on Chromosorb W. The response factors experimentally determined for enones were used in estimating the quantity of the corresponding lumiketone formed. Under an identical set of irradiation conditions the relative quantum yield (ϕ_{rel}) is related to the molar ratio of the amounts of lumiketone formed from the enone to the lumiketone formed from 4,4-dimethylcyclohex-2-en-1-one. The results are summarized in Table V.

Acknowledgment. J.M.R. thanks the Council of Scientific and Industrial Research, New Delhi, India, for a leave of absence. This work was supported by a grant from the National Science Foundation (CHE-7819750). The ^{13}C and 100-MHz ^1H NMR spectra and mass spectra were obtained by Mr. Charles Strom, whose assistance is gratefully acknowledged.

Registry No. 1, 13395-74-6; 2, 17299-41-1; 3, 1073-13-8; 5, 28354-98-5; 7, 23438-77-9; *cis*-9, 76514-89-1; *trans*-9, 76514-90-4; 10, 76514-91-5; 11, 76514-92-6; 12, 76514-93-7; 15, 76514-94-8; 16, 76514-95-9; 17, 76514-96-0; 18, 1846-48-6; 1-(2-methyl-1-propene)-pyrrolidine, 2403-57-8; ethyl vinyl ketone, 1629-58-9; 3-methylbutan-2-one, 563-80-4; methyl vinyl ketone, 78-94-4.

Structures of the Decomposition Products of Chlorozotocin: New Intramolecular Carbamates of 2-Amino-2-deoxyhexoses

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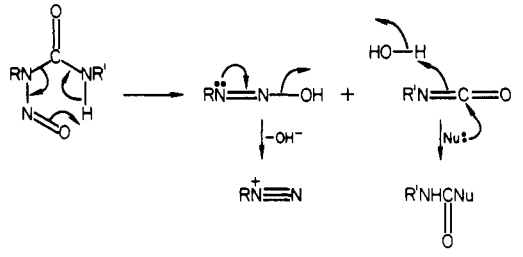
Six intramolecular five-membered-ring carbamate sugars were obtained from the decomposition of chlorozotocin (2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-D-glucose, **2**) in phosphate and triethylammonium bicarbonate buffers at pH 7.4. The structures were proven by spectroscopic methods and chemical inference to be 2-(carboxyamino)-2-deoxy-α-D-glucopyranose intramolecular 2,1-ester (**3**), 2-(carboxyamino)-2-deoxy-α-D-glucofuranose intramolecular 2,1-ester (**7**), "cis dimer" **11** (1*R*-[1α(1*S**,2*R**),5α,5aα,6α(1*S**,2*R**),10α,10aα]-5,10-dihydroxytetrahydro-1,6-bis[1,2,3-trihydroxypropyl]-1*H*,3*H*,5*H*,8*H*-dioxazolo[3,4-*a*:3′,4′-*d*]pyrazine-3,8-dione), "trans dimer" **12** (1*R*-[1α(1*S**,2*R**),5β,5aα,6α(1*S**,2*R**),10α,10aα]-5,10-dihydroxytetrahydro-1,6-bis[1,2,3-trihydroxypropyl]-1*H*,3*H*,8*H*-dioxazolo[3,4-*a*:3′,4′-*d*]pyrazine-3,8-dione), 2-(carboxyamino)-2-deoxy-α-D-mannofuranose intramolecular 2,3-ester (**13**), and the "monomer", which is either 2-(carboxyamino)-2-deoxy-D-glucopyranose intramolecular 2,3-ester (**15**) or its open-chain aldehyde hydrate **16**. It was shown that the latter "monomer" was in equilibrium with the "cis dimer" and "trans dimer" and was probably the precursor to **13**, whose formation by epimerization was catalyzed by silica gel. Acetate and *O*-trimethylsilyl derivatives were prepared of all compounds except the "monomer", which gave only "cis and trans dimer" derivatives. The acetate derivatives of compounds **7** and **11** have been found previously from the decomposition of the related streptozotocin (**1**).[30]

N-Nitrosoureas are believed to decompose in aqueous solution at pH 7–7.4 to produce diazo hydroxides and isocyanates.[2-8] These further react to form an active



alkylating agent and a carbamate or urea, depending on the nature of the nucleophile (Nu:). The alkylating side of the decomposition has been studied extensively by many research groups and is thought to be the cause of the antitumor activity of many of the *N*-nitrosoureas.[3,5,9-17] The ability of the presumed isocyanate to carbamoylate nucleophiles is determined in vitro by incubating the urea with radioactively labeled lysine.[18] An important biological role has been suggested for nitrosourea carbamoylating activity from data derived from in vitro studies. This includes the prolongation of the S phase of cell synthesis,[19] inhibition of repair X-irradiated and alkylated DNA,[20,21] inhibition of nucleolar and nucleoplasmic RNA,[22] and inhibition of DNA polymerase II.[23] The 2-amino-2-deoxy-D-glucose nitrosoureas streptozotocin (**1**) and chlorozotocin (**2**, 2-[[[(2-chloroethyl)nitrosoamino]carbonyl]-amino]-2-deoxy-D-glucose) show significantly reduced carbamoylating activity and bone marrow toxicity relative

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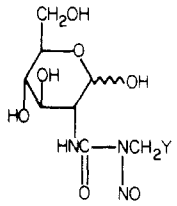
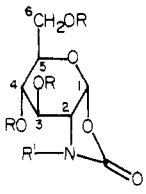


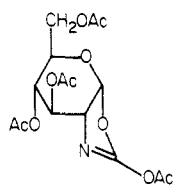
1, Y = H
2, Y = CH_2Cl

to nitrosoureas with unsubstituted carrier alkyl groups.[18,24] Nevertheless, full antitumor activity is retained by nitrosoureas of low carbamoylating potential, suggesting that alkylation is the principal mediator of cytotoxic activity against neoplastic cells.[21,25,26]

In 1967, Herr, Jahnke, and Argoudelis[27] of Upjohn reported that the reaction of 1 with 2 N NaOH gave 3, whose tetraacetyl derivative was assigned structure 6 based on spectral data. In 1978, about a year after we[28] pointed



3, R = R′ = H
4, R = R′ = Ac
5, R = Ac; R′ = H



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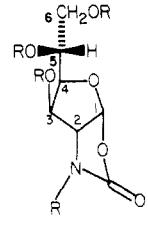
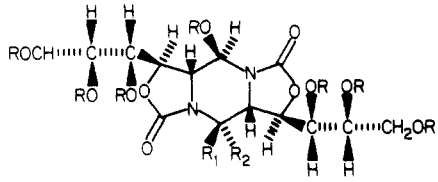


7, R = H
8, R = Ac

out that the data did not fit the enol acetate 6, we were informed[29] that an X-ray study of the tetraacetate showed the structure to be 8. This established that the base decomposition product was really 7 and not 3 as was originally suggested. This structure, along with other reactions of 1, was published in 1979.[30] The decomposition of 1 in pH 4.6 saline solution followed by acetylation gave a dimer whose structure was shown to be 9 mainly on spectroscopic data.[30]



9, R = Ac, R_1 = OAc; R_2 = H
10, R = Ac; R_1 = H; R_2 = OAc
11, R = R_2 = H; R_1 = OH
12, R = R_1 = H; R_2 = OH

Chlorozotocin (2) is presently undergoing clinical trials in the Division of Medical Oncology at the Georgetown University Hospital under the direction of one of us (P. S.S.). In connection with these trials, several studies[25,26,31-34] have shown that the 2-deoxy-2-D-glucosamine

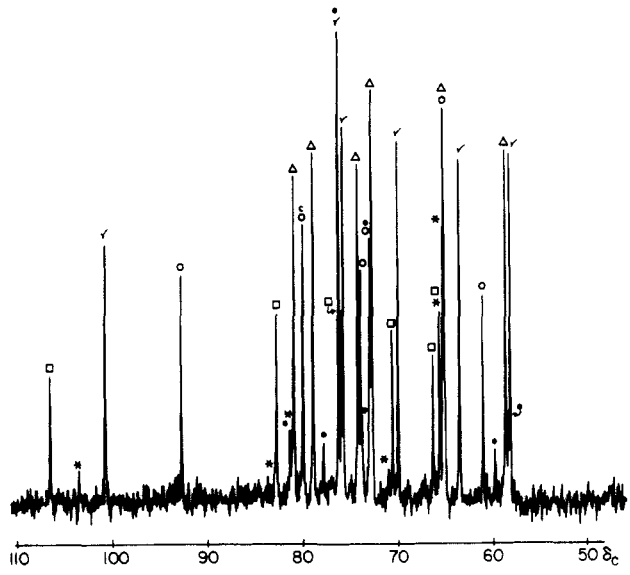
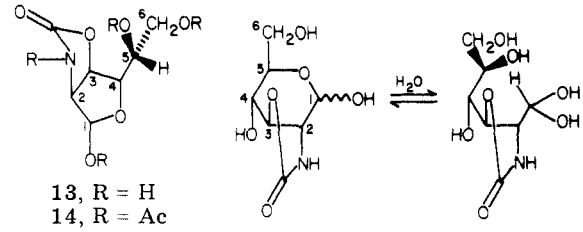


Figure 1. ^{13}C NMR spectrum in D_2O of a decomposition mixture of 2 in triethylammonium bicarbonate buffer at pH 7.4. [The carbonyl region is not shown. The symbols show the assignment of each peak: 3 ( ), 7 (□), 11 (△), 12 (●), 13 (*) and 15 (○).]

moiety of 2 seems to reduce the bone marrow toxicity of the nitrosourea cytotoxic group. Therefore it was decided to conduct a careful study of the decomposition products of 2 at physiological pH (buffered at pH 7.4) in the hope that the low carbamoylating activity, full antitumor activity, and reduced hematologic toxicity could be explained. The results of this study are reported here.

