C; IR (KBr) 1755 and 1720 cm^{-1}; ^1H NMR (D_2O) δ 2.05 (2 H, m, H_3), 3.69 (2 H, m, H_5), 3.95 (1 H, m, H_4), and 4.56 (1 H, dd, H_2, J = 4.5 and 8.0 Hz).

[*S*-(*R,*S**)]-5-(2,3-Dihydroxypropyl)hydantoin (30).** The second band was concentrated to dryness to give a residue which on crystallization from absolute alcohol gave 12 mg of **30:** mp 143–145 °C; IR (KBr) 1775 and 1730 cm^{-1}; ^1H NMR (D_2O) δ 2.15 (2 H, m, H_3), 3.66 (2 H, m, H_5), 4.10 (1 H, m, H_4), and 4.50 (1 H, t, H_2, J = 4.5 Hz).

Spermidine, β-Hydroxycinnamamide (7). The resin (8 mL) from a second hydrolysis of **1** (1 g) was washed with water and then eluted with 2 N ammonium hydroxide (50 mL). Concentration to dryness under reduced pressure at 40 °C gave 500 mg of a gummy residue. This was chromatographed over a 50-mg Celite partition column with the system heptane/1-butanol/water (10:100:25). The main band was concentrated to dryness under reduced pressure at 40 °C. Trituration of the residual gum with ethanol gave 70 mg of crystals of **7:** mp 232–235 °C; IR (KBr) 3425, 2985, 2855, 2565, 2445, 1650, and 1605 cm^{-1}; λ_{max} (0.1 N NaOH) 345, 310 sh, and 224 nm (ε 17 700, 9900, and 10 000); ^1H NMR (D_2O) δ 1.79 (4 H, m, H_5 and H_6), 1.95 (2 H, m, H_2), 3.14 (6 H, m, H_3, H_4, and H_7), 3.37 (2 H, t, H_1), 6.56 and 7.55 (1 H, d's, J = 16 Hz, trans vinyl H's), 7.06 and 7.63 (2 H, d's, J = 9 Hz, Ar H's); mass spectrum *m/e* 274.1677 (M − 17) (calcd for $C_{16}H_{22}O_2N_2$, 274.1688).[27] Field desorption molecular weight: calcd for $C_{16}H_{25}O_2N_3$, 291; found, 292 (M + 1).

X-Ray Structure Determinations. Intensities for each crystal were measured in the range $3° < \theta < 60°$ using the $\theta/2\theta$ scan method on an Enraf-Nonius CAD-3 computer-controlled diffractometer. Nickel-filtered Cu Kα radiation from a fine focus tube was used with pulse height analysis of the diffracted beam to provide further wavelength discrimination. After correction for Lorentz and polarization effects normalized structure factors $E(hkl)$ were computed for each

crystal. The structures were solved by the direct phase determination method, using MULTAN[28] in all cases except for **9** for which TANGEN of the XRAY 72 system[29] was used. More detailed descriptions of the crystallographic studies will be published elsewhere.

Square tabular crystals of methyl *p*-(2,4,6-trideoxy-2-guanidino-4-ureido-α-D-glucopyranosyloxy)benzoate hydrochloride (**5**), $C_{16}H_{23}N_5O_6 \cdot HCl \cdot H_2O$, grown by evaporation from cyclohexane/ethyl acetate/methanol solution are orthorhombic, a = 32.347 (8) Å, b = 8.785 (2) Å, c = 7.797 (2) Å, space group $P2_12_12_1$. The calculated density, assuming one molecule per asymmetric unit, is 1.31 g cm^{-3}; the measured value by flotation in a bromobenzene/benzene mixture is 1.29 g cm^{-3}. Three-dimensional data collection yielded 1955 independent reflections of which only 950 were classified as observed by the criterion $I > 1.0\sigma(I)$. The small number of observed data may be attributed partly to the small size of the crystal, 150 × 120 × 20 μm, and partly to poor crystal quality. An E map was observed which contained peaks corresponding to all the nonhydrogen atoms of the chemical structure proposed for **5**.

Preliminary least-squares refinement of the model treating all nonhydrogens as carbons gave R = 0.23 where $R = [\Sigma||F_o| - |F_c||/\Sigma|F_o|]$. Temperature parameters at this stage were used, in conjunction with the proposed structure, to establish atomic types. However, it was not possible to distinguish between guanidine and urea; for further refinement both groups were included as guanidine.

