Nicotinic Acid 1-Oxide. Nicotinic acid 1-oxide was prepared by the oxidation of nicotinic acid with hydrogen peroxide (30%) and glacial acetic acid: mp 260–261° dec (lit.[4] mp 254–255°).

2-Chloronicotinic Acid. Nicotinic acid 1-oxide on treatment with phosphorus oxychloride gave 2-chloronicotinic acid: mp 174–178° dec.[4]

2-[α-(Nitrophenyl)hydrazino]nicotinic Acids (3–5). A mixture of 2-chloronicotinic acid (1, 7 mmol) and appropriate nitrophenylhydrazine (2, 7 mmol) in 15 ml of absolute ethanol was refluxed on a steam bath for 8 hr. The solid mass which separated out on removing the excess of ethanol under reduced pressure and on cooling was collected by filtration, washed with water, and recrystallized from ethanol. These hydrazinonicotinic acids were characterized by their sharp melting points and elemental analyses (Table II).

3-Hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines (6–8). An appropriate 2-[α-(nitrophenyl)hydrazino]nicotinic acid (2 mmol) was added to 15 ml of water containing 1.5 ml of concentrated hydrochloric acid and the mixture was refluxed on a sand bath for 6 hr. The reaction mixture was cooled, filtered, and concentrated. The crude product which separated out was collected by filtration and recrystallized from dilute hydrochloric acid. The pyrazolopyridines thus synthesized were characterized by their sharp melting points and elemental analyses (Table II).

Biochemical Studies. Normal healthy albino rats (100–150 g) kept on *ad libitum* diet were sacrificed by decapitation. Livers were immediately removed and homogenized in 0.25 *M* ice-cold sucrose (1:9 ratio) in a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 100,000g for 30 min and the clear supernatant (nonparticulate or soluble fraction) thus obtained was used for the assay of the purine-catabolizing enzymes. Adenosine deaminase activity was determined by the estimation of the disappearance of adenosine at 265 nm. Guanosine deaminase activity was determined by estimation of the disappearance of guanosine at 245 nm and guanine deaminase activity was determined by the estimation of the disappearance of guanine at 245 nm. Xanthine oxidase activity was determined by the estimation of uric acid formed from the oxidation of hypoxanthine at 290 nm. Details of the assay procedure are as reported earlier.[1]

Protein Estimation. Protein estimations were carried out by following the method of Lowry, *et al.*,[5] using bovine serum albumin as the standard. Readings were taken at 750 nm.

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Cycloserine Carbamates

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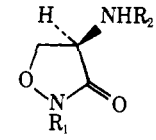
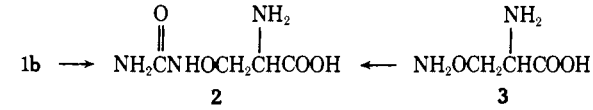
Cycloserine[1] (**1a**) is a broad-spectrum antibiotic which is known to inhibit cell wall synthesis in certain bacteria. It is a cyclic hydroxylamine which upon carbamoylation forms a derivative of *N*-hydroxyurea (HU), a known[2] anticancer agent. β-Aminooxy-D-alanine, the hydrolysis product of **1a**, and cycloserine dimer **6** are both *O*-alkylhydroxylamines which upon carbamolyation lead to HU derivatives which might eliminate molecular HU *in vivo*. It was, consequently, of interest to prepare these carbamoyl compounds and several others for screening by the National Cancer Institute.



1a, $R_1 = H$; $R_2 = H$ — **d**, $R_1 = H$; $R_2 = CONH_2$
b, $R_1 = CONH_2$; $R_2 = H$ — **e**, $R_1 = CONH_2$; $R_2 = Z$
c, $R_1 = CONH_2$; $R_2 = CONH_2$ — **f**, $R_1 = Z$; $R_2 = CONH_2$

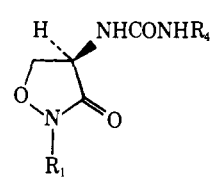
Z = carbobenzyloxy

Our earlier work[3] on carbobenzyloxy (Z) derivatives of **1a** allowed us to prepare the appropriately blocked derivatives so that carbamoylation would lead to **1e** and **1f**. Deblocking of **1e** and **1f** with HBr–HOAc and HF, respectively, afforded the desired products. The carbamoylations were carried out using potassium cyanate in aqueous alcoholic solution at pH 4 and were routinely screened of salts (KCl) by ion-exchange chromatography. Control of the pH during the reaction was crucial, since both **1b** and **1d** were sensitive to low pH and 1b was destroyed at pH >5. The 2-carbamoyl derivative **1b** underwent very rapid ($t_{1/2} \sim$ 40 min) hydrolysis even in the cold at pH <4 to form β-ureidooxy-D-alanine (**2**). This hydroxyurea derivative was