Results and Discussion

Clinical samples of chlorozotocin (2) were added to flasks containing water buffered by phosphate or triethylammonium bicarbonate at pH 7.4 and placed in an oil bath maintained at 37 ± 1 °C. Bubbles, presumably nitrogen gas, were formed immediately and after 3 h, 2 had completely dissolved. The heating was continued for a total of 24 h with occasional swirling. Charcoal column chromatography with 2% 2-propanol in water as the eluent was used to remove the phosphate salts of the samples decomposed in phosphate buffers. For the other buffer, three lyophilizations and finally a small column containing a mixed-bed ion-exchange resin (H^+, OH^-) removed the last traces of triethylamine without significant loss of the carbohydrates. ^{13}C NMR spectra in D_2O were run at each stage of the workup. A sample spectrum of the original mixture from a triethylammonium bicarbonate buffered decomposition is shown in Figure 1. Ultimately six sugar derivatives, 3, 7, 11, 12, 13, and 15 or possibly 16, were



13, R = H
14, R = Ac

15

16

identified. Table I gives the approximate distributions of

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Table I. Approximate Distribution[a] of Decomposition Products of Chlorozotocin (2)

mixture	buffer[b]	% 3	% 7	% 11	% 12	% 13	% 15/16
1	PO_4^{3-}	37	⩽10[c]	32	⩽12[c]	⩽5[c]	32
2[d]	PO_4^{3-}	35	5	17	6	9	27
3	PO_4^{3-}	40	12	20	8[e]	12	8
4	Et_3NH^+	30	14	30	10	⩽3[c]	15
5	Et_3NH^+	27	12	33	10	⩽3[c]	19

[a] Distribution is based on moles of aminosugar monomer or moles of reactant. Each mole of 11 or 12 contains 2 mol of glucosamine. Unless otherwise indicated the distribution of products was estimated by comparing the peak heights of the following carbons (mainly the anomeric C) of the ^{13}C NMR spectra of the mixtures: δ 106.4 (7), 103.5 (13), 100.8 (3), 92.7 (15/16), 79.0 (11), 77.9 (12). The peak height of δ 77.9 was doubled as this contained one-half of the two glucosamine moieties in 12. The percent of 11 and 12 may be different than the actual amounts due to a different relaxation time for these carbons. See Figure 1 for a typical ^{13}C NMR spectrum. [b] Buffered at pH 7.4. [c] Presumably in the noise of the spectrum and not included in the total distribution (to determine 100%). [d] The following ^{13}C NMR peaks were used: 58.4 (3), 70.8 (7), 58.7 (11), 77.9 (12), 71.2 (13), 61.1 (15/16). [e] ^{13}C NMR peak at δ 59.8 was used.

the products as determined by ^{13}C NMR. Summaries of the ^{13}C NMR data and ^1H NMR data for these six compounds and their acetate derivatives are given in Tables II and III, respectively.

Numerous attempts at separating the products by preparative TLC and column chromatography with a variety of solid phases and solvent systems gave, at best, three fractions that were still mixtures as shown mainly by ^{13}C NMR spectra. While reversed-phase high-pressure LC (C-18 column) also gave a different set of three fractions, separation into five fractions was obtained on a BioRad Aminex Carbohydrate HPX-87 column (7.8 × 300 mm), using water (0.6 mL/min) as the eluent: fraction A (t_R 10 min, 11 and 12), fraction B (12 min, 15 or 16), fraction C (16 min, 3), fraction D (18–20 min, 13), and fraction E (24 min, 7). These fractions were collected from the semipreparative column mentioned above, lyophilized, and stored in a freezer at −20 °C until used for the structural studies. The acetate derivatives of each were also prepared as well as the *O*-trimethylsilyl derivatives for GC–MS studies. The remainder of this paper is concerned with the structure proofs of these six compounds.

Fraction E (7). Fraction E was shown to be 2-(carboxyamino)-2-deoxy-α-D-glucofuranose intramolecular 2,1-ester (7) by conversion to the tetraacetyl derivative 8 [2-(acetylcarboxyamino)-2-deoxy-α-D-glucofuranose intramolecular 2,1-ester 3,5,6-triacetate], which was identified by comparison of its ^1H NMR spectrum and other physical data (IR, melting point, $[\alpha]_D$) with those previously reported by Upjohn.[30] All possible spectral data (IR, ^1H NMR, ^{13}C NMR, mass spectra) were obtained on 7 and 8 for comparison with the other compounds.

Fraction C (3). The IR spectrum of fraction C exhibited a carbamate carbonyl band at 1742 cm^{-1} and the tetraacetyl derivative contained IR bands at 1793 (carbonyl of the acetylated carbamate), 1749 (*O*-acetate carbonyls), and 1712 (*N*-acetate carbonyl) cm^{-1}. These are very similar to the corresponding IR bands of 7 (1743 cm^{-1}) and 8 (1788, 1744, and 1707 cm^{-1}) and indicated the presence of a five-membered carbamate ring. The chemical shift of the anomeric hydrogen (H_1) in the ^1H NMR spectrum of fraction C (δ 6.07 in D_2O) did not change much upon acetylation (δ 6.00 in $CDCl_3$) and the large coupling constant $^1J_{C_1H_1}$ of the anomeric carbon (192 Hz compared to

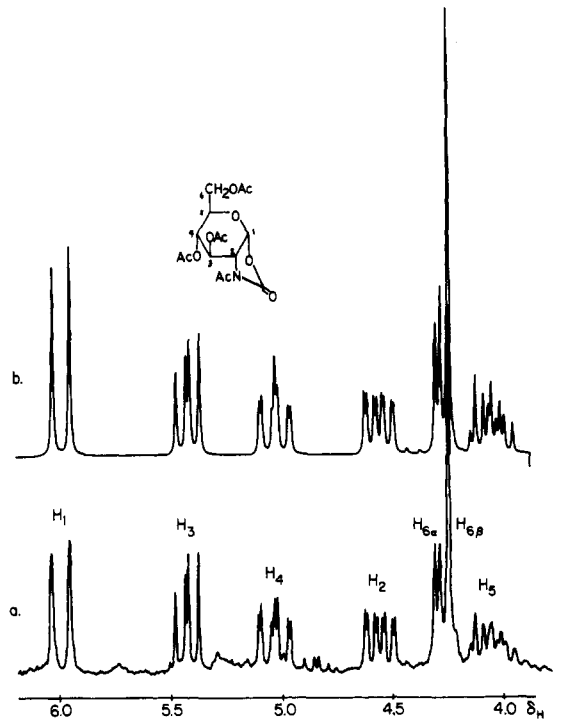

Figure 2. Experimental (a) and calculated (b) ^1H NMR spectra of 4 (excluding the acetate methyl region) in $CDCl_3$ at 90.0 MHz.

170 and 161–163 Hz in α- and β-2-(acetoxyamino)-2-deoxy-D-glucose[35,36] showed that the carbamate function (instead of OH) was attached to the anomeric carbon atom. A pyranose ring form was established because H_4 moved considerably downfield (δ 3.6–4.1 vs. δ 5.04; see Table III) upon acetylation, whereas H_5 did not change significantly, indicating that O_5 is part of the acetal (pyranose) ring and O_4 is free to be acetylated (opposite to the situation observed in the ^1H NMR of 7 and 8, which are furanose ring systems). The chemical-ionization (CI) mass spectrum, using NH_3 as the reagent gas, of fraction C was the same as that of 7; that is, it exhibited peaks at *m/e* 223 ($M + NH_4^+$), 206 ($M + H^+$), 162 ($M + H^+ - CO_2$), and 144 ($M + H^+ - CO_2 - H_2O$), consistent with a monosaccharide derivative having a formula of $C_7H_{11}NO_6$. The electron-impact (EI) mass spectral data of the tris(*O*-trimethylsilyl) derivatives of fraction C and 7 were also similar (M^+, *m/e* 421; M − 15, *m/e* 406; M − 90 (Me_3SiOH), *m/e* 331). Taking all the above data into account, fraction C was assigned to structure 3 [2-(carboxyamino)-2-deoxy-α-D-glucopyranose intramolecular 2,1-ester], whose tetraacetate is assigned to 4 [2-(acetylcarboxyamino)-2-deoxy-α-D-glucopyranose intramolecular 2,1-ester 3,4,6-triacetate].

Thus 3 and 4 have the ring system originally proposed for 8. 3 was independently synthesized by the reaction of 2-amino-2-deoxy-D-glucose with *p*-nitrophenyl chloroformate (PNPCF) by the method of Umezawa et al.[37] for the preparation of carbamate derivatives of aminosugars. In addition to 3 (40%), a 15% yield of a mixture of fractions A and B along with 45% unreacted starting glucosamine was obtained (as determined by ^{13}C NMR). It is interesting that 7 is not formed in this rapid derivatization reaction.

The remaining question of structure 3 for fraction C was the configuration (α or β) of the anomeric carbon (C_1). An

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Table II. ^{13}C NMR Chemical Shifts and $^{1}J_{CH}$ Couplings[a] of Chlorozotocin (2) Decomposition Products,[b] Their Acetates,[c] and Some Model Compounds[b]

compd (symbol)[d]	C_1	C_2	C_3	C_4	C_5	C_6	carbamate C=O[e]	*N*-acetate C=O	*N*-acetate CH_3	*O*-acetate C=O	*O*-acetate CH_3
α-D-glucosamine·HCl[b]	91.3	56.6	71.9	71.9	73.9	62.8					
β-D-glucosamine·HCl[b]	94.9	59.1	74.2	71.9	78.4	62.8					
α-D-mannosamine·HCl[b]	92.5	56.7	69;1	68.5	74.1	62.8					
β-D-mannosamine·HCl[b]	93.2	57.9	71.7	68.4	78.3	62.8					
N-acetyl-α-D-glucosamine[b]	93.3	56.5	73.2	72.6	74.0	63.2		176.8	24.4		
N-acetyl-β-D-glucosamine[b]	97.4	59.2	76.3	72.4	78.4	63.2		177.1	24.7		
3[b] (√)	100.8 (185)	58.4 (154)	76.0[f] (~151)	70.2[f] (~147)	76.5[f] (~137)	63.7 (145)	161.9 (0)				
7[b] (□)	106.4 (192)	66.5 (157)	76.1[f] (156)	82.8[f] (143)	70.8[f] (146)	65.9 (149)	162.3 (0)				
9 [C_6D_6][c,g]	79.7[h]	56.4	77.4[h]	69.6[i]	70.8[i]	61.7	154.7			170.6, 170.4, 170.1, 169.7	20.6, 20.3, 20.1 (2)
9 [acetone-d_6][c,g]	79.8[h]	56.2	77.5[h]	69.6[i]	71.1	62.4	155.0			171.3, 170.0, 170.7 (2)	20.8 (2), 20.5 (2)
10 [C_6D_6][c,g]	78.4[h]	54.6	72.9[h]	68.6	*j*	*k*	*j*			*j*	*j*
	75.2[h]	*j*	70.1[h]	*j*	*j*	*k*	*j*			*j*	*j*
10 [acetone-d_6][c,g]	78.7[h]	56.6	73.5[h]	68.9	70.1	*k*	*j*			*j*	*j*
	77.7[h]	55.2	71.4[h]	*j*	*j*	*k*	*j*			*j*	*j*
11[b,g] (△)	80.9[h] (157)	58.7 (149)	79.0[h] (159)	74.3[i] (140)	72.8[i] (145)	65.2 (143)	160.6 (0)				
12[b,g] (●)	81.4[h]	59.8 (151)	77.9[h] (155)	73.9[i]	73.2[i]	*k*	163.5 (0)				
	80.0[h]	58.4	76.3[h] (~163)	*j*	*j*	*k*	159.5 (0)				
13[b] (*)	103.5 (176)	65.2 (156)	82.9 (168)	81.2 (151)	71.2 (144)	65.7 (143)	163.2 (0)				
15/16[b,l] (○)	92.7 (163)	61.1 (147)	80.0[f]	73.0[f]	74.0[f]	65.2 (143)	162.1 (0)				

