Cocaine hydrochloride produced a marked numbness. β-Cocaine hydrochloride produced essentially no effect.

β-Cocaine was evaluated in parallel with cocaine in the guinea pig intradermal wheal test as described by Bülbring and Wajda.[6] The average threshold anesthetic concentration (TAC_5) was obtained from the dose–effect curve (semilogarithmic plot of duration in minutes *vs.* dosage) as described by Luduena and Hoppe.[8] Experimental values are furnished in Table I. β-Cocaine was approximately one-third as active as cocaine.

Acknowledgment. We thank Dr. Eugenio F. Bogado for the analgesic testing done on guinea pigs.

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Synthesis of Chlorozotocin, the 2-Chloroethyl Analog of the Anticancer Antibiotic Streptozotocin

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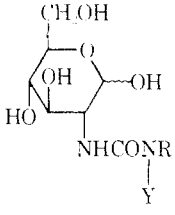
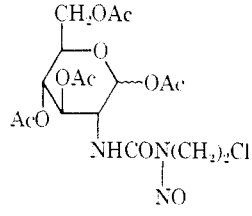
Streptozotocin (1) is a natural glucose-substituted *N*-methyl-*N*-nitrosourea,[1] a broad-spectrum antibiotic,[2] and an experimental anticancer agent[3] that has shown diabetogenic activity in animals[4] and clinical activity in the treatment of malignant insulinomas in man.[5] Prior synthesis of congeners of 1 has emphasized variation of the glucose moiety,[6-9] which resulted in enhancement of activity against leukemia L1210 in some cases judged on a comparable basis;[9] limited testing of the ethyl and butyl analogs of 1 indicated inactivity.[3] A moderate increase in the antileukemic activity of 1 was observed when the diabetogenic activity was suppressed with nicotinamide.[3,10] The structure and properties of 1 suggested some time ago the replacement of the methyl group with a 2-chloroethyl group, a modification demonstrated[11] to enhance markedly the effectiveness of a number of *N*-methyl-*N*-nitrosoureas against leukemia L1210.

The principal previous attempt to synthesize 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (3, "chlorozotocin") involved deacetylation of the tetraacetate 4,[12] a method modeled after the original synthesis of 1[1] which has since been superseded by other methods.[13,14] Interest in the synthesis of 3 was recently revived in view of the observation that, in mice, the tetraacetate **4**, like 1,[15] showed reduced bone-marrow toxicity and was, unlike 1, nondiabetogenic.[16] Myelosuppression has been reported to be the limiting toxicity in the clinical use of the *N*-(2-chloroethyl)-*N*-nitrosoureas BCNU[17] [*N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea] and CCNU[18] [*N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea, **5a**].



1. R = Me; Y = NO
2. R = $(CH_2)_2Cl$; Y = H
3. R = $(CH_2)_2Cl$; Y = NO



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Chemistry. The (2-chloroethyl)urea 2 required for the synthesis of 3 was prepared by the addition of 2-chloroethyl isocyanate to D-glucosamine in water and later in anhydrous *N*,*N*-dimethylformamide (DMF). The latter yield-improving variation was tried as a model for similar conversions in which hydrolysis of the isocyanate would be a significant competitive reaction in an aqueous system. The liberation of D-glucosamine from its hydrochloride with a basic ion-exchange resin was also a convenient innovation.

The nitrosation of 2 with dinitrogen trioxide (chosen, as in the preparation of 1,[14] to simplify the separation of water-soluble 3) failed in a number of attempts with conventional media: (1) in formic acid, which would direct nitrosation to the desired position,[11] unwanted formylation (of presumably the primary hydroxyl group and possibly others) occurred; (2) in water, the preferred medium for the preparation of 1,[14] and in dilute formic acid, the reaction was exceedingly slow and incomplete after several hours; (3) in dilute hydrochloric acid, the reaction was somewhat faster but gave at least two products as indicated by tlc. In concentrated hydrochloric acid, the reaction was essentially complete in less than 1 hr; recrystallization of the resulting precipitate produced an analytically pure sample of 3. The pmr spectrum showed no evidence of random nitrosation and indicated a predominance of the α anomer. In aqueous solution, however, anomers of 3 would be expected to equilibrate as do various lots of crystalline 1.[16] In a typical subsequent run, dinitrogen trioxide was introduced at a moderately fast rate until the reaction solution became red and intermittently thereafter until an appreciable amount of 3 had precipitated; precipitation was completed by the addition of a proportionately large volume of ether. Unrecrystallized 3 was suitable (melting point, ir, and tlc) for biological testing.

