Studies on Synthesis and Anticancer Activity of Selected *N*-(2-Fluoroethyl)-*N*-nitrosoureas

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An activated carbamate, 2-nitrophenyl (2-fluoroethyl)nitrosocarbamate (**3**), was used to advantage in the synthesis of the water-soluble (2-fluoroethyl)nitrosoureas **6a–d** from 2-aminoethanol, (1α,2β,3α)-2-amino-1,3-cyclohexanediol, *cis*-2-hydroxycyclohexanol, and 2-amino-2-deoxy-D-glucose. In a variation of this method, 2,4,5-trichlorophenyl (2-fluoroethyl)carbamate (**4**) was used to prepare the urea from which the essentially water-insoluble *N'*-(2,6-dioxo-3-piperidinyl)-*N*-(2-fluoroethyl)-*N*-nitrosourea (**6e**) was derived. The anticancer activity of these nitrosoureas was determined against the murine tumors B16 melanoma and Lewis lung carcinoma and found to be significant and comparable to their chloroethyl counterparts. On the basis of results from both systems, the dihydroxycyclohexyl derivative **6b** may be the most effective.

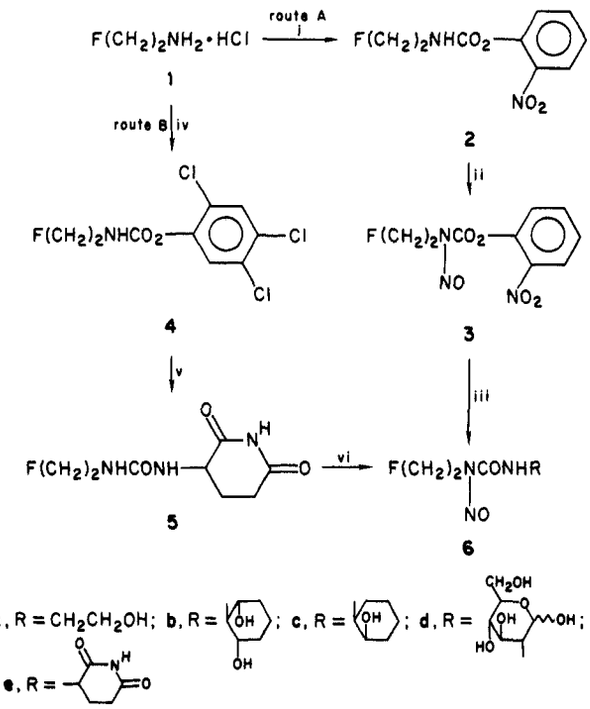
The (2-haloethyl)nitrosoureas, especially the chloro, have been studied extensively as part of the National Cancer Institute's drug development program. The (2-chloroethyl)nitrosoureas are among the most effective antitumor agents in preclinical animal tumor models and have also demonstrated some activity in human lymphomas, leukemias, and a few solid tumors.[1] According to Kohn and to Lown, the activity of (2-chloroethyl)nitrosoureas is due, in part, to their ability to form DNA interstrand cross-links both in vitro[2,3] and in cells.[4] The formation of these cross-links in thought to involve, first, the formation of a monoadduct of the 2-chloroethyl group at a nucleophilic site and, second, the nucleophilic displacement of chlorine. There is a delay of a few hours between the first alkylation and the second.

The presence of a 2-haloethyl group on the N-1 of the urea has been shown to be essential for significant antitumor activity.[5] To date, all the clinically evaluated (2-haloethyl)nitrosoureas have contained chlorine as the leaving group. The (2-fluoroethyl)nitrosoureas, however, have demonstrated antitumor activity against the experimental L1210 leukemia model equal to that of the chloro compounds.[5–7] They also form DNA cross-links, but the rate of interstrand cross-linking for the fluoro compounds is greatly reduced.[2–4] The decrease in the rate of cross-linking is due to the fact that chlorine is a better leaving group than fluorine, which indicates that (2-fluoroethyl)-nitrosoureas may exert their antitumor activity by a different mechanism.