[a] Coupling constants in hertz were determined by gated decoupling and are placed in (#) after the corresponding δ. [b] Parts per million from internal CH_3CN in D_2O corrected to parts per million (δ) relative to $(CH_3)_3Si(CH_2)_3SO_3Na$ in D_2O via a separate spectrum. [c] Parts per million (δ) relative to internal tetramethylsilane in the solvent indicated in [#] after the compound number, which refers to structures given in the text. [d] Symbol used to indicate peak assignments in Figures 1 and 5. [e] δ values of model carbamate carbonyls, 2-oxazolidinone and tetrahydro-2*H*-1,3-oxazin-2-one, were observed at 161.2 and 155.0 ppm in $CDCl_3$ and 165.1 and 159.2 ppm in D_2O, respectively. [f] Assignments of C_3, C_4, and C_5 are tentative. [g] The spectra of the cis and trans dimers were obtained in the same solutions. [h] Assignment of C_1 and C_3 may be reversed. [i] Assignment of C_4 and C_5 may be reversed. [j] The unobserved carbons are thought to be hidden under the corresponding atoms of the cis dimer **9** or **11**. [k] Both C_6 and C_6' are thought to be under C_6 of the cis dimer **9** or **11**. [l] The spectrum of **15/16** was obtained in the same solutions with varying amounts of **11** and **12** as these compounds are in slow equilibrium.

Table III. ^{1}H NMR Chemical Shifts of Chlorozotocin (2) Decomposition Products[a] and Their Acetate Derivatives[b]

compd [solvent][a,b]	CH protons H_1	H_2	H_3	H_4	H_5	$H_{6\alpha}$	$H_{6\beta}$	OH protons H_1	H_3	H_4	H_5	H_6	NH	*N*-Ac CH_3	*O*-Ac CH_3
3 [D_2O][a]	6.07	3.6					4.1								
[Me_2SO-d_6][b]	5.82	3.1					3.7		5.28[c]	5.19[c]		4.70	8.00		
4 [$CDCl_3$][b,d]	6.00	4.57	5.43	5.04	4.06	4.30	4.25							2.52	2.11, 2.10, 2.04
[C_6D_6][b]	5.05	3.92	5.45	5.11	3.66	4.21	4.08							2.30	1.68, 1.67, 1.49
5 [$CDCl_3$][b]	5.96	3.88	4.90	5.05	4.08	4.33	4.20						5.99		2.11, 2.09, 2.08
7 [D_2O][a]	6.27	4.38	3.6				4.3								
[Me_2SO-d_6][b]	6.06	3.1					4.1		5.32[e]		4.72[e]	4.51	8.01		

8 $[CDCl_3]^{b,d}$	6.16	4.66	5.66	4.32	5.21	4.57	4.09				2.57	2.09 (2), 2.00	
$[C_6D_6]^b$	5.29	~3.95	5.66	~4.03	5.35	4.67	~3.95				2.16	1.77, 1.70, 1.63	
$[Me_2SO\text{-}d_6]^b$	6.29	4.62	5.58	~4.5	5.09	4.45	4.02				2.40	2.01, 2.00, 1.93	
9 $[CDCl_3]^{b,f}$	6.11	3.58	5.16	5.30	5.30	4.30	4.30					2.15, 2.14, 2.12, 2.06	
10 $[CDCl_3]^{b,f}$	6.54, 6.33	———		———	*g*	———	———					*g*	
11 $[D_2O]^{a,h}$	5.31	4.07	5.01	3.6	———	———	4.0						
$[Me_2SO\text{-}d_6]^{b,h}$	5.3	———		*g*	———	———	3.1	6.78	———	*g*	———		
12 $[D_2O]^{a,h}$	5.42, 5.32	*g*	4.84–5.09[i]	———	———	*g*	———						
$[Me_2SO\text{-}d_6]^{b,h}$	5.3	———		*g*	———	———	3.1	6.78	———	*g*	———		
13 $[D_2O]^a$	5.36	4.35	5.36	4.25	4.0	———	3.6		4.89	4.43	7.92		
$[Me_2SO\text{-}d_6]^b$	5.05	3.96	5.05	3.7	———	———	3.1	6.55				2.55	2.12, 2.09, 2.06
14 $[CDCl_3]^{b,d}$	6.27	4.81	5.11	4.42	5.26	4.59	4.16					2.12	1.70, 1.66, 1.51
$[C_6D_6]^b$	6.38	4.15	4.15	4.15	5.38	4.69	4.06						
15/16 $[D_2O]^{a,h}$	4.84–5.09[i]	*g*	4.84–5.09[i]	———	———	*g*	———						
$[Me_2SO\text{-}d_6]^{b,h}$	5.3	———		*g*	———	———	3.1	6.45–6.7, 6.00–6.1	*g*	*g*	7.4–7.55		

[a] Parts per million from internal CH_3CN corrected to parts per million (δ) relative to $(CH_3)_3Si(CH_2)_3SO_3Na$ in D_2O via a separate spectrum. [b] Parts per million (δ) from internal tetramethylsilane in the solvent indicated. [c] Assignment of O-H_3 and O-H_4 may be reversed. [d] Assignments were made via a spin simulation of the CH protons and/or selective homonuclear decoupling experiments. [e] Assignment of O-H_3 and O-H_5 may be reversed. [f] The spectra of **9** and **10** were obtained from the same solution of the fraction A acetylation mixture; see Figure 4 for the low-field portion of the spectrum. [g] Not assignable—mostly buried under the corresponding peaks of the cis dimer. [h] The spectra of **11**, **12**, and **15/16** were obtained from the same solutions of fractions A or B. [i] H_3 and H_3' of **12** overlap H_1 and H_3 of **15/16**.

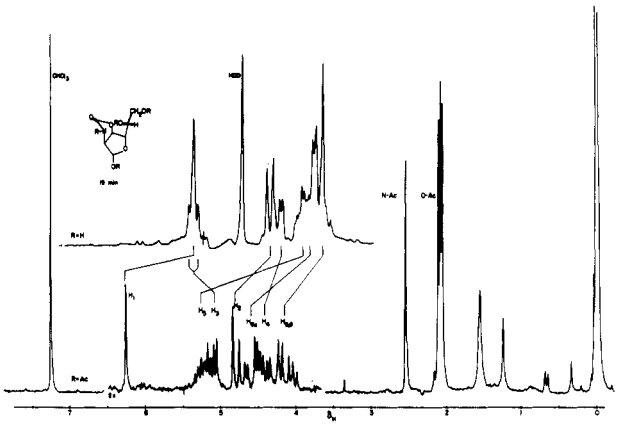

Figure 3. ^1H NMR spectra of fraction D at 90.0 MHz: (upper) **13** in D_2O; (lower) **14** in $CDCl_3$.

examination of molecular models, in agreement with the literature,[38] revealed that the α configuration having a cis fusion of a cyclic carbamate with both five- and six-membered sugar rings is clearly more stable than a trans fusion (β anomer). One point of experimental evidence in strong agreement with the α configuration was the observation of a long-range coupling ($^4J_{H_2H_4}$ = 1.06 Hz, determined experimentally and by agreement with a spin simulation of the line frequencies in the spectrum of **4**) between H_2 and H_4. Figure 2 shows the ^1H NMR experimental and calculated spectra of **4** and Table IV gives the observed proton–proton coupling constants of all the compounds (except **15/16**) discussed in this paper. The models indicated that the β-anomer cannot exist in a conformation where the H_2–C_2–C_3–C_4–H_4 frame is essentially coplanar, the so-called W configuration necessary for a four-bond coupling of >0.8 Hz in saturated systems.[39-42] The α-anomer, however, has more than one conformation placing the five atoms in the planar W configuration. (A more detailed discussion of the coupling constants and conformations of **4**, **8**, and **14** along with various Karplus relationships will be presented in another paper.) A final point of evidence for the α-anomers for **3** and **4** was the preparation of the known tri-*O*-acetyl derivative **5**,[43,44] using conditions (pyridine/acetic anhydride at 0 °C for 2.5 h) that did not acetylate the carbamate nitrogen. The melting point (171.0–172.5 °C) agreed well with the literature values (170 °C[43] and 174–5 °C[44]).

Fraction D (13). Examination of the IR data of fraction D (carbamate carbonyl at 1740 cm^{-1}) and its tetraacetyl derivative (acetylated carbamate carbonyl at 1790 cm^{-1}) indicated the presence of another five-membered-ring carbamate. The CI mass spectral data was the same as that found for **3** and **7**, consistent with a formula of $C_7H_{11}NO_6$, showing that it was a monosaccharide also. The ^1H NMR chemical shift behavior of H_4 (δ 4.25 → 4.42) and H_5 (δ 3.6–4.0 → 5.26) of fraction D compared to that of its tetraacetyl derivative showed that the sugar ring was in the furanose form (see Figure 3 and Table III). Addition, H_1 of fraction D was at the normal chemical shift (δ 5.36

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(42) Hall, L. D.; Manville, J. F.; Tracey, A. *Carbohydr. Res.* **1967**, *4*, 514.
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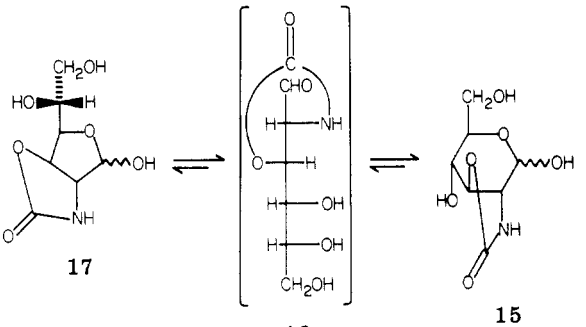
(44) Konstas, S.; Photaki, I.; Zervas, L. *Chem. Ber.* **1959**, *92*, 1288.