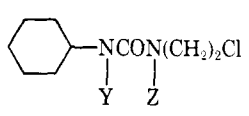
The enhanced rate of nitrosation in concentrated hydrochloric acid was attributed to *in situ* generation of nitrosyl chloride in analogy to the standard preparation involving the addition of a concentrated aqueous solution of sodium nitrite to concentrated hydrochloric acid.[19] The observed selective nitrosation was surpising, however, until the nitrosation of *N*-(2-chloroethyl)-*N*′-(*trans*-4-methylcyclohexyl)urea (**6**) under the same conditions (except that ethanol was added for solubilization) was demonstrated to give a nitrosourea that was identical with homogeneous samples of MeCCNU (**7**). Furthermore, a 1-hr treatment of a 2:1 mixture of **5a** and isomeric **5b** with ethanolic concentrated hydrochloric acid resulted in a high-yield transformation to a nitrosourea that was very nearly identical with homogeneous samples of CCNU. These results parallel the selective nitrosations and nitroso group migrations previous-

Table I. Activity against Leukemia L1210[a]

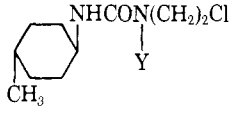
Compd	Diluent	Dosage,[b] mg/kg/dose	Schedule	60-day survivors/total	Median day of death	% ILS	Cell kill
Control					11.0		
1	Citrate buffer	50	qd 1–9	0/10	14.5	31	43%/dose
4	Citrate buffer	7.5	qd 1–9	1/10	15.0	36	47%/dose
3	Citrate buffer	2.5	qd 1–9	0/10	18.0	63	67%/dose
Control					8.5		
4	CMC[c]	20	Day 1 only	4/10	17.0	100	≥5 logs
3	Saline	30	Day 1 only	9/10			≥6 logs

[a] BDF_1 mice inoculated with 10^5 L1210 cells intraperitoneally (ip) and treated ip. [b] Maximum nontoxic dose as judged by concurrent toxicity controls in normal mice. [c] Carboxymethylcellulose sodium salt, 0.4% solution in saline.

ly observed in essentially anhydrous formic acid[11,12] and illustrate for the first time these effects in an aqueous system. Thus, rearrangement may occur by abstraction of nitroso groups as nitrosyl chloride in one case and nitrosyl formate (formyl nitrite) in the other, both a source of nitrosonium ion for renitrosation in a more stable position.



5a, Y = H; Z = NO
b, Y = NO; Z = H



6, Y = H
7, Y = NO

Biologic Evaluation. The prediction of superior activity for chlorozotocin (3) against leukemia L1210 has been corroborated by experiment (see Table I). Groups of leukemic mice were treated daily for 9 days with 3, its tetraacetate 4, and streptozotocin (1), respectively, in a single experiment. On this schedule, the maximum increase in life span, based on the median day of death, produced by 3 was more than twice that produced by either of the other two nitrosoureas, proving the clear superiority of 3. The schedule used in this experiment (qd 1–9) was chosen because it is the optimal schedule for 1,[20] but it is not optimal for 3 or 4. In another single experiment on the optimal schedule (single dose) for 3 and 4, 3 killed 6 logs or greater of L1210 cells, curing nine of ten animals; 4 killed about 5 logs and cured four animals, increasing the life span of the dying animals 100%. Given in this way 1 is said to be inactive.[20] In addition to its high-level L1210 activity, 3 showed reduced bone-marrow toxicity[21] and has the advantage of water solubility over 4.† On the basis of activity and low bone-marrow toxicity, 3 is now undergoing pharmacologic evaluation preliminary to clinical trials.

Experimental Section

Melting points with a range were determined with a Mel-Temp apparatus and are uncorrected; those without range, with a Kofler Heizbank. Ir spectra were determined with Perkin-Elmer 521 and 621 spectrophotometers and pmr spectra with a Varian XL-100-15 spectrometer. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Elemental analysis of 3 was performed by Galbraith Laboratories, Knoxville, Tenn.; spectral determinations and elemental analysis of 2 were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr.; and biological evaluation was performed in the Cancer Screening Division of Southern Research Institute under the direction of Dr. W. R. Laster, Jr.