To further assess the potential of (2-fluoroethyl)-nitrosoureas as anticancer agents, the fluoro analogues **6a–e** and **7** of selected (2-chloroethyl)nitrosoureas, four of which have been clinically evaluated, were chosen for evaluation in preclinical models. The (2-chloroethyl)-nitrosoureas **8–11** were used for comparative evaluation.

Chemistry. Previous synthesis of (2-fluoroethyl)-nitrosoureas[5–7] involved the preparation of nitrosatable (2-fluoroethyl)ureas by one of the following general methods: (1) the reaction of 2-fluoroethylamine with an isocyanate or an isocyanate generated in situ by aqueous decomposition of a methyl- or (2-chloroethyl)nitrosourea and (2) the reaction of a primary amine with 2-fluoroethyl isocyanate generated in situ by aqueous decomposition of *N'*-(2-fluoroethyl)-*N*-methyl-*N*-nitrosourea. These methods failed in prior attempts to prepare fluorozotocin (**6d**) and *N'*-(2,6-dioxo-3-piperidinyl)-*N*-(2-fluoroethyl)-*N*-nitrosourea (**6e**).[8]

Scheme I[a]



[a] i = 2-$O_2NC_6H_4OCOCl$ in Et_2O, KOH in H_2O; ii = NOCl, AcOH, Ac_2O, AcOH, P_2O_5; iii = RNH_2, EtOH, THF, PhH, CO_2; iv = 2,4,5-$Cl_3C_6H_2OCOCl$ in Et_2O, KOH in H_2O; v = 2-aminoglutarimide hydrobromide, $(i\text{-Pr})_2NEt$, EtOH; vi = N_2O_3, AcOH.

The one-step process developed as an improved synthesis of streptozotocin[9] and later extended to streptozotocin analogues[10] and certain (2-chloroethyl)nitrosoureas[11]

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Table I. Antitumor Activity of (2-Fluoroethyl)nitrosoureas against Intraperitoneally Implanted B16 Melanoma[a]

compd	MTD,[b] mg/kg	experiment 1[c] MST,[d] days	experiment 1[c] optimal % ILS[e]	experiment 1[c] day-60 survivors	experiment 2 MST, days	experiment 2 optimal % ILS	experiment 2 day-60 survivors
vehicle-treated controls		21.6		0/40	17.1		0/40
7	60	37	71	1/10	nt[g]		
6a	60	50	131	3/10	42.8	150	3/10
6b	60	57	163	4/10	*f*	*f*	5/10
6c	60	48	122	3/10	35.8	109	0/10
6d	60	50	131	3/10	36	110	2/10
6e	60	45.3	109	1/10	40.3	133	3/10

[a] Tests were conducted according to the NCI protocol previously described (Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 11). BDF_1 (experiment 1) or $B6C3F_1$ (experiment 2) mice were inoculated intraperitoneally with 0.5 mL of 1:10 B16 melanoma brei on day 0 and treated with intraperitoneally injected nitrosourea on day 2. A series of logarithmically spaced dosages were tested and each level was administered to 10 mice (40 control mice received only vehicle). [b] Maximally tolerated dose. [c] Data from experiment 1 have appeared in abstract form (see ref 19). [d] Median survival time including day-60 survivors. [e] Increased life span [(100 × MST treated mice/MST control mice) − 100]. [f] MST and optimal % ILS not attained, 5/10 mice still alive at termination of experiment on day 60. [g] Not tested.