Table IV. Proton Coupling Constants[a] of Chlorozotocin (2) Decomposition Products and Their Acetate Derivatives

compd (solvent)	$^3J_{12}$	$^3J_{23}$	$^4J_{24}$	$^3J_{34}$	$^3J_{45}$	$^3J_{56\alpha}$	$^3J_{56\beta}$	$^2J_{6\alpha 6\beta}$
3 (D_2O)	6.45	-	-	-	-	-	-	-
4 ($CDCl_3$)[b]	7.03	3.96	1.06	5.28	6.75	5.78	2.88	-12.0
7 (D_2O)	5.57	0.0	-	-	-	-	-	-
8 ($CDCl_3$)[c]	5.86	0.0	-	3.05	9.39	2.44	4.98	-12.25
9 ($CDCl_3$)[d]	6.16	3.52	-	-	-	-	-	-
10 ($CDCl_3$)[d]	8.22, 1.76	-	-	-	-	-	-	-
11 (D_2O)	8.2	3.5	-	~0	-	-	-	-
13 (D_2O)	0.0	7.32	-	3.66	~8.5	-	-	-
14 ($CDCl_3$)[e]	0.0	7.36	-	3.51	8.30	3.17	4.75	-12.46
21 (D_2O)[f]	0.0	6.5	-	3.2	9.0	-	-	-

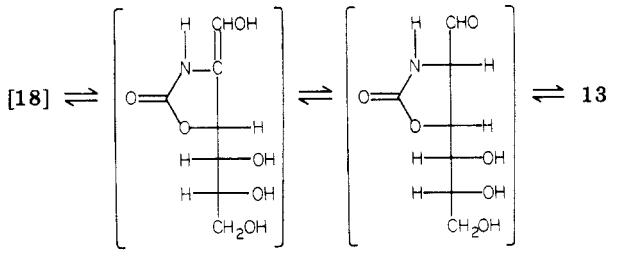
[a] Coupling constants are reported in hertz and were determined by direct observation and, when possible, by spin simulation and an iterative fit of the experimental line frequencies (- means the couplings were not observable). [b] Treated as a seven-spin case (see Figure 2). [c] Treated as a partial five-spin case on all line frequencies and couplings involving H_3 through $H_{6\beta}$ because $^3J_{23}$ was not resolvable (<0.25 Hz). [d] Obtained directly from the same spectrum (see Figure 3) of a mixture of 9 and 10. [e] Treated as a six-spin case since $^3J_{12}$ was not resolvable (<0.25 Hz). [f] D-Mannofuranose 2,3-carbonate couplings reported: Perlin, A.S. *Can. J. Chem.* **1964**, *42*, 1365.

in D_2O) of free sugars[45,46] but moved considerably downfield upon acetylation (δ 6.27 in $CDCl_3$). On the other hand, H_3 of fraction D was quite downfield (δ 5.36 in D_2O) compared to underivatized hexoses and did not move significantly upon acetylation (δ 5.11 in $CDCl_3$), strongly indicating that fraction D contained a 2,3-carbamate. The possibility that it was the glucofuranose derivative **17** was ruled out because this is a highly strained trans fusion of two five-membered rings and would certainly convert to the more stable glucopyranose **15** via the open-chain aldehyde **18** as shown below. This problem led us to the



17 18 15

thought that fraction D was possibly a 2-amino-2-deoxy-D-mannose derivative **13** that could be formed by epimerization via enolization of the open-chain aldehyde **18** through **19** and **20** as shown below.



19 20

Indeed, when 2-amino-2-deoxy-D-mannose was derivatized with PNPCF as described above, the major product (ca. 50%) was identical via ^{13}C NMR with fraction D (no glucosamine product was observed). Consequently, fraction D was assigned to structure **13** [2-(carboxyamino)-2-deoxy-α-D-mannofuranose intramolecular 2,3-ester]. This

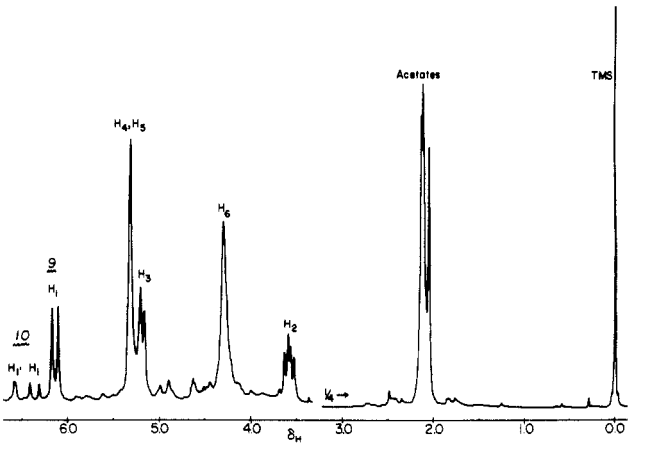
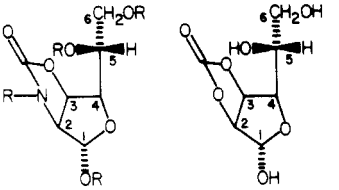


Figure 4. ^1H NMR spectrum of the acetylated fraction A mixture (**9** and **10**) in $CDCl_3$ at 90.0 MHz.

structure has the very stable cis fusion[47-49] of two five-membered rings and may be the driving force for the epimerization. By analogy, the tetraacetyl derivative is **14** [2-(acetylcarboxyamino)-2-deoxy-α-D-mannofuranose intramolecular 2,3-ester 1,5,6-tri-*O*-acetate]. Further evidence for these assignments was provided by the very similar proton couplings, $^3J_{12}$, $^3J_{23}$, $^3J_{34}$, and $^3J_{45}$, of **13** and **14** (see Table IV) with those of α-D-mannofuranose 2,3-carbonate (**21**).[47] In both **13** and **21**, $^3J_{12}$ is smaller than



13, R = H
14, R = Ac

21

the resolution of the instruments used (<0.2 Hz) and is consistant with the α-anomer in which a dihedral angle of near 90° is expected while the corresponding angle of the β-anomer would be 0–30° and should be easily observable. Probably both α- and β-anomers of **13** are present under some conditions because the high-pressure LC peak

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(48) Angyal, S. J. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 157.
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sometimes separated into two small peaks (R_f 18.5 and 19.8 min), a behavior typical of α- and β-anomers on the HPX-87 column used.[50]

Fraction A (11, 12). A comparison of the two remaining high-pressure LC fractions (A and B) showed that peak A was significantly broader than peak B. Since A has the smaller retention time, fraction A was thought to contain at least two components with nearly identical retention times. Indeed, the ^{1}H NMR spectrum (see Figure 4) of the acetylation products of fraction A revealed three sets of anomeric hydrogen doublets at δ 6.54 ($^3J_{12}$ = 1.76 Hz), 6.33 ($^3J_{12}$ = 8.22), and 6.11 ($^3J_{12}$ = 6.16) in a ratio of about 0.5:0.5:3.0, respectively. The doublet at δ 6.11 plus the remainder of the ^{1}H NMR spectrum of the acetylation mixture was very similar to the published spectrum of the cis dimeric product **9** from the decomposition of **1**.[30] All attempts to separate the original fraction A mixture or the acetylated mixture by a wide variety of chromatographic methods (column, TLC, and high-pressure LC) were completely unsuccessful. (Further comments regarding this problem will be made later.) GC–MS of the trimethylsilylation mixture of fraction A, however, did separate two compounds. The two peaks eluted with much longer retention times than the corresponding derivatives of **3**, **7**, and **13**. EI mass spectral data showed that both compounds were dimers with high mass peaks at *m/e* 986 corresponding to the octakis(*O*-trimethylsilyl) derivatives of **11** and **12**, to which structures the two components of fraction A were assigned. It should be noted that CI mass spectroscopy did not give the peaks at *m/e* 411 (M + H$^+$) or 428 (M + NH$_4^+$) expected for the dimers **11** and **12**. In fact, only very small peaks at *m/e* 206 and 223 (M + H$^+$ and M + NH$_4^+$ of **3**, **7**, and **13**) were found. Since **3**, **7** and **13** gave strong peaks at these masses, the CI mass spectral data did suggest that fraction A was not similar. The relatively large molecular weight and eight highly polar hydroxyls probably make **11** and **12** so nonvolatile that the heat required to vaporize them causes extensive decomposition.

A comparison of the ^{1}H NMR chemical shifts of **11** vs. **9** again provides proof that the carbamate ring is connected to C_3 since acetylation causes very little change on H_3 and $H_{3'}$ (δ 5.01 → 5.16) and that the anomeric hydroxyls are acetylated because H_1 (and $H_{1'}$) changes from δ 5.31 to 6.11 (see Table III). In addition, the nitrogens are not acetylated because no CH_3CO peak appeared at δ 2.40–2.52 in the ^{1}H NMR spectrum of **9**, and **11** did not have an NH proton in the dimethyl-d_6 sulfoxide (Me_2SO-d_6) spectrum. This is also confirmed in the ^{13}C NMR spectrum (see Table II). Since four acetyl groups (×2) are observed and the NH and O_3 are not acetylated, it follows that C_1, C_4, C_5, and C_6 hydroxyls and their equivalent prime positions of the dimer must be present and are acetylated. At this point, the structure of **9** was published[30] and we concurred with Upjohn's structure. We, however, observed a second dimer, which was assigned to the trans dimer **10** and **12** structures because both ^{1}H and ^{13}C NMR data showed two different anomeric hydrogens (δ 6.54, 6.33) and carbons (δ 81.4, 80.0), respectively. In addition, it was possible to identify a total of 10 of the expected 14 carbons present in **12** (see Table II). The Upjohn[30] group had assigned **9** to the cis dimer they isolated because only seven carbons (neglecting the acetate groups) were observed in the ^{13}C NMR spectrum and, in addition, proposed that the center ring was present in a boat conformation so that the molecule had a center of symmetry. In the case of **10** (or **12**), a model of the trans dimer in a boat conformation agrees nicely with the ^{1}H NMR spectrum (Figure 4), in which the two anomeric hydrogens are observed with different $^3J_{12}$ values of 8.22 and 1.76 Hz (see Table IV) that correspond well with one large dihedral angle of ca. 180° and a small dihedral angle of ca. 60° between H_1 and H_2, and $H_{1'}$ and $H_{2'}$, respectively. The four carbons that are not observed in unique positions are $C_{4'}$, $C_{5'}$, C_6, and $C_{6'}$, all of which are on the propyl side chains. Each of these is probably hidden under the corresponding peaks of the cis dimer; indeed, three of them might be expected to have nearly identical δ values.