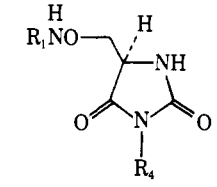


1b ⟶ $NH_2CNHOCH_2CHCOOH$ (**2**) ⟵ $NH_2OCH_2CHCOOH$ (**3**)

prepared more conveniently by direct carbamoylation of β-aminooxy-D-alanine[4] (**3**) at pH 5. The urea **1d**, which was nitroprusside† positive, was also converted into another compound at pH <4 as shown by the rapid formation of another spot (nitroprusside red) on thin-layer chromatography (tlc). Earlier workers[6] had shown that the carbamate **1g** was readily converted by HCl into the hydantoin **4b**; consequently, we expected that **1d** might form the unsubstituted hydantoin **4a** under acidic conditions with loss of the nitroprusside (blue) sensitive isoxazolidone ring.



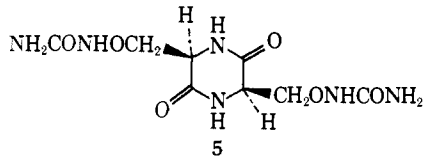
1d, $R_1 = H$; $R_4 = H$
g, $R_1 = H$; $R_4 = C_6H_5$



4a, $R_1 = H$; $R_4 = H$
b, $R_1 = H$; $R_4 = C_6H_5$
c, $R_1 = CONH_2$; $R_4 = H$

At pH >5, **1b** dimerized to give the dicarbamoylcycloserine dimer **5**. This compound, another hydroxyurea derivative, was prepared very readily by direct carbamoylation of cycloserine dimer[7] **6**. The dimerization of **1b** is analogous to the spontaneous dimerization of 2-carbobenzyloxy-D-cycloserine previously observed in these laboratories.[8]

†Both **1b** and **1c** also give a blue nitroprusside test.[5] These ring carbamylated derivatives are the only 2-substituted cycloserines so far encountered which react with this reagent.



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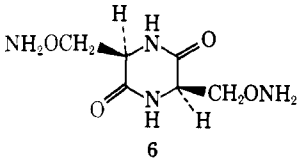

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The synthesis of the dicarbamoylcycloserine derivative 1c proved to be much more difficult to accomplish. When 1a was treated with only 1 equiv of cyanate, tlc showed the rapid formation of a product which was both ninhydrin- and nitroprusside-positive. This result is reasonably explained by assuming the rapid formation of **1b** which then more slowly carbamylated at the amino group to form 1c. This result indicated that the hydroxylamine ring nitrogen is more nucleophilic toward cyanic acid than is the amino group.‡ Treatment of **1a** with excess cyanate lead to a ninhydrin-negative nitroprusside-positive product homogeneous by tlc which was converted to another substance during attempted desalting on a Dowex-50 resin. On standing in solution for short periods, solutions of 1c gave several products (tlc), one of which was apparently the hydantoin **4c** and its decomposition products. The use of ion-retardation chromatography separated 1c from a major portion of the KCl present, but the product isolated still contained KCl. Several passes of this purified product through an ion-retarding resin gave no further separation of 1c from KCl as shown by elemental analysis. Apparently 1c chelates potassium ion with considerable tenacity. The structure of 1c is secured by spectroscopic data and its synthesis from *both* monocarbamates **1b** and **1d** as shown by tlc analysis of their reactions with cyanate. When 1c (containing KCl) was dissolved in trifluoroacetic acid, it was converted into a stable trifluoroacetate salt of the hydantoin **4c**. An aqueous solution of this salt slowly precipitated pure **4c** free of KCl. The fact that **4c** was obtained from KCl-containing dicarbamoylcycloserine 1c also secures the structure of 1c.

The anticancer screening data are summarized in Table I. The diketopiperazine derivative **5** was the only compound which showed significant activity. Compounds **1b**, **2**, and **5** were inactive against mouse lymphocytic leukemia (P388) and lymphoid leukemia (L1210) *in vivo* at a dose of 100 mg/kg.