involved displacements of azide ion from appropriate, potentially explosive nitrosocarbamic azides. This method was also used in unambiguous preparations of cycloalkylnitrosoureas.[12] Similarly, the displacement of the corresponding 2-substituted phenol from 2-nitrophenyl or 2-cyanophenyl (2-chloroethyl)nitrosocarbamate by amino derivatives of glucose gave chlorozotocin and related compounds in high yield without the need of chromatographic purification.[13] We adapted the latter method as shown in route A of Scheme I for preparative-scale synthesis of **6d** and other water-soluble (2-fluoroethyl)nitrosoureas (**6a–c**). It is a method that minimized problems of isolation and purification peculiar to water-soluble nitrosoureas. The utility of this approach was subsequently enhanced by the use of other activated nitrosocarbamates, including the (2-chloroethyl)nitrosocarbamic ester of *N*-hydroxysuccinimide.[14] The latter reagent enabled the reported synthesis of the *N'*-nitroso isomer of CCNU (**8**), which is water-insoluble and inaccessible in pure form by direct nitrosation.[15]

A different route was chosen for the synthesis of **6e** primarily in order to avoid the last-step formation of a blue-green contaminant that would result from the use of 3-amino-2,6-dioxopiperidine as the free base, which was used in our previous synthesis of the chloro analogue **11**.[5] The reaction of 3-amino-2,6-piperidinedione with 2,4,5-trichlorophenyl (2-fluoroethyl)carbamate (**4**) as indicated in route B of Scheme I afforded a good yield of the required urea (**5**) for last-step nitrosation. In view of favorable results with this sequence, we would explore the use of 2,4,5-trichlorophenyl (2-fluoroethyl)nitrosocarbamate in future synthesis of **6a–d** and related compounds instead of the 2-nitrophenyl analogue **3**, which underwent gradual denitrosation even when stored cold and dry. Moreover, an attempted vacuum distillation of crude 2-nitrophenyl chloroformate on a 2-mol scale led to uncontrolled decomposition and consequent tedious purification of the derived carbamate **2**. Aside from these disadvantages, **2** should also be an effective reagent in route B.

A standard method[16] was used for derivation of **2** and **4** from 2-fluoroethylamine hydrochloride (**1**), which was purified by distillation of the amine liberated from commerical hydrochloride. The nitrosation of **2** was accomplished with excess nitrosyl chloride in a mixture of acetic acid, acetic anhydride, dry potassium acetate, and phosphoric anhydride, which was reported to be effective in the nitrosation of aromatic carbamates where other procedures failed.[17] Nitrosyl chloride in pyridine, however, proved to be equally effective in analogous cases.[14] The nitrosocarbamate **3** reacted readily with 2-aminoethanol, (1α,2β,3α)-2-amino-1,3-cyclohexanediol, and *cis*-2-aminocyclohexanol to produce satisfactory yields of the essentially pure, corresponding nitrosoureas **6a–c**, but the reaction of **3** with 2-amino-2-deoxy-D-glucose was inexplicably sluggish and the yield of **6d** considerably diminished by recrystallization.

Antitumor Activity. The antitumor effects of *N'*-cyclohexyl-*N*-(2-fluoroethyl)-*N*-nitrosourea[5] (**7**) and the five new (2-fluoroethyl)nitrosoureas **6a–e** were compared in mice bearing the intraperitoneally implanted B16 melanoma (Table I). Following intraperitoneal administration on day 2, compounds **6a–e** demonstrated good activity, increasing the life span of the tumor-bearing mice by at least 100% and producing one or more day-60 survivors at their optimal doses. Compound **7** was slightly less ef-

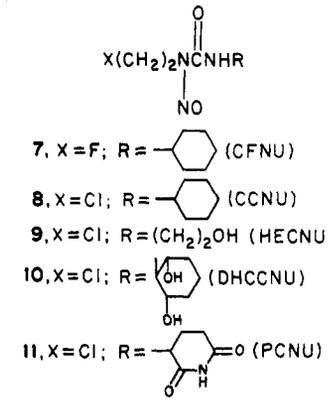

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Table II. Effect of (2-Haloethyl)nitrosoureas on Intravenously Implanted Lewis Lung Carcinoma[a]

compd	optimal dose, mg/kg	MST,[b] days postimplant	optimal % ILS[c]	day-60 survivors
vehicle-treated controls		19.2		0/40
8	32	32.0	66	1/10
7	20	44.0	129	3/10
9	6.25	*d*	*d*	9/10
6a	5	45.8	138	3/10
10	5	33.3	73	3/10
6b	10	*d*	*d*	7/10
6c	20	*d*	*d*	6/10
11	8	*d*	*d*	8/10
6e	20	*d*	*d*	9/10