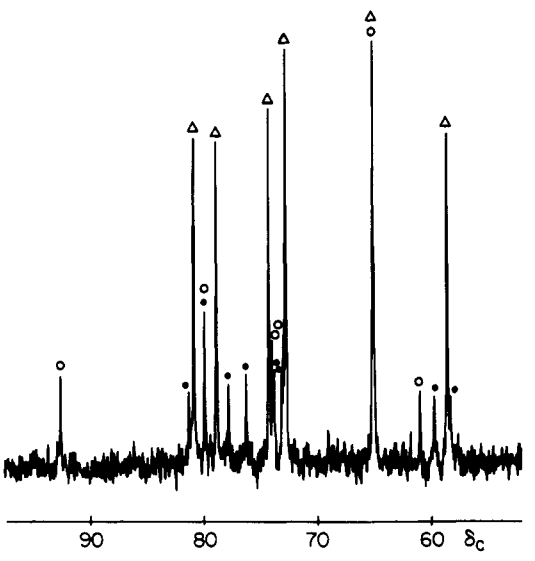


Figure 5. ^{13}C NMR spectrum in D_2O of fraction A a few hours after collection from the high-pressure LC. [Note that fraction B (**15/16**) (○) has already begun to form. Fraction A originally contained only the peaks of **11** (△) and **12** (●)].

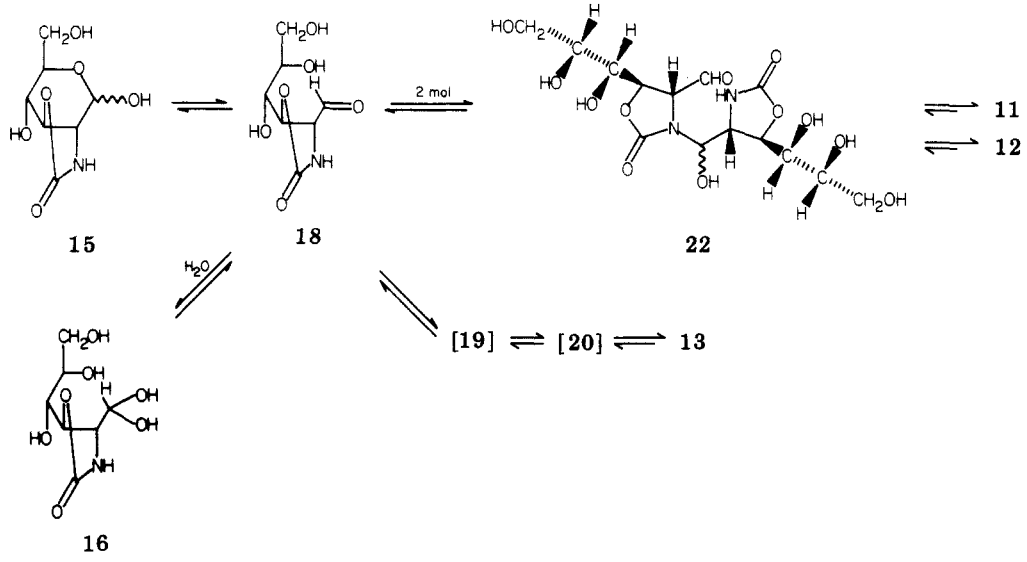
That the dimers might be mannosamine derivatives like **13** is considered only remotely possible because they were not observed in the mannosamine–PNPCF reaction described above, but **11** and presumably **12** (in the noise of the ^{13}C NMR spectrum) were observed in the glucosamine–PNPCF reaction in low yield.

Fraction B (15/16). The situation of fraction A is somewhat more complicated than has been presented thus far. Whenever fraction A, collected from high-pressure LC, was allowed to stand in aqueous solution, or when the freeze-dried fraction was redissolved in water, reinjection in the high-pressure LC always gave varying amounts of both fractions A and B, the latter of which increased with time. Fraction B also changes to the same mixture in aqueous solutions. While the rate of conversion (4–48 h) was not reproducible, the high-pressure LC peaks A and B always approached nearly equal intensities, which we assume must be the equilibrium mixture. This means that all spectra of fraction A always contained B (and vice versa), the amount of which depended on how long after sample collection the spectra were run. Figure 5 shows the ^{13}C NMR spectrum of a sample of fraction A taken a few hours after collection from the high-pressure LC. The peaks for **11**, **12**, and fraction B (**15/16**) are labeled with the symbols shown in Table II (△, ●, and ○, respectively).

All attempts to make a derivative of fraction B resulted in only derivatives of the two dimers, **11** and **12**, and not of the third component of the mixtures. Therefore, the structure determination of fraction B was very elusive until the reversible equilibrium process was realized. The facile conversion of the components in fractions A and B, however, limits the possible structures for B to the same cyclic 2,3-carbamate present in **11** and **12**. Thus, the reasonable

(50) Cummings, Larry; BioRad Laboratories, private communication, 1979.

Scheme I



15 18 22 16

structures for B appeared to us to be the cyclic glucopyranose hemiacetal **15**, the open-chain aldehyde **18** or its hydrate **16**, and the half-aldehyde-half-hemiacetal dimer **22**, all of which can be in equilibrium, along with the epimerized product **13** (TLC data will be shown later supporting the latter conversion also) as shown in Scheme I. The equilibria shown in Scheme I explain the formation of all of the cyclic 2,3-carbamates found.

The problem now is to decide which of these species predominates in fraction B. The presence of high concentrations of **18** and **22** could be ruled out in aqueous solution because the ^1H and ^{13}C NMR spectra (D_2O) did not exhibit an aldehydic proton (δ 9–10) or an aldehydic carbonyl (ca. δ 200), respectively. The other two possibilities are more difficult to distinguish because somewhat conflicting data were obtained. Since it was not possible to obtain a derivative and since the ^1H NMR spectra of fraction B were always confused by the presence of the dimers **11** and **12**, it was not possible to observe the coupling constants of the protons or obtain good δ_H data. The anomeric hydrogen of **15** and the hydrated aldehyde hydrogen of **16** would both be expected in the δ 5–5.5 region[51–53] and are therefore not very useful. ^{13}C NMR is somewhat more useful since the chemical shift of the hydrated carbon of **16** should be 5–6 ppm upfield from the anomeric carbon of **15** based on the results of Bock and Pederson[54] on α-streptomycin vs. β-streptomycin. The low-field sugar carbon of fraction B was found at δ 92.7, which is between the positions of the anomeric carbons of the α- and β-anomers of D-glucosamine and D-mannosamine (δ 91.3–94.9; see Table II). Thus, the ^{13}C NMR data favor the pyranose **15** over the hydrate **16**.

In a final attempt to obtain definitive information, CI mass spectral data were obtained on several lyophilized mixtures of fractions A and B. As mentioned earlier, CI mass spectroscopy of a sample of A, which was lyophilized quickly after collection and contained very little B, gave only small peaks at m/e 206 and 223 (and no peaks at m/e 411 and 428 expected for the dimers). CI mass spectroscopy of two samples of fraction B, one of which contained

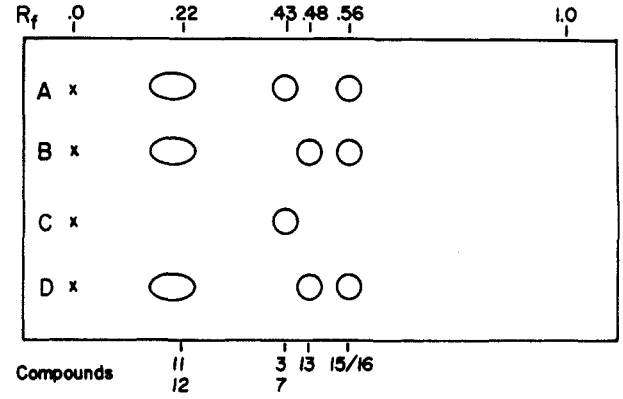


Figure 6. Analytical TLC of preparative TLC fractions (using 9:1 1-butanol–boric acid (0.03 M), where X = origin, A = original decomposition mixture, B = preparative TLC band at R_f 0.63, C = preparative TLC band at R_f 0.50, and D = preparative TLC band at R_f 0.22).

a significant amount of A by ^{13}C NMR, gave a small peak at m/e 206 but exhibited a large peak at m/e 223 (**3**, **7**, and **13** gave strong peaks at both masses). The relative absence of the m/e 206 peak suggests some fundamental but as yet unidentified difference between the structure of B from **3**, **7**, and **13**. This seems to favor **16**, if dehydration occurs in the mass spectrometer probe, or even possibly the aldehyde **18**, which could easily hydrate in the D_2O used as the NMR solvent, thus explaining the ^1H NMR results (no aldehyde H), although an ^1H NMR spectrum in Me_2SO-d_6 also did not show an aldehydic proton. The dehydrated aldehyde might capture NH_4^+ preferentially to H^+ in CI mass spectroscopy using NH_3 and give a large m/e 223 ion relative to m/e 206.

Thus, the data on fraction B prohibit assigning one definitive structure. Structure **15** is favored but **16** is still a possibility until more information or some type of a derivative that does not disturb the equilibrium appreciably can be obtained. Whatever the exact structure of fraction B, it appears to be the key intermediate to the other three cis-fused 2,3-carbamate products. It is in equilibrium with the two dimers **11** and **12** and is the precursor for the epimerized product **13**. In our earlier work before high-pressure LC became available to us, we had observed that the amount of **13** increased sharply using TLC on silica gel plates. For example, preparative TLC of the original mixture gave three separable bands

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at R_f 0.22 (elongated), 0.50, and 0.63, using 9:1 1-butanol–boric acid (0.03 M) as the eluent. Removal and extraction of these bands from several plates gave samples on which ^{13}C NMR spectra could be obtained. The R_f 0.22 band contained 11 and **12**, the R_f 0.50 band contained **3** and **7**, and the R_f 0.63 band corresponded to Fraction B with **13** also present (note that no **13** was observed in the original sample on the TLC plate or the amount present was not discernable). Respotting the three TLC fractions and the original mixture on an analytical silica gel plate gave the results shown in Figure 6. The original mixture showed the expected three spots at R_f 0.22, 0.43, and 0.56; however, the preparative TLC 0.22 and 0.63 fractions were identical and showed, in addition to the R_f 0.22 and 0.56 spots, a new spot at R_f 0.48. Only the preparative TLC 0.50 fraction remained unchanged, showing a single spot at R_f 0.43. Mildly stated, we were very bewildered by these results, but now they are easily understood. The interconverting components are the cyclic 2,3-carbamates. The equilibrium between **15/16** (R_f 0.56) and the dimers **11** and **12** (R_f 0.22) explains their similar TLC behavior, and the new R_f 0.48 compound, which was subsequently shown to be **13**, is produced from them at an enhanced rate on the silica gel plates. Apparently the epimerization of B to **13** is catalyzed by silica gel. Small amounts of **13** are present in the original mixture as the quantities given in Table I were obtained on samples that had not been in contact with any separating medium (which is why ^{13}C NMR data were used even with its own set of quantitative difficulties).