2-[3-(2-Chloroethyl)ureido]-2-deoxy-D-glucopyranose (2). A. Preparation in Water. A cold (5°), stirred solution of β-D-glucosamine[22] [mp 110–111° (lit.[23] mp 110–111°); 4.00 g, 22.3 mmol] in H_2O (25 ml) was treated slowly with 2-chloroethyl isocyanate (1.97 ml, 22.3 mmol; Eastman Kodak Co.). After the addition, the reaction solution was stirred at 5° for 1 hr. The white precipitate that formed was collected, washed with two 5-ml portions of EtOH, and dried overnight *in vacuo* over P_2O_5: yield of 2 4.50 g (71%); mp 160–161° dec; ir (KBr) 3600–3000 (OH, NH), 3000–2800 (CH), 1620 (C=O), 1590 cm^{-1} (CNH, amide II); homogeneous by tlc (silica gel, 3:1 $CHCl_3$–MeOH, charring after NH_4SO_4–H_2SO_4 spray). *Anal.* ($C_9H_{17}ClN_2O_6$) C, H, N.

B. Preparation in DMF. A stirred suspension of β-D-glucosamine (300 mg, 1.67 mmol) in DMF (4 ml) was treated with $Cl(CH_2)_2NCO$ (0.144 ml, 1.67 mmol), all the suspended solid having dissolved after ~15 min. The solution was stirred for 1 hr, during which time a precipitate formed. The suspension was diluted with ether (40 ml), stirred for 30 min, and chilled. The product was collected, washed successively with ether (two 20-ml portions), EtOH (two 10-ml portions), and again ether (two 20-ml portions), and dried overnight *in vacuo* over P_2O_5: yield 450 mg (94%); mp 159–160° dec; ir identical with that of the analytically pure sample described above.

D-Glucosamine (by Ion Exchange). A stirred solution of D-glucosamine hydrochloride (1.00 g, 4.64 mmol) in H_2O (25 ml) was treated at intervals with Dowex 1-X2 (OH^-) ion-exchange resin until the pH was 9–10. The resin was removed by filtration and washed with two 10-ml portions of H_2O; the filtrate and washings were combined and evaporated to dryness under reduced pressure with the aid of added EtOH (20 ml) and then ether (20 ml). The residual D-glucosamine was further dried *in vacuo* over P_2O_5 [yield 650 mg (78%), mp ~98° dec] and converted, in H_2O, to 2, mp 159–160° dec, in 57% yield (ir identical with that of the analytical sample of 2).

2-[3-(2-Chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (3). N_2O_3 was bubbled into a cold (0–5°), stirred solution of 2 (4.60 g, 16.2 mmol) in concentrated HCl (46 ml) at a moderately fast rate until the solution became red and then intermittently until an appreciable amount of solid had formed (~1.25 hr). Ether (400 ml) was added and the mixture was stirred at 0° for 20 min. The precipitate was collected on a filter, washed with two 20-ml portions of ether, and dried overnight *in vacuo* over P_2O_5: yield 2.67 g (53%); mp 140–141° dec. This sample was identical (melting point, ir, and tlc) with the analytical sample obtained from a previous, 45-min nitrosation and recrystallized from EtOH: ir (KBr) 3600–3100 (OH, NH), 3000–2800 (CH), 1695 (C=O), 1540 (CNH, amide II), 1490 cm^{-1} (N=O); pmr (DMSO-d_6–TMS, chemical shifts quoted as either ranges or approximate centers) δ 3.0–3.9 [m, CH_2OH, H-5, H-4, H-3, H-2, overlapping upfield half of A_2B_2 system due to $N(CH_2)_2Cl$], 4.12 [pseudo t from downfield half of A_2B_2 system due to $N(CH_2)_2Cl$], 3.9–5.2 (3 OH's, apparently exchanging with small amount of H_2O in solvent), 4.67 (d, $J_{1,2} \approx 9$ Hz, H-1 of β anomer;[24] obscured by OH's until after addition of D_2O), 5.14 (d, $J_{1,2} \approx 2$ Hz, H-1 of α anomer;[24] coupling measured after addition of D_2O, which eliminated coupling with OH), 6.6 (br m, OH probably at C-1), 7.8 (br d, NH of α anomer), 8.5 (br d, NH of β anomer). [Pmr indicated an α:β anomeric ratio of ~10:1, but 3 appeared homogeneous by tlc on silica gel with 5:1 $CHCl_3$–MeOH and detection by uv and char-