[a] Nitrosoureas, prepared before each injection, were administered intraperitoneally on days 5–13 to $B6C3F_1$ mice inoculated intravenously with 10^6 Lewis Lung tumor cells on day 0. A series of logarithmically spaced dosages were tested and each level was administered to 10 mice; 40 control mice received vehicle injections. Characteristics of the iv model have been described previously (Ovejera, A. A.; Johnson, R. K.; Goldin, A. *Cancer Chemother. Rep., Part 2* **1975**, *5*, 111). [b] Median survival time including day-60 survivors. [c] Increased life span [(100 × MST treated mice/MST control mice) − 100]. [d] MST and optimal % ILS not attained, more than half the mice alive at termination of experiment on day 60.

fective, producing a maximum increase in life span of 71% and one long-term survivor in experiment 1. In both experiments, **6b** appeared to be the most active analogue on the basis of the number of day-60 survivors. The maximally tolerated dose for all six compounds was the same, 60 mg/kg. This was in contrast to the corresponding (2-chloroethyl)nitrosoureas where changing the substituent on N-3 altered the therapeutic dosage range.[18]

In order to assess further the potential therapeutic effectiveness of the fluoroethyl derivatives, **6a–c**, **6e**, and **7** were tested against the established intravenously implanted Lewis lung carcinoma in direct comparison with their corresponding clinical chloroethyl analogues (Table II). Following daily intraperitoneal administration on days 5–13, both the fluoroethyl and chloroethyl compounds demonstrated good activity, either producing increased life spans greater than 60% with at least one of ten long-term survivors or causing over half the mice to survive until the experiment was terminated on day 60. As a class, the fluoroethyl derivatives were as effective as the chloroethyl compounds although differences were noted. While **6b** and **7** were more active than **10** and **8**, respectively, **6e** and **11** gave a similar number of day-60 survivors, and **6a** was less active than **9**. The most active of the (2-fluoroethyl)-nitrosoureas appeared to be **6e** and **6b**, which gave nine of ten and seven of ten long-term survivors, respectively. Thus, on the basis of their activity in experimental tumor models in vivo, the (2-fluoroethyl)nitrosoureas are as effective as the chloro compounds and would appear to be good candidates for further development if significant differences from the chloro compounds can be established, such as reduced bone marrow toxicity, which has already been observed with **7**.[19] It would also be interesting to investigate whether the difference in the rate of DNA cross-linking that has been observed between the fluoroethyl and chloroethyl analogues in human cell lines would significantly alter the therapeutic effectiveness of nitrosoureas in the clinic.

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Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected, IR spectra with a Nicolet Fourier-transform MX-1 spectrometer, and NMR spectra with a Varian XL-100-15 spectrometer. HPLC assay was performed with a Waters ALC-242 chromatograph equipped with a 254-nm UV detector and Hewlett-Packard 3380 integrator on a μ-Bondapak C_{18} column, 300 × 4 mm. TLC was performed on Analtech silica gel GF. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Elemental analyses of **6a**, **6c**, and **6e** were performed by Galbraith Laboratories, Knoxville, TN; other elemental analyses and spectral determinations were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. William C. Coburn, Jr. Biological testing was done at Arthur D. Little, Inc., Cambridge, MA, and Southern Research Institute.

Evaporations were carried out under reduced pressure (water aspirator) with a rotary evaporator, and solutions of unstable compounds were evaporated without heat. Intermediates and final products and all analytical samples were dried in vacuo over phosphoric anhydride. Nitrosoureas were stored cold (freezer) and dry.