Conclusion. We have shown that the decomposition of chlorozotocin (**2**) at physiological pH gives compounds **3**, **7**, **11**, **12**, **13**, and **15/16**, all of which are intramolecular five-membered-ring carbamate derivatives of 2-amino-2-deoxy-D-glucose or 2-amino-2-deoxy-D-mannose. The intramolecular carbamoylation provides an explanation for the low in vitro carbamoylating activity of chlorozotocin since the intramolecular attack on the carbonyl moiety of the nitrosourea by the glucose hydroxyls at C_1 and C_3 is entropy favored over intermolecular carbamoylation by the lysine amino groups. The results also suggest that the antitumor activity and toxicity found in in vivo studies of **2** must be due to the *N*-(β-chloroethyl)-*N*-nitroso (or alkylating) portion of the molecule rather than the sugar carrier portion.

Experimental Section

Melting points were measured on a Gallenkamp melting-point apparatus and are corrected. Optical rotations were determined with an O. C. Rudolph and Sons No. 479 polarimeter at the Na_D line. Infrared spectra were obtained with neat films (from evaporation of methanol or chloroform solutions on a KBr plate) on a Perkin-Elmer Model 457 grating infrared spectrometer. Chemical-ionization mass spectra were kindly determined by Mr. Noel Whittaker of NIH on a Finnigan Model 1015D mass spectrometer, using ammonia (1 torr) as the reagent gas at a source temperature of 120–150 °C. Electron-impact mass spectra (direct probe) and GC–MS data were kindly determined by Mr. William Comstock of NIH on a LKB-9000 (at 70 eV).

NMR spectra were taken on a Bruker WH/HFX-90 FT NMR spectrometer equipped with QPD and a 20K BNC-12 Nicolet computer using a deuterium internal lock. Tetramethylsilane was used as an internal standard in nonaqueous solvents while acetonitrile was used as an internal standard in D_2O and corrected to Tier's salt [$(CH_3)_3Si(CH_2)_3SO_3Na$] in D_2O (δ_{CH_3CN} = 2.054 ppm at 90.02 MHz for ^1H spectra and δ_{CH_3CN} = 3.28 ppm at 22.628 MHz for ^{13}C spectra). The estimated errors in reported chemical shifts are less than 0.01 and 0.1 ppm for ^1H and ^{13}C NMR, respectively. Spin simulations and iterative fitting calculations were done by using the NMRCAL and ITRCAL programs developed by the Nicolet Instrument Corporation, Madison, WI, on the Bruker BNC-12 computer system.

Thin-layer chromatography was performed on Analtech silica gel GF plates, 250 μm thick for analytical TLC and 1000 μm thick, 20 × 20 cm, for preparative TLC. Compounds were detected on analytical plates by spraying with 10% sulfuric acid in methanol and heating about 5 min at 110 °C. On preparative plates the bands were detected on each edge by spraying with $AgNO_3$ solution followed by alcoholic KOH.

High-pressure liquid chromatography was performed with a Waters Model 6000 A solvent delivery system having an R-401 differential refractometer detection unit. High-pressure LC grade water (Alltech) and high-pressure LC grade acetonitrile (Fisher Scientific Co.) were filtered and degassed prior to use. All samples were filtered through 0.45-μm Millipore filters prior to injection.

Materials. Deuterated solvents were obtained from Aldrich (C_6D_6), Merck & Co. (D_2O, Me_2SO-d_6), and Norell Chemical & Co., Inc. ($CDCl_3$). All other solvents for reactions and chromatography were Baker Analyzed reagent grade. All organic reagents were obtained from Aldrich except the following: acetic anhydride (J. T. Baker), D-mannosamine hydrochloride (ICN Pharmaceuticals, Inc.), hexamethyldisilazane and silylation-grade pyridine (Pierce Chemicals). Inorganic reagents including Dowex IX-2 resin were obtained from J. T. Baker. Darco G-60 charcoal was obtained from Ruger Chemical Co., Inc. Celite 545 and Rexyn 300 (H^+, OH^-) resin were obtained from Fisher Scientific Co.

General Acetylation Procedure of Sugar Derivatives. Peracetate derivatives were prepared by stirring the carbohydrate (5–10 mg) with pyridine (0.30 mL, stored over molecular sieves, 5 Å) and acetic anhydride (0.30 mL, stored over molecular sieves, 5 Å) for 16–24 h at room temperature. The solution was then diluted with benzene, frozen, and concentrated under high vacuum (<1 mmHg). The residual sirup was dissolved in benzene, frozen, and concentrated under high vacuum. This was repeated twice more to yield white powders.

Trimethylsilylation Procedure of Sugar Derivatives. Trimethylsilyl derivatives were prepared by stirring the carbohydrate (5–10 mg) with pyridine (0.50 mL, Pierce silylation grade) for about 1 min at room temperature to ensure solution and then consecutively adding hexamethyldisilazane (0.30 mL) and trimethylchlorosilane (0.10 mL). Stirring was continued for 2 h at which time the solution was diluted with cyclohexane, frozen, and concentrated under high vacuum. The residue was mixed with cyclohexane and filtered through 0.45-μm Millipore filters. The filtrate was frozen and concentrated under vacuum, and the residue was dissolved again for a third concentration. The final residues were either white powders (from **11** and **12**) or colorless oils (from **3**, **7**, and **13**). The derivatives were then dissolved in either cyclohexane or hexane for GC–MS analysis using a 1% OV-17 column (6-ft length) with temperature programming from 165 to 300 °C at 12 °C/min.

Synthesis of 2-Oxazolidinone. 2-Oxazolidinone (or ethylene carbamate) was prepared according to the procedure of Homeyer[55] by the reaction of 2-aminoethanol with diethyl carbonate: mp 85–86 °C (lit.[55] 87–89 °C); ^1H NMR ($CDCl_3$) δ 6.68 (NH, br s, 1 H), 4.47 (H_5, t, ^3J = 8, 2 H), 3.63 (H_4, t, ^3J = 8, 2 H); ^{13}C NMR ($CDCl_3$) δ 161.2 (C_2), 65.1 (C_5), 40.8 (C_4); ^{13}C NMR (D_2O) δ 165.1 (C_2), 68.8 (C_5), 43.1 (C_4); IR (film) 3000, 2920 (CH), 1766 (carbamate C=O), 1690 (amide C=O), 1465, 1382, and 1357 (CH_2), 1298 (C–N), 1212, 1140, and 1030 (COC, C–O), 953 cm^{-1}; IR ($CHCl_3$) 3027, 2925 (CH), 1785 (carbamate C=O), 1702 (amide C=O), 1481, 1382 and 1362 (CH_2), 1312 (C–N), 1230, 1140, 1057, and 1039 (COC, C–O), 988, 955 cm^{-1}.

Synthesis of Tetrahydro-2*H*-1,3-oxazin-2-one. Tetrahydro-2*H*-1,3-oxazin-2-one (or propylene carbamate) was prepared by the reaction between 3-amino-1-propanol and ethylene carbonate according to the procedure of Najer et al.:[56] mp 78.9–79.9 °C (lit.[60] mp 80 °C); ^1H NMR ($CDCl_3$) δ 7.12 (NH, br s, 1 H), 4.30 (H_6, t, ^3J = 5.4, 2 H), 3.35 (H_4, d of t, ^3J = 5.8, 2, 2 H), 1.95 (H_5, pentet, ^3J = 6, 2 H); ^{13}C NMR ($CDCl_3$) δ 155.0 (C_2), 67.0 (C_6), 39.6 (C_4), 21.2 (C_5); ^{13}C NMR (D_2O) δ 159.2 (C_2), 70.4 (C_6), 41.5 (C_4), 22.8 (C_5); IR (film) 3255 (NH), 2910 (CH), 1683 (C=O), 1474 and 1420 (CH_2), 1290 (C—N), 1220, 1124, 1113, and 1072 (COC,

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C—O), 943, 906, 808, 762, 740, 679 cm^{-1}.

Decomposition of Chlorozotocin (2) in Phosphate Buffer. Chlorozotocin (ca. 2.0 g, 6.4 mmol, ≥98% pure by UV, TLC, [α], and elemental analysis) was added to 0.100 M phosphate buffer (4.312 g of Na_2HPO_4 and 1.198 g of KH_2PO_4 per liter, pH 7.40, 400 mL) in a flask immersed in an oil bath maintained at 37 ± 1 °C. Bubbles (presumably N_2) formed immediately. The flask was occasionally swirled and the stopper removed periodically to allow the gas(es) to be released. Within about 3 h, all **2** had dissolved. Heating was continued for 24 h and the solution lyophilized. Charcoal column chromatography (according to the method of Whistler[57,58]) with 2% 2-propanol in water as the eluent was used to remove the phosphate salts from the carbohydrates. The carbohydrates could also be separated from the phosphate salts by several extractions with warm pyridine (50 °C).

Decomposition of 2 in Triethylammonium Bicarbonate Buffer. **2** (ca. 2.5 g, 8.0 mmol) was added to 0.1 M triethylammonium bicarbonate buffer[59] (500 mL, pH 7.4) in a flask in an oil bath at 37 ± 1 °C. Bubbles formed immediately and swirling and periodic removal of the stopper were done as in the case of the phosphate buffer. After 24 h at 37 °C, the solution was lyophilized at least three times but some triethylamine (or one of its salts) still remained (as shown by ^1H and ^{13}C NMR). The triethylamine component was removed by passing the mixture through a small mixed-bed ion-exchange resin (Rexyn 300, H^+–OH^- forms). See Table I for the distribution of products in phosphate or triethylammonium bicarbonate buffer.

Purification of Decomposition Products of Chlorozotocin (2) by High-Pressure Liquid Chromatography. Injection of the mixture of products from the decomposition of **2** onto a semipreparative Aminex carbohydrate HPX-87 column (7.8 × 300 mm, BioRad Laboratories) with water (0.6 mL/min) as the eluent resulted in the following five fractions: fraction A (t_R 10 min, **11** and **12**), B (12 min, **15** or **16**), C (16 min, **3**), D (18–20 min, **13**), E (24 min, **7**). The peak at 18–20 min was usually broad with a shoulder but occasionally separated into two peaks. By use of numerous 8–12-mg injections of the decomposition mixtures, fractions A–E were collected and lyophilized to give white solids (usually gummy). Reinjection of fractions C–E (**3**, **13**, **7**) showed these components were >95% pure. Fractions A and B were found to convert with time (4–48 h) to a mixture with peaks A and B of approximately equal areas.