In the difference electron density an isolated peak was found, large enough to correspond to an oxygen atom. The position of the peak with respect to the chloride ion, the hydroxyl group, and the guanidine/urea groups was appropriate for hydrogen bond formation. The peak was assumed, therefore, to represent the oxygen atom of a water of crystallization.

Further isotropic refinement gave R = 0.142 and it was then possible to distinguish between urea and guanidine on the basis of bond lengths and hydrogen bonding.

No attempt was made to refine the structure beyond this point because the ratio between number of observed intensities and the number of variables in anisotropic refinement (≈3.5:1) was considered too small to produce a meaningful result.

Crystals of **5α-(hydroxymethyl)-4β-(2-oxo-4-imidazolin-4-yl)-2-oxazolidinone (8)**, $C_7H_9N_3O_4$, obtained from wet ethyl acetate/acetone mixtures, were initially transparent but became opaque on standing in mother liquor. A small, relatively clear fragment, cut from a larger crystal, was found to be orthorhombic, space group $P2_12_12_1$, with a = 11.290 (3), b = 10.277 (3), c = 7.465 (2) Å. The density measured by flotation in a bromobenzene/*s*-tetrabromoethane mixture was found to be 1.52 g cm^{-3}; calculated density, 1.53. Data collection yielded 792 independent reflections of which 495 were classified as observed by the criterion $I > 1.5\sigma(I)$. Preliminary isotropic refinement of a trial structure in which all atoms were treated as carbons gave R = 0.15. Atomic type assignments were made initially on the basis of isotropic temperature parameter values and at a later stage with the additional help of the observed bond lengths. The structure was refined isotropically to R = 0.112 and then anisotropically to R = 0.090. As a further confirmation of the x-ray structure peaks were found, in a final difference map, in positions appropriate for hydrogen atoms. Since the number of data collected was relatively small (≈35 reflections/atom), and of rather poor quality, no attempt was made to include hydrogen atoms for further refinement. An attempt to distinguish the correct hand of the structure by means of the anomalous dispersion effects of oxygen led to inconclusive results.

Columnar crystals of **(1*S*,2*R*,3*R*,4*R*)-1,2-dideoxy-1,2-ureylene-D-xylofuranose (9)**, $C_6H_{10}N_2O_4$, grown from wet ethyl acetate are orthorhombic, space group $P2_12_12_1$, a = 10.704 (1) Å, b = 14.878 (2) Å, c = 4.821 (1) Å, d_c = 1.51 g cm^{-3} assuming one molecule in the asymmetric unit, d_o = 1.51 g cm^{-3} (by flotation in heptane/bromobenzene/1,1,2,2-tetrabromoethane mixture). A crystal with approximate dimensions 320 × 120 × 64 μm was used to collect intensity data. Of the 715 independent reflections measured, 219 were classified as unobserved by the criterion $I < 2.0\sigma(I)$. The trial structure, assuming all nonhydrogen atoms to be carbon, gave R = 0.25. Values of temperature parameters obtained after one cycle of isotropic refinement were used to distinguish nitrogen and oxygen atoms from carbons. Isotropic refinement after atom type assignment gave R = 0.102. Anisotropic refinement then gave R = 0.070 at which stage hydrogen atoms (excluding those of the hydroxyl groups) were located in a difference map. Further anisotropic refinement after including hydrogens (but not refining them) gave a final value of R = 0.049.

The anomalous dispersion effects of oxygen (for Cu Kα $\Delta f_o'$ = 0 $\Delta f_o''$ = 0.1) may, in principle, be used to determine the absolute configuration of the molecule. To do this structure factors with $\Delta f_o''$ = +0.1 and $\Delta f_o''$ = −0.1 were computed and the reliability indices R_+ = 0.04976, R_- = 0.05029 were compared using the Hamilton significance test.[31] For these data the values of R are significantly different at the 98% confidence level so that the structure may be assigned as (1*S*,2*R*,3*R*,4*R*)-1,2-dideoxy-1,2-ureylene-D-xylofuranose.

Prismatic crystals of **12**, $C_{13}H_{21}N_3O_8$, grown from ethyl acetate/hexane solution are monoclinic, space group $P2_1$ with a = 8.087 (3) Å, b = 12.018 (7) Å, c = 9.144 (4) Å, β = 103.04 (3)°. Assuming one molecule in the asymmetric unit the calculated density is 1.33 g cm^{-3}; the measured value, by flotation in bromobenzene/benzene mixture, is 1.323 g cm^{-3}. Three-dimensional data collection gave 1453 independent reflections of which 959 were classified as observed by the criterion $I > 2.0\sigma(I)$. A partial structure (15 atoms) was obtained by an application of MULTAN. The remainder of the structure was subsequently found in electron density difference syntheses.