2-Fluoroethylamine Hydrochloride (1). The commercial hydrochloride (Aldrich, mp 90–92 °C) was purified on a 2.5-mol scale by distillation of the free amine[20] through a 25-cm Vigreux column and reconverted to 1 with ethanolic hydrogen chloride. Fractions boiling up to 62 °C and condensed with circulating ice water were combined on the basis of refractive index (lit.[20] n^{25}_D 1.3690, bp 60–62 °C) and yielded 1 (158 g, 63%) in two batches, mp 100–101.5 and 104 °C (lit.[20] mp 95 °C dec). A fraction boiling at 63–69 °C gave low-melting 1 (11 g). The best yield of distilled amine on the same scale was 73%. An aqueous solution of the free amine was best converted to 1 by acidification with concentrated hydrochloric acid, evaporation to dryness in vacuo, and extraction into hot ethanol.

2-Nitrophenyl Chloroformate. A solution of sodium methoxide (108 g, 2 mol) in methanol (1 L) was slowly added to a solution of 2-nitrophenol (278 g, 2 mol; Eastman) in methanol (2 L) with exclusion of moisture. The resulting mixture was cooled for 2 h, and the red sodium 2-nitrophenoxide was collected in three crops (by successive concentrations), washed lightly with methanol, and dried in a vacuum oven at 130 °C: yield 277 g (86%). To a stirred solution of phosgene in toluene (950 mL), saturated at −20 °C, was added at 0 °C over a period of 3.5 h the above sodium salt (277 g, 1.72 mol) in small portions while a slow flow of phosgene was maintained.

The mixture was slowly stirred under nitrogen overnight while warming to ambient temperature, and excess phosgene was removed with a stream of nitrogen (2 h). The mixture was filtered and the filtrate concentrated in vacuo at 40 °C: yield 315 g (91%), which was used as such in conversion to the carbamate **2**. The chloroformate produced in a pilot run was distilled and redistilled, bp 106–107.5 °C (0.9 mm) [lit. bp 131.5–132.5 °C (7 mm),[21] 105 °C (2 mm)[22]], but attempted distillation on a larger scale (1.8 mol) resulted in uncontrolled decomposition.

2-Nitrophenyl (2-Fluoroethyl)carbamate (2). A mixture, quickly prepared at 0–5 °C, of 0.220 mol each of distilled 2-nitrophenyl chloroformate (44.3 g), dissolved in ether (300 mL), and 1 (21.9 g), dissolved in water (180 mL), was stirred and kept below 5 °C while a precooled solution of potassium hydroxide (24.7 g, 0.440 mol) in water (220 mL) was added dropwise. After 45 min, the ether layer was separated and the aqueous layer extracted further with ether (2 × 500 mL). The ether layers were combined, dried ($MgSO_4$), treated with Norit, and filtered through Celite. The filtrate was concentrated to near dryness and chilled. The resulting solid was collected, washed with a little cold ether and hexane, dried, and recrystallized by dissolving in boiling hexane with a few drops of ethanol and cooling: yield 29.4 g (59%); mp

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99–100 °C; TLC (99:1 $CHCl_3$–MeOH, silica gel, UV) single spot, R_f 0.53.

Undistilled 2-nitrophenyl chloroformate gave on a 22-mmol scale 3.28 g (65%) of **2**, mp 98–99 °C, which was judged (TLC) suitable for nitrosation, but **2** (80 g, mp 89–90 °C) obtained on a 1.0-mol scale was appreciably contaminated by, as identified by TLC and melting point, bis(2-nitrophenyl) carbonate,[23] *N,N*-bis(2-fluoroethyl)urea,[5] 2-nitrophenol, and other impurities. Additional crude **2** (51.3 g, mp 92–94 °C) was obtained by extracting the original aqueous layer with dichloromethane (2 × 1 L) and cooling after concentration to near dryness. The crude products were combined and recrystallized from benzene–hexane (yielding 125 g) and then from dichloromethane–hexane in 13 crops. Only those crops (total 89 g) with melting point ≥98 °C (Kofler Heizbank) and favorable TLC were combined for the next step, but all crops showed contamination of varying degrees. A smaller run (0.248 mol) with undistilled chloroformate produced 28.6 g (51%) of satisfactory **2** (mp, TLC), isolated as follows. The solid present in the cold reaction mixture was collected and dissolved in ether, and the filtered solution was evaporated, giving 15.0 g, mp 99 °C. The combined ether extracts yielded 10.5 g, mp 99–100 °C, and 3.1 g, mp 98 °C.