Spectral and Physical Data of High-Pressure LC Fractions and Their Acetate Derivatives. Fraction E: 2-(Carboxyamino)-2-deoxy-α-D-glucofuranose Intramolecular 2,1-Ester (7). Lyophilization of fraction E yielded **7** as an amorphous white solid: mp 164–170 °C dec (lit.[30] mp 165–168 °C dec); ^1H NMR (D_2O) and ^1H NMR (Me_2SO-d_6), see Tables III and IV; ^{13}C NMR (D_2O), see Table II; **IR** (neat film) 3365 (OH), 2925 (CH), 1743 (C=O), 1403 (OH), 1295, 1242, 1198, 1107, 1080, and 1020 (COC, CO), 988, 943, 884, 788, 762, 725 cm^{-1}; mass spectrum (CI), m/e 223 ($M + NH_4^+$), 206 ($M + H^+$), 162 ($M + H^+ - CO_2$), 144 ($M + H^+ - CO_2 - H_2O$), 126 ($M + H^+ - CO_2 - 2H_2O$); mass spectrum (EI) of tris(*O*-trimethylsilyl) derivative, m/e 406 (M − 15), 331 (M − 90), 318 (M − 103).

2-(Acetylcarboxyamino)-2-deoxy-α-D-glucofuranose Intramolecular 2,1-Ester 3,5,6-Triacetate (8). **8** was prepared from **7** by using the general acetylation procedure described above: mp 172–176 °C (lit.[30] mp 169–172 °C); $[\alpha]^{27}_D$ − 49° ± 6° (*c* 1, $CHCl_3$) [lit.[30] $[\alpha]^{27}_D$ −46° (*c* 1, EtOH)]; ^1H NMR ($CDCl_3$) δ 6.16 (H_1, d, ^3J = 5.86, 1 H), 5.66 (H_3, d, ^3J = 3.05, 1 H), 5.21 (H_5, ddd, ^3J = 9.39, 4.98, 2.44, 1 H), 4.66 (H_2, d, ^3J = 5.86, 1 H), 4.57 ($H_{6\alpha}$, dd, ^2J = −12.25, ^3J = 2.44, 1 H), 4.32 (H_4, dd, ^3J = 9.39, 3.05, 1 H), 4.09 ($H_{6\beta}$, dd, ^2J = −12.25, ^3J = 4.98, 1 H), 2.57 (CH_3CON, s, 3 H), 2.09 (2 CH_3COO, s, 6 H), 2.00 (CH_3COO, s, 3 H). ^1H NMR (Me_2SO-d_6), see Table III for chemical shifts (multiplicities same as those in $CDCl_3$); ^1H NMR (C_6D_6) δ 5.66 (H_3, d, ^3J = 3.22, 1 H), 5.35 (H_5, m, 1 H), 5.29 (H_1, d, ^3J = 5.29, 1 H), 4.67 ($H_{6\alpha}$, dd, ^2J = −12.3, ^3J = 2.35, 1 H), ~4.03 (H_4, dd, ^3J = 10, 3.22, 1 H), ~3.95 (H_2 and $H_{6\beta}$, m, 2 H), 2.16 (CH_3CON, s, 3 H), 1.77, 1.70, 1.63 (3 CH_3COO, 3 s, 9 H); IR (film) 2930 (CH), 1788 (carbamate C=O), 1744 (ester C=O), 1707 (amide C=O), 1417 (CH_2 and CH_3), 1368 (CH_3), 1325 (CH), 1293 (C–N), 1240 and 1217 (COC), 1160, 1100, and 1040 (COC, C–O), 997, 974, 776, 750 cm^{-1}.

Fraction C: 2-(Carboxyamino)-2-deoxy-α-D-glucopyranose Intramolecular 2,1-Ester (3). Lyophilization of fraction C yielded **3** as an amorphous white solid. **3** was also prepared by the reaction of 2-amino-2-deoxy-D-glucose with *p*-nitrophenyl chloroformate (see later): $[\alpha]^{27}_D$ +26° ± 3° (*c* 3.2, methanol); ^1H NMR (D_2O) and ^1H NMR (Me_2SO-d_6), see Tables III and IV; ^{13}C NMR (D_2O), see Table II; IR (film) 3350 (OH and NH), 2905 (CH), 1742 (carbamate C=O), 1373 (OH), 1247, 1189, 1073, 1043, and 1031 (COC, C–O), 965, 882, 840, 763, 733 cm^{-1}; mass spectrum (CI, direct probe), m/e 223 ($M + NH_4^+$), 206 ($M + H^+$), 162 ($M + H^+ - CO_2$), 144 ($M + H^+ - CO_2 - H_2O$), 126 ($M + H^+ - CO_2 - 2H_2O$) (m/e 287 appeared at widely varying intensities in different spectra); mass spectrum (EI) on tris(*O*-trimethylsilyl) derivative, m/e 421 (M^+), 406 (M − 15), 331 (M − 90), 316 (M − 105).

2-(Acetylcarboxyamino)-2-deoxy-α-D-glucopyranose Intramolecular 2,1-Ester 3,4,6-Triacetate (4). **4** was prepared from **3** by using the acetylation procedure described above: ^1H NMR ($CDCl_3$) δ 6.00 (H_1, d, ^3J = 7.03, 1 H), 5.43 (H_3, dd, ^3J = 3.96, 5.28, 1 H), 5.04 (H_4, ddd, ^3J = 6.75, 5.28, ^4J = 1.06, 1 H), 4.57 (H_2, ddd, ^3J = 7.03, 3.96, ^4J = 1.06, 1 H), 4.30 ($H_{6\alpha}$, dd, ^2J = −12.0, ^3J = 5.78, 1 H), 4.25 ($H_{6\beta}$, dd, ^2J = −12.0, ^3J = 2.88, 1 H), 4.06 (H_5, ddd, ^3J = 6.75, 5.78, 2.88, 1 H), 2.52 (CH_3CON, s, 3 H), 2.11, 2.10, 2.04 (3 CH_3COO, 3 s, 9 H); ^1H NMR (C_6D_6) δ 5.45 (H_3, dd, ^3J = 5.86, 4.40, 1 H), 5.11 (H_4, ddd, ^3J = 7.33, 5.86, ^4J = 0.88, 1 H), 5.05 (H_1, d, ^3J = 7.03, 1 H), 4.21 ($H_{6\alpha}$, dd, ^2J = −12.3, ^3J = 4.98, 1 H), 4.08 ($H_{6\beta}$, dd, ^2J = −12.3, ^3J = 3.22, 1 H), 3.92 (H_2, ddd, ^3J = 7.03, 4.40, ^4J = 0.88, 1 H), 3.66 (H_5, ddd, ^3J = 7.33, 4.98, 3.22, 1 H), 2.30 (CH_3CON, s, 3 H), 1.68, 1.67, 1.49 (3 CH_3COO, 3 s, 9 H); IR (film) 2930 (CH), 1793 (carbamate C=O), 1749 (ester C=O), 1712 (amide C=O), 1422 (CH_2, CH_3), 1370 (CH_3), 1284 (C–N), 1226, 1158, 1130, 1092, and 1043 (COC, CO), 987, 913, 893, 783, 754 cm^{-1}.

2-(Carboxyamino)-2-deoxy-α-D-glucopyranose Intramolecular 2,1-Ester 3,4,6-Triacetate (5). **3** (120 mg, 0.59 mmol), pyridine (2.5 mL), and acetic anhydride (2.5 mL) were stirred in an ice bath for 2.5 h. The solution was worked up as described earlier in the acetylation procedure to give a white powder. Recrystallization from acetone–ether and then from acetone–cyclohexane gave white crystals: mp 171.0–172.5 °C (lit. mp 170 °C[43] or 174–175 °C[44]); $[\alpha]^{27}_D$ +23° ± 5° (*c* 2.0, $CHCl_3$), (lit. $[\alpha]^{27}_D$ +33°[43] or +50.3°[44]), ^1H NMR ($CDCl_3$) δ 5.99 (NH, s, 1 H), 5.96 (H_1, d, ^3J = 7.03, 1 H), 5.05 (H_4, dd, ^3J = 5.86, 8.65, 1 H), 4.90 (H_3, dd, ^3J = 3.81, 5.86, 1 H), 4.33 ($H_{6\alpha}$, dd, ^2J = −12.45, ^3J = 5.28, 1 H), 4.20 ($H_{6\beta}$, dd, ^2J = −12.45, ^3J = 2.8, 1 H), 4.08 (H_5, ddd, ^3J = 8.65, 5.28, 2.8, 1 H), 3.88 (H_2, dd, ^3J = 7.03, 3.81, 1 H), 2.11, 2.09, 2.08 (3 CH_3COO, 3 s, 9 H). The four peaks in the doublet of doublets at δ 3.88 (H_2) were broad and two of the four peaks in the doublet of doublets at δ 5.05 (H_4) were further split into doublets (J = 0.88 Hz), indicating a long-range coupling between H_2 and H_4.

Fraction D: 2-(Carboxyamino)-2-deoxy-α-D-mannofuranose Intramolecular 2,3-Ester (13). Lyophilization of fraction D yielded **13** as an amorphous solid. **13** was also synthesized by the reaction of 2-amino-2-deoxy-D-mannose with *p*-nitrophenyl chloroformate (see below): ^1H NMR (D_2O) and ^1H NMR (Me_2SO-d_6), see Tables III and IV; ^{13}C NMR (D_2O), see Table II; IR (film) 3350 (OH, NH), 2927 (CH), 1740 (carbamate C=O), 1410 (OH bend), 1295 [1218, 1077, and 1027 (COC, C–O)], 965, 856, 757 cm^{-1}; mass spectrum (CI, direct probe), m/e 223 ($M + NH_4^+$), 206 ($M + H^+$), 162 ($M + H^+ - CO_2$), 144 ($M + H^+ - CO_2 - H_2O$), 126 ($M + H^+ - CO_2 - 2H_2O$); m/e 287 and 250 peaks of varying intensities appeared in different spectra; mass spectrum (EI) on tris(*O*-trimethylsilyl) derivative, m/e 406 (M − 15), 318 (M − 103), 316 (M − 105).