The assignment of atom types in this structure was made on the basis of the proposed chemical structure taken in conjunction with refined isotropic temperature parameter values and bond distances. The structure did not refine well; the final discrepancy factor at the end of isotropic refinement was R = 0.14. Further anisotropic refinement (excluding the atoms of terminal acetyl groups) gave a final R = 0.11. The isotropic temperature parameters for the terminal atoms of the acetyl groups were three or four times larger than the average value for the remaining atoms. This was taken as an indication of disorder in these regions of the crystal structure. However, attempts to define the nature of the disorder and improve the refinement were not successful.

Although the molecule contains eight oxygen atoms and is therefore a candidate for absolute configuration determination by the anomalous dispersion effect, the poor refinement did not leave any possibility that the hand could be determined successfully.

Prismatic crystals of **13**, $C_7H_{15}O_4N_3$, grown from methanol/ethanol/water mixture are orthorhombic, space group $P2_12_12_1$ with a = 22.939 (9), b = 9.451 (4), c = 9.198 (4) Å; there are two molecules in the asymmetric unit. The calculated density is 1.367 g cm^{-3}; the measured value by flotation in CCl_4/acetone mixture is 1.375 g cm^{-3}. Three-dimensional data collection gave 1750 reflections of which 1176 were classified as observed by the criterion $I > 2.0\sigma(I)$. Isotropic refinement of the trial structure with all atoms treated as carbon gave R = 0.13. At this stage atom type assignments were made on the basis of the chemical structure taking into account also the refined values of temperature parameters. Further anisotropic refinement gave a final R value of 0.095. Of the 30 hydrogen atoms in the structure 26 were located in a difference map and were included (and refined isotropically) in the final least-squares calculations. It was considered unlikely that an attempt to determine the absolute configuration of the structure (using anomalous dispersion effects of oxygen) would be successful.

Crystals of **[*S*-(*R**,*R**)]-5-(2,3-dihydroxypropyl)hydantoin (29)**, $C_6H_{10}N_2O_4$, grown from methanol are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions a = 23.498 (4), b = 7.327 (1), c = 4.672 (1) Å. The calculated density based on one molecule per asymmetric unit is 1.44 g cm^{-3}; the observed density, by flotation in carbon tetrachloride/benzene mixture, is 1.457 g cm^{-3}. Three-dimensional data collection gave 772 independent reflections of which 70 were classed as unobserved with $I < 2\sigma(I)$. Initial least-squares refinement of the trial structure treating all atoms as carbon led to R = 0.16. Atom types were then assigned on the basis of the refined values of the isotropic thermal parameters and chemical information. Further isotropic refinement reduced R to 0.11; thereafter anisotropic refinement gave R = 0.073. Peaks for all ten hydrogen atoms were found in a difference electron density map. Further refinement of the nonhydrogen atoms after including hydrogens gave R = 0.049; after isotropic refinement of hydrogen atoms the final discrepancy factor was R = 0.045. A structure factor calculation for the (*R*,*R*) configuration gave R_+ = 0.0456; for the (*S*,*S*) configuration R = 0.0448. Application of the Hamilton significance test[30] indicates that the (*R*,*R*) configuration may be rejected at the 1% significance level. Further refinement of the (*S*,*S*) configuration gave a final R factor of 0.0445. From this analysis, therefore, on the basis of anomalous dispersion effects of oxygen, the structure is established as [*S*-

(*R**,*R**)]-5-(2,3-dihydroxypropyl)hydantoin.

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(16) At this point we had no more pure γ_2 to rerun its ^{13}C NMR spectrum in Me_2SO to compare with a spectrum of the water-insoluble **15** in Me_2SO.
(17) Elution of the resin from the methanolysis of **2** with strong acid (after removal of the neutral **11**) gave **9.** However, after elution of **13** from the resin used for the methanolysis of **1,** a similar strong acid treatment did not give **9.** Neither did acetic acid hydrolysis of **1,** although a considerable effort was made to try to obtain **9** from **1** in both these reactions. This most likely reflects some lability of the precursor **10** to the more prolonged methanolysis conditions necessary to liberate **13** or, in the case of acetic acid hydrolysis, the greater stability of the terminal glycosidic linkage in **1** compared with **2.**
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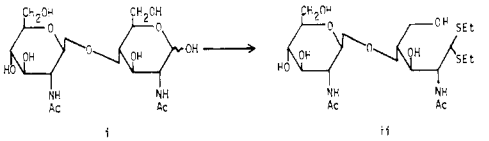

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