2-Nitrophenyl (2-Fluoroethyl)nitrosocarbamate (3). Dry potassium acetate (130 g, 1.33 mol) and phosphoric anhydride (~2 g) were added to a mechanically stirred solution of **2** (121 g, 0.530 mol) in acetic acid (1.7 L) and acetic anhydride (850 mL). To this solution, kept at 5–10 °C, was added dropwise a solution of nitrosyl chloride (86.8 g, 1.33 mol) in acetic anhydride (130 mL). After 1 h, the absence of **2** was indicated by TLC (99:1 $CHCl_3$–MeOH, silica gel, UV). The mixture was poured into ice–water (6.5 L), stirred several minutes, and let stand for several minutes. The yellow oil that separated was dissolved in dichloromethane (1.5 L) and the solution washed successively with cold water (300 mL), 6% sodium carbonate solution (300 mL), saturated sodium bicarbonate solution (2 × 300 mL), and cold water (2 × 300 mL). The dichloromethane layer was dried ($MgSO_4$) and evaporated in vacuo to constant weight. The residual yellow oil, partially solidified in a freezer, was cooled in a dry ice bath and, while cold, triturated in ice water (total of 1 L); the hard mass was broken up, collected, and dried in vacuo: yield 101 g (74%); mp 29–31 °C. This material, essentially homogeneous by TLC, was kept dry and in a freezer until used.

(1α,2β,3α)-2-Nitro-1,3-cyclohexanediol. The reported method[24] was modified as in the preparation of DHCCNU (**10**).[25] The reaction mixture obtained by sodium carbonate promoted condensation of glutaraldehyde (100 mL of 25% aqueous solution, 0.25 mol; Aldrich) with nitromethane in cold methanol–water was let stand at room temperature for 4 h, neutralized with acetic acid (~80 mL), and concentrated in vacuo at 30–35 °C to yield a residual dark-brown mixture, which was extracted with ether (8 × 200 mL). Concentration of the dried (Na_2SO_4) and decolorized extracts (Norit) under reduced pressure left the diol, which was twice stirred in cold ethyl acetate and collected after chilling to −10 °C: yield 22.7 g (56%); mp 162–163 °C (lit.[26] mp 161.5–163.5 °C dec); ^1H NMR (Me_2SO-d_6) δ 1.0–2.0 [m, 6, $(CH_2)_3$], 3.5–3.95 (m, 2, C*H*OH), 3.95–4.25 (t, 1, $CHNO_2$), 5.3–5.5 (d, 2, CHO*H*, exchanged with D_2O). Larger runs in which addition times were longer and reaction mixtures were stirred 4 h at room temperature produced lower yields: e.g., 44% from a threefold scale-up. The yield from a 1.5-fold scale-up with an 18-h reaction time was 11% from 4:1 EtOH–PhH, mp 165.5–167 °C).