†The log *P* value of 3, as determined by Dr. W. J. Haggerty of Midwest Research Institute, Kansas City, from the octanol-water partition coefficient and transmitted by Dr. R. R. Engle of Drug Research and Development, National Cancer Institute, is −1.02. Reported[9] values for other streptozotocin analogs range from −0.82 to −1.57 (streptozotocin −1.45).

ring after $(NH_4)_2SO_4$-H_2SO_4 spray.] *Anal.* ($C_9H_{16}ClN_3O_7$) C, H, N.

Nitrosation of *N*-(2-Chloroethyl)-*N'*-(*trans*-4-methylcyclohexyl)urea (6) in Ethanolic Hydrochloric Acid. N_2O_3 was bubbled into a cold (0–5°), stirred solution of **6**[12] (125 mg, 0.570 mmol) in concentrated HCl (2 ml) and EtOH (1 ml) at a moderate rate for 15 min. Kept at 0–5°, the reaction mixture was stirred for 5 min before and after dilution with cold H_2O (20 ml). The light-yellow MeCCNU (**7**) that separated was collected and dried *in vacuo* over P_2O_5: yield 105 mg (74%); mp 70° (lit.[12] mp 70°); identity with an authentic sample established by ir (KBr) and pmr ($CDCl_3$-TMS).

Nitroso Group Migration in Ethanolic Hydrochloric Acid: CCNU (5a). An isomeric mixture[11] (200 mg), mp 69° dec, of **5a** (~65%) and **5b** (~35%) was stirred in suspension in a cold (0–5°) solution of EtOH (2 ml) in concentrated HCl (3 ml) for 1 hr. The mixture was diluted with cold H_2O (20 ml) and stirred at 0° for 20 min more. The light-yellow solid was collected, washed with cold H_2O (5 ml), and dried *in vacuo* over P_2O_5: yield 190 mg (95%); mp 89° (lit.[11] mp of CCNU 90°); pmr ($CDCl_3$-TMS) showed possibly a trace of **5b**.

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Synthesis and Antimicrobial Evaluation of *N*-D-Alanyl-1-aminoethylphosphonic Acid

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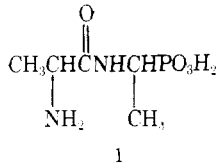
A number of antibiotic substances are known to exert antimicrobial activity by virtue of their ability to inhibit the synthesis of bacterial cell wall material.[1] Some of the compounds included in this class are vancomycin, ristocetin, penicillins, cephalosporins, phosphonomycin, and cycloserine (oxamycin). The mode of action of cycloserine is probably known in most detail and involves the inhibition of the enzyme that racemizes L-alanine and the enzyme that synthesizes D-alanyl-D-alanine.[2] Since the dipeptide D-alanyl-D-alanine is the terminal feature of the various peptides from which bacteria build their cell walls, it seemed to us that this would represent a possible point of attack on murein biosynthesis. It was with this purpose in mind that we undertook to prepare and evaluate the antimicrobial activity of the dipeptide *N*-D-alanyl-1-aminoethylphosphonic acid (**1**) which is a phosphonic acid analog of D-alanyl-D-alanine (**2**).



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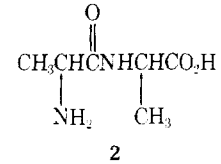

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The dipeptide **1** was prepared as outlined in Scheme I using conventional procedures which have been shown to be applicable to the synthesis of peptides derived from α-aminobenzylphosphonic acid.[3-5] 1-Aminoethylphosphonic acid (**3**) was prepared by basically the same method as described by Chambers and Isbell[6] from triethyl 2-phosphonopropionate. In order to avoid any possible complications during the succeeding esterification step, the basic amine function of **3** was blocked by conversion to the carbobenzoxy derivative **4** using standard Schotten–Baumann conditions. In order to block the phosphonic acid portion, **4** was converted to the diethyl ester **5** by heating