Experimental Section

All melting points were performed on a Nagle-Kopfler micro hot stage. Infrared spectra were recorded on a Perkin-Elmer Model 621. The nmr spectra were obtained using a Varian T-60 spectrometer. Optical rotations were determined with a Rudolph Model 80 polarimeter. Thin-layer chromatography was performed in three solvent systems: (1) 1-butanol-acetic acid-water, 4:1:5 (BAW); (2) methyl ethyl ketone-pyridine-water, 20:5:8 (MPW); (3) *tert*-butyl alcohol-methyl ethyl ketone-water 9:9:2 (TBK). Detection was with 1% iodine in methanol (I), 0.3% ninhydrin in 95% ethanol (N), 4% sodium nitroprusside-4 *N* NaOH, 1:1 (NP, blue), fluorescence quenching (uv), and with acidic 3% $FeCl_3$ (Fe). All new compounds showed the correct C, H, and N analyses.

2-Carbobenzyloxy-α-*N*-carbamoyl-D-cycloserine (1f). To a stirred solution of 0.160 g (2 mmol) of KCNO in 5 ml of water was added 0.317 g (1 mmol) of 2-carbobenzyloxy-D-cycloserine hydrobromide over 5 min. The pH of the reaction mixture was maintained with a pH stat at 4 for 1 hr and the precipitated **1f** was recovered by centrifugation, washed once with 1.5 ml of water, and dried overnight *in vacuo* giving 0.195 g (70%), mp 141–143°. The precipitate was recrystallized from methanol (4.4 ml) and washed once with 1 ml of absolute ethanol and dried overnight *in vacuo* giving 0.114 g (63%) of **1f**: mp 143–145°; ir (KBr) 3460 (NH), 1795 (C=O), 1660 cm^{-1} (C=O); nmr (TFA) δ 7.50 (s, 5 H, Ph), 6.85 (s, H, NH), 5.55 (s, 2 H, $-CH_2Ph$), 4.8 ppm (m, 3 H, $-CH_2CH-$); R_f^{TBK} 0.63 (uv +, I −, N −); $[\alpha]^{26}D$ +29.8° (*c* 0.5, MeOH).

α-*N*-Carbamoyl-D-cycloserine (1d). Liquid HF was poured from a precooled HF cylinder into a 50-ml polyethylene centrifuge tube and the inorganic residue allowed to settle. The acid (10 ml) was decanted into a second 50-ml polyethylene centrifuge tube containing 0.558 g (2 mmol) of **1f** and 0.432 g (4 mmol) of anisole. The reaction was continued for 30 min at 0° with frequent stirring and the HF was removed in a stream of nitrogen. The residue was washed three times with 10 ml of anhydrous ether and dried *in vacuo* overnight giving a crude yield of 0.290 g. The brown solid was extracted three times with 14 ml of methylene chloride giving 0.279 g of pure **1d** (96%): mp 167–170° dec; ir (KBr) 3460 (NH), 3320 (NH), 1730 (C=O), 1680 cm^{-1} (C=O); nmr (TFA) δ 7.00 (s, H, -NHCO-), 4.85 ppm (m, 3 H, $-CH_2CH-$); R_f^{MPW} 0.45 (NP +, N −, I +, Fe +); $[\alpha]^{26}D$ + 38.7° (*c* 1, 1 *N* HCl).

α-*N*-Carbobenzyloxy-2-carbamoyl-D-cycloserine (1e). A solution of 0.945 g (4 mmol) of *N*-carbobenzyloxy-D-cycloserine in 32 ml of 50% ethanol-water was cooled to 10° and adjusted to pH 5, and 0.36 g (4.5 mmol) of KCNO was added slowly with stirring. The pH was maintained at 5 for 4 hr during which time 1e crystallized from solution. With the temperature maintained at 10°, 32 ml of water was added to the suspension over 1 hr. The white crystalline product was recovered by filtration, washed twice with 10 ml of water, and dried *in vacuo* overnight. The yield was 0.80 g (72%): mp 120–122°; ir (KBr) 3425 (NH), 3290 (NH), 1735 (C=O), 1690 cm^{-1} (C=O); nmr (TFA) δ 7.45 (s, 5 H, -Ph), 7.10 (m, 3 H, -NH-, $-NH_2$), 5.35 (s, 2 H, $-CH_2Ph$), 4.90 ppm (m, 3 H, CH_2CH-); $[\alpha]^{20}D$ +48.3° (*c* 2, MeOH).

2-Carbamoyl-D-cycloserine Hydrobromide (1b). A solution of 2.79 g (10 mmol) of 1e in 42 ml of glacial acetic acid was added to 42 ml of 1 *N* HBr in glacial acetic acid with stirring. After 5 hr, the mixture was poured into 420 ml of anhydrous ether and stirred 10 min, and the precipitate was recovered by filtration. The brown solid was washed once with 10 ml of ether and dried overnight *in vacuo* giving 2.05 g (95%) of crude product. The product was recrystallized from 80 ml of methanol by the slow addition of 125 ml of ether. The product was washed once with 25 ml of ether-methanol (9:1) giving 1.29 g (63%) of **1b**: mp 181–183° dec; ir (KBr) 3420 (NH), 3260 (NH), 1740 (C=O), 1710 (C=O), 1690 cm^{-1} (C=O); nmr (TFA) δ 7.10 (s, 2 H, $-CONH_2$), 5.15 ppm (m, 3 H, $-CH_2CH-$); R_f^{MPW} 0.41 (NP +, N +, I +); $[\alpha]^{20}D$ +37.2° (*c* 2, MeOH).