(1α,2β,3α)-2-Amino-1,3-cyclohexanediol. The hydrogenation (Parr shaker, 50 psi) of the above nitrocyclohexanediol (24.9 g, 0.154 mol) in ethanol (200 mL) over Raney nickel was modeled after that already reported[26] and gave 14.2 g (70%) of the amine, mp 195–196 °C (lit.[26] mp 191.5–193.5 °C). The product, which was collected after concentration of the filtered reaction mixture to near dryness, was washed with cold ethanol.

cis-2-Aminocyclohexanol. The preparation of *trans*-2-aminocyclohexanol hydrochloride from cyclohexene oxide and its three-step inversion to the cis hydrochloride followed reported procedures.[27,28] Sodium hydroxide solution (1 N, 283 mL) was added dropwise to a cold (ice-salt bath), stirring suspension of the cis hydrochloride (42.9 g, 0.283 mol) in ethanol (500 mL) so that the mixture was at ~10 °C. The resulting solution was filtered and evaporated to dryness in vacuo at ~35 °C with an addition of ethanol. The residue was ground up, dried further, and extracted with chloroform (2 × 300 mL). The extract was filtered and evaporated to dryness in vacuo, leaving the free base: yield 30.1 g (92%); mp 76.5–78.5 °C [lit. mp: 71–72 °C;[28] 72–73 °C;[29] 68–70 °C initially and, after numerous recrystallizations from dry ether, 107–108 °C;[30] and 73–75 °C[31]].

Typical Procedure: N-(2-Fluoroethyl)-N′-(2-hydroxyethyl)-N-nitrosourea (6a). Dry ice (~17 g, small uncrushed pieces) was added all at once to a cold (0.5 °C), stirring solution of 2-aminoethanol (4.90 mL, 81.2 mmol; Fluka) in absolute ethanol (300 mL). Thirty-milliliter portions of this solution, kept cold in an ice-salt bath, were removed under nitrogen and added dropwise but rapidly to a cold (−6 to −3 °C), stirring solution of **3** (25.0 g, 97.2 mmol) in an anydrous mixture of tetrahydrofuran (300 mL) and benzene (80 mL) over a period of 70–80 min. The storage flask and dropping funnel were rinsed with ethanol (~20 mL) and the rinsings added to the stirring reaction mixture, which was then allowed to warm to room temperature. Aliquots were tested for unchanged 2-aminoethanol at 1-h intervals by TLC (9:1 $CHCl_3$–MeOH, silica gel, ninhydrin). After 2 h, the reaction mixture was concentrated in vacuo (water aspirator and then oil pump at room temperature) to an oil, which was stirred and shaken with 500- and 250-mL portions of hexane until solid and free of 2-nitrophenol, one of two UV-absorbing contaminants detected by TLC. The solid was dried in vacuo and then triturated once with 300 mL and twice with 200 mL of carbon tetrachloride until free of **2**, the second contaminant: yield of **6a** 10.4 g (72%); mp 39.5–42.5 °C dec; IR (KBr) 3600–3100 (OH, NH), 3100–2800 (CH), 1710 (C=O), 1540 (CNH), 1473 (N=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.2–3.7 (m, 4, CH_2CH_2OH), 3.9–4.3 (m, 2, FCH_2CH_2), 4.1–4.7 (m, 2, FCH_2CH_2), 4.74 (t, 1, OH; exchanged with D_2O), 8.57 (t, 1, NH; exchanged with D_2O); TLC (9:1 $CHCl_3$–MeOH, silica gel, UV) single spot, R_f 0.57. Anal. ($C_5H_{10}FN_3O_3$) C, H, N.

N′-[(1α,2β,6α)-2,6-Dihydroxycyclohexyl]-N-(2-fluoroethyl)-N-nitrosourea (6b). The reaction of (1α,2β,3α)-2-amino-1,3-cyclohexanediol (5.8 g, 52 mmol; dry ice, 11 g) with **3** (16 g, 62 mmol) as in the preparation of **6a** was complete after 2 h at room temperature (TLC, 9:1 $CHCl_3$–MeOH, silica gel, ninhydrin) and, after an additional hour, was evaporated to dryness and the solid residue triturated and washed with ether to remove much of the yellow color: yield 10.1 g (78%); mp 128–129 °C dec; IR (KBr) 3700–3100 (OH, NH), 3050–2800 (CH), 1690 (C=O), 1555 (CNH), 1500 (N=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.0–2.0 [m, 6, $(CH