2-(Acetylcarboxyamino)-2-deoxy-α-D-mannofuranose Intramolecular 2,3-Ester 1,5,6-Triacetate (14). **14** was prepared from **13** by using the general acetylation procedure described above: $[\alpha]^{28}_D$ −48° ± 5° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.27 (H_1, s, ~0.8 H), 5.26 (H_5, ddd, ^3J = 8.30, 4.75, 3.17, 1 H), 5.11 (H_3, dd, ^3J = 7.36, 3.51, 1 H), 4.81 (H_2, d, ^3J = 7.36, 1 H), 4.59 ($H_{6\alpha}$, dd, ^2J = −12.46, ^3J = 3.17, 1 H), 4.42 (H_4, dd, ^3J = 8.30, 3.51,

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1 H), 4.16 ($H_{6\delta}$, dd, ^2J = −12.46, ^3J = 4.75, 1 H), 2.55 (CH_3CON, s, 3 H), 2.12, 2.09, 2.06 (3 CH_3COO, 3 s, 9 H) (if area of other small peaks in the δ 6.0–6.6 region is included with the area of H_1, the total area is 1 H; ^{1}H NMR (C_6D_6) δ 6.38 (H_1, s, 1 H), 5.38 (H_5, m, 1 H), 4.69 ($H_{6\alpha}$, dd, ^2J = −12.3, ^3J = 3.22, 1 H), 4.15 (H_2, H_3 + H_4, m, 3 H), 4.06 ($H_{6\beta}$, dd, ^2J = −12.3, ^3J = 4.69, 1 H), 2.12 (CH_3CON, s, 3 H), 1.70, 1.66, 1.51 (3 CH_3COO, 3 s, 9 H); IR (film) 2930 (CH), 1790 (carbamate C=O), 1746 (ester C=O), 1710 (amide C=O), 1426 (CH_2, CH_3), 1371 (CH_3), 1313 (CH), 1293 (C–N), 1217, 1149, 1085, and 1046 (COC, C–O), 1016, 975, 850, 773, 752 cm^{-1}; mass spectrum (EI), m/e 331 (M − CH_2CO), 314 (M − CH_3COO·), 272 (M − CH_3COO· − CH_2CO), 236 (metastable 314 → 272, calcd 235.6).

Mixtures of Fraction A (11,12) and B (15/16). Mixture I. Fraction A was collected such that most of the solution stood at room temperature for only a few hours (to limit conversion to fraction B): ^{1}H NMR (D_2O) and ^{1}H NMR (Me_2SO-d_6), ≥90% peaks were **11** and **12** (see Tables III and IV for δ and J values); IR (film) 3365 (OH), 2915 (CH), 1734 (C=O), 1420 (OH), 1225 and 1075 (COC, CO), 875, 760 cm^{-1}; GC/MS of the trimethylsilyl derivatives revealed two isomers (eluted from 1% OV-17 column at 276 and 283 °C) with the same m/e values corresponding to the octakis(*O*-trimethylsilyl) derivatives of **11** and **12**, mass spectrum, m/e 983 (M^+, calcd 986), 968 (M − 15, calcd 971), 897 (M − 90, calcd 896), 883 (M − 105, calcd 881) (the estimated error in measuring the m/e values is about 3 mass units at these high masses); mass spectrum (CI), m/e (% base peak) 223 (2), 206 (1), 162 (100), 144 (62) (other peaks observed above m/e 206 were 386 (<1), 345 (<1), 316 (1), 282 (2), 269 (1), 243 (4), 226 (2); the 223 peak (M + NH_4^+) was thought to be due to **15/16** because it was much larger in mixtures II and III described below).

Mixture II. Fraction B was collected over a period of several days such that at least part of the fraction stood at room temperature for up to 12 h: ^{1}H NMR (D_2O) δ 5.25–5.40 (H_1 and $H_{1'}$, of **11** and **12**, m), 4.8–5.1 (H_3's of **11**, **12**, **15/16**, and H_1 of **15/16**, m), 3.6–4.5 (H_2, H_4, H_5, H_6's of **11**, **12**, **15/16**, m); ^{1}H NMR (Me_2SO-d_6) δ 6.78 (1-OH and 1′-OH of **11** and **12**, d, ^3J = 5.6), 7.4–7.55 (NH of **15/16**, m, ~40% of area of δ 6.78 peak), 6.45–6.7 and 6.0–6.1 (anomeric OH's of **15/16**, 2 m, each m has ~30% of area of δ 6.78 peak), 3.1–5.3 (all other protons of **11**, **12**, and **15/16**, m); ^{13}C NMR (D_2O) of a fresh solution of lyophilized sample gave approximately a 4:3 mixture of **15/16** (δ 92.7, 80.0, 74.0, 73.0, 65.2, 61.1) and **11** (δ 80.9, 79.0, 74.3, 72.8, 65.2, 58.7) with **12** presumably in the noise. After standing in the NMR tube for 4 months at room temperature the ratio of **15/16** to **11** reduced to 1:1. GC/MS of trimethylsilyl derivatives gave the same results as mixture I: (CI) m/e (% base peak) 223 (100), 206 (6), 162 (60), 144 (40), 126 (21); other peaks observed above m/e 206 were 287 (40), 269 (6), 251 (2), 241 (2), 233 (1), 215 (8).

Mixture III. Fraction B was trapped in dry ice to freeze it as soon as it exited the high-pressure LC. The sample was lyophilized without thawing, and the residue was dissolved in a small amount of water for about 15 s and immediately frozen for lyophilization in a vial. A fluffy white solid was obtained. GC/MS of trimethylsilyl derivatives gave the same results as mixture I: (CI) m/e (% base peak) 287 (4), 223 (33), 206 (2), 162 (100), 144 (32); no other peaks were observed.

Acetylation of Mixtures of Fractions A and B. Acetylations of mixtures I, II, and III containing various amounts of **11**, **12**, and **15/16** with pyridine and acetic anhydride at 0 and 25 °C all produced essentially the same mixture of products as indicated by ^{1}H NMR. The major product (**9**) formed had only one type of anomeric hydrogen [δ 6.11 (d, ^3J = 6.16)], in agreement with published data.[30] For the area to equal 2 H, however, the areas of the two other observed downfield doublets of H_1 and $H_{1'}$ for **10** [δ 6.54 (^3J = 1.76) and δ 6.33 (^3J = 8.22)] had to be included: ^{1}H NMR ($CDCl_3$) δ 6.54 ($H_{1'}$ of **10**, d, ^3J = 1.76), 6.33 (H_1 of **10**, d, ^3J = 8.22), 6.11 (H_1 and $H_{1'}$ of **9**, d, ^3J = 6.16), 5.30 (H_4, $H_{4'}$, H_5, $H_{5'}$, s, 4 H), 5.16 (H_3, $H_{3'}$, d, ^3J = 3.52, 2 H), 4.30 (2 H_6, 2 $H_{6'}$, s, 4 H), 3.58 (H_2, $H_{2'}$, dd, ^3J = 6.16, 3.52, 2 H), 2.15, 2.14, 2.12, 2.06 (8 CH_3COO, 4 s, 24 H), the areas of peaks at δ 6.54, 6.33, and 6.11 add up to 2 H. The assignments for the protons (other than H_1 and $H_{1'}$) are the same for **9** and **10** except for two small peaks observed outside the doublet of doublets at δ 3.58 which were assigned to H_2 and $H_{2'}$ of **10**. ^{13}C NMR gave a ca. 3:1 mixture of **9** and **10**. For chemical shifts of **9** and **10** in C_6D_6 or acetone-d_6 see Table II. IR (film) 1776 (carbamate C=O), 1742 (ester C=O), 1400 and 1364 (CH_2, CH_3), 1212, 1040, and 1010 (COC, C–O), 960, 850, 743 cm^{-1}; mass spectrum (EI), m/e 686 (M^+ − CH_3COOH), 642 (M^+ − CH_3COOH − CO_2), 626 (M^+ − 2CH_3COOH), 571 (metastable, 686 → 626, calcd 571.25).

Reaction of 2-Amino-2-deoxy-D-glucose with *p*-Nitrophenyl Chloroformate (PNPCF). The procedure of Umezawa et al.[37] was used. A solution of 2-amino-2-deoxy-D-glucose hydrochloride (3.46 g, 0.016 mol) in water (70 mL) was stirred with wet Dowex 1X-2 resin (130 mL, OH^- form, 200–400 mesh) in an ice–water bath for 15 min. An ice-cold solution of PNPCF (8.00 g, 0.0397 mol) in acetone was added over a 15-min period, resulting in a viscous yellow slurry. The mixture was removed from the ice bath and stirred at room temperature for 1.25 h. The slurry was stirred with an equal volume of ether, the ether removed, and the slurry filtered. The residue was washed with hot water (ca. 100 mL) and the washing combined with the filtrate to give a solution (ca. 300 mL) whose pH was adjusted to 3.0 by addition of 4 M HCl. The solution was extracted with ether (5 × 200 mL and 3 × 100 mL) until the extract gave no yellow color upon addition of NaOH solution. The aqueous solution was then neutralized with Dowex IX-2 resin (OH^-) and lyophilized to give 2.7 g of a light yellow solid. ^{13}C NMR of this solid revealed a mixture of approximately 40% **3**, 15% of a mixture of fractions A and B, and 45% unreacted glucosamine.

Reaction of 2-Amino-2-deoxy-D-mannose with PNPCF. By the same procedure described above, 2-amino-2-deoxy-D-mannose hydrochloride (1.26 g, 5.83 mmol) in water (30 mL) with wet Dowex 1X-2 resin (45 mL, OH^- form, 200–400 mesh) was reacted with PNPCF (3.06 g, 15.2 mmol) in acetone (50 mL). Workup of the mixture as above followed by lyophilization and ^{13}C NMR revealed the major product (50.1% of mixture) had peaks at δ 163.2, 103.5, 82.9, 81.2, 71.2, 65.7, and 65.2. These peaks are identical with those of the high-pressure LC fraction D (**13**). Unreacted 2-amino-2-deoxy-D-mannose was 25.4% of the mixture at δ 93.5 and 92.9. Three other peaks (δ 106.7, 106.2, 99.7) presumably represent the anomeric carbons of three unidentified products (4.7, 6.4, 12.7% of mixture, respectively). Other unidentified peaks were observed at δ 84.2, 82.5, 82.0, 79.1, 71.8, 71.5, 69.7, 64.3, 63.8, 61.8, 60.2, and 59.1.

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Registry No. **2**, 54749-90-5; **3**, 7103-39-1; **3** tris(TMS), 76481-06-6; **4**, 76481-07-7; **5**, 76548-23-7; **7**, 68107-80-2; **7** tris(TMS), 76481-08-8; **8**, 68050-71-5; **9**, 68050-76-0; **10**, 76548-24-8; **11**, 76481-09-9; **11** octakis(TMS), 76481-10-2; **12**, 76548-25-9; **12** octakis(TMS), 76548-26-0; **13**, 76581-86-7; **13** tris(TMS), 76481-11-3; **14**, 76481-12-4; **15**, 76581-87-8; **16**, 76481-13-5; **21**, 76548-27-1; α-D-glucosamine HCl, 14131-62-5; β-D-glucosamine HCl, 14131-63-6; α-D-mannosamine HCl, 14131-65-8; β-D-mannosamine HCl, 14131-67-0; *N*-acetyl-α-D-glucosamine, 10036-64-3; *N*-acetyl-β-D-glucosamine, 14131-68-1; 2-oxazolidinone, 497-25-6; tetrahydro-2*H*-1,3-oxazin-2-one, 5259-97-2; 2-amino-2-deoxy-D-glucose HCl, 66-84-2; *p*-nitrophenyl chloroformate, 7693-46-1; 2-amino-2-deoxy-D-mannose, 5505-63-5.