***N*,2-Dicarbamoyl-D-cycloserine (1c).** A. From D-Cycloserine (1a). To a solution of 1.01 g of KCNO (12.5 mmol) in 15 ml of water at pH 4 was added 0.51 g (5 mmol) of 1a. The addition was completed within 5 min while the pH was allowed to rise to 5.5. The reaction was allowed to continue for 50 min after which time the reaction mixture was passed through a Bio-Rad AG11A8 ion retardation column (1.8 × 45 cm). The product was found between 60 and 80 ml of effluent as indicated by external titration with $FeCl_3$ (10% in 1% HCl) and the effluent was lyophilized giving 0.64 g of crude 1c (68%). The crude 1c was dissolved in 54 ml of water and diluted with 120 ml of absolute ethanol followed by 240 ml of anhydrous ether, and the resulting precipitate was recovered by centrifugation and washed once with ether-ethanol (2:1). The white powder was dried overnight *in vacuo* giving 0.49 g of 1c (52%): mp 175–178° dec; ir (KBr) 3480 (NH), 3310 (NH), 1665 (C=O), 1632 (C=O), 1565 cm^{-1} (C=O); nmr (TFA) δ 7.05 (s, H, -NHCO-), 4.85 ppm (m, 3 H, $-CH_2CH-$); R_f^{MPW} 0.34,

Table I. Anticancer Screening Data[a]

Compd[b]	Slope[c]	ED_{50}[d]
1a	−0.67	42
Hydroxyurea	−1.05	2.4
1b	−0.70	49
5	−1.07	3.8

[a] Screening data obtained by the Drug Research and Development, National Cancer Institute. [b] Compounds were run in cell culture of human epidermoid carcinoma of the nasopharynx. [c] Change of response *vs.* change of log dosage. [d] μg/ml.

‡Similar results were obtained when cycloserine was treated with *p*-nitrobenzaldehyde; *cf.* ref 9.

R_f^{BAW} 0.45 (NP +, I +, Fe +, N −). Elemental analysis showed the presence of KCl.

B. From 2-Carbamoyl-D-cycloserine Hydrobromide (1b). To a solution of 0.51 g of KCNO (6.3 mmol) in 15 ml of water was added 1.13 g (5 mmol) of **1b**. The work-up procedure which followed was identical with that in part A and the final yield of **1c** was 0.41 g (44%): mp 174–177° dec; R_f^{MPW} 0.36, R_f^{BAW} 0.45 (NP +, I +, Fe +, N −); identical spectrally with **1c** prepared in part A.

C. From *N*-Carbamoyl-D-cycloserine (1d). To a solution of 0.51 g of KCNO (6.3 mmol) in 15 ml of water was added 0.74 g (5 mmol) of **1d**. The work-up procedure which followed was identical with that in part A and the final yield of **1c** was 0.47 g (50%): mp 174–177° dec; R_f^{MPW} 0.36, R_f^{BAW} 0.45 (NP +, I +, Fe +, N −); identical spectrally with **1c** prepared in part A.

***O*-Ureido-D-serine (2). A. From β-Aminooxy-D-alanine (3).** A solution of 6.24 g (40 mmol) of β-aminooxy-D-alanine in 17 ml of water was cooled to 10°, adjusted to pH 5, and mixed slowly with 3.60 g (44.5 mmol) of KCNO. The pH of the solution was maintained at 5 for 20 min and the reaction allowed to proceed for 4 hr. Immediately thereafter, the solution was acidified to pH 2.5, filtered, and percolated through a Dowex 50W-X8 (39 × 1.7 cm, H^+ form) column. After a preliminary water wash, the initial effluent from a 4 *N* NH_3 wash was collected and the sample recovered by lyophilization yielding 4.72 g (70%) of **2**. The crude product was redissolved in 14 ml of water and precipitated by the slow addition of 700 ml of ethanol followed by 700 ml of ether. The suspension was stirred for 2 hr to clarify the supernatant and the product was recovered by centrifugation and washed twice with 50-ml portions of ether. This product was recrystallized from water–ethanol and dried overnight *in vacuo* affording 2.70 g (40%): mp 198–200° dec; ir (KBr) 3380 and 3200 (NH), 1655 cm^{-1} (C=O); nmr (TFA) δ 4.72 (s, 3 H, $-CH_2CH-$), 8.00 ppm (s, 3 H, $-NHCONH_2$); $[\alpha]^{28}D$ 12.4° (*c* 2, 1 *N* HCl); R_f^{BAW} 0.26 (N), R_f^{TBK} 0.17 (N).

B. From 2-Carbamoyl-D-cycloserine Hydrobromide (1b). A 1% solution of **1b** in water was stirred at room temperature for 12 hr and samples for chromatography were taken at varying time intervals and compared with authentic *O*-ureido-D-serine (**2**). It was found that **1b** gradually turns into a ninhydrin-positive nitroprusside-negative substance of R_f 0.25 in BAW and R_f 0.17 in TBK, identical with **2** as prepared in part A.

***cis*-3,6-Bis(ureidooxymethyl)-2,5-piperazinedione (5). A. From D-Cycloserine Dimer 6.** To a solution of 8 g (30 mmol) of **6** in 23 ml of water was added 8 g (99 mmol) of KCNO followed by 8.16 ml of concentrated HCl. The reaction mixture was stirred for 15 hr at 10°, warmed to 30°, and recooled to 4°, and the white precipitate was recovered by centrifugation. The product was washed once with 240 ml of 90% ethanol and dried overnight *in vacuo* giving 7.96 g (70%) of **5**. A portion of this compound (5.91 g) was dissolved in 160 ml of 0.8% acetic acid at 75°, clarified by centrifugation, and finally cooled to 4°. The white precipitate was recovered by centrifugation, washed once with 90% ethanol, and dried overnight *in vacuo* giving 4.88 g (82%): mp 219–220° dec; ir (KBr) 3400 and 3290 (NH), 1670 cm^{-1} (C=O); nmr (TFA) δ 8.45 (s H, -NH-), 4.65 ppm (d, 3 H, $-CH_2CH-$); $[\alpha]^{23}D$ +71.6° (*c* 0.5, H_2O); R_f^{BAW} 0.43 (I).

B. From 2-Carbamoyl-D-cycloserine Hydrobromide (1b). A solution of 0.226 g (1 mmol) of **1b** in 11 ml of water was stirred for 15 hr at pH 6. The white precipitate was isolated by centrifugation, washed with 5 ml of 90% ethanol, and dried for 10 hr *in vacuo* giving 0.094 g (65%) of **5**. The compound exhibited an R_f^{BAW} of 0.42 (I) identical with **1b** prepared in part A.

D-5-(Ureidooxymethyl)hydantoin (4c). A 0.5-g (2.66 mmol) sample of **1c** containing some residual KCl was added to 5 ml of trifluoroacetic acid (TFA) and stirred at ambient temperature 12 hr. The reaction mixture was concentrated *in vacuo* at 50°. Isopropyl alcohol (15 ml) was added to the residue and the mixture refluxed for 0.5 hr and filtered, and the filtrate, when cooled, gave a white precipitate which was collected by centrifugation and dried overnight *in vacuo* to yield 0.22 g (29%) of the trifluoroacetate salt of **4c**: mp 72–80° dec; R_f^{BAW} 0.56 (uv −, I +, NP + red); mass spectrum *m/e* (rel intensity) 112 (52), 76 (5), 69 (24), 45 (88), 44 (68), 43 (56), 41 (100), 64 (33), 28 (52), and 27 (23). The 2-propanol insoluble solid can be again treated with TFA to afford, after 2-propanol extraction, another 30% of product. Further repetition of this procedure increased the yield somewhat.

The trifluoroacetate salt was dissolved in a minimal amount of water (~1 ml) and allowed to stand at ambient temperature for 3 hr during which time a white precipitate formed which was collected by centrifugation and washed twice with absolute ethanol and dried overnight *in vacuo* to give 0.13 g (91%) of **4c**: mp 92–105° dec; ir (Nujol) 3175 (NH), 1798 (C=O), 1720 (C=O), 1678 cm^{-1} (C=O); pmr (TFA) δ 3.6 (m, 2 H, $=CH_2$), 3.82 (m, 1 H, -CH), 6.64 (s, 1 H, -CONHCO-), 8.55 (s, 1 H, NH), 9.33 (s, 1 H, -ONH), 11.26 ppm (s, 2 H, $-CONH_2$); R_f^{BAW} 0.56 (uv −, I +, NP + red); $[\alpha]^{26}D$ +14.4° (*c* 1, H_2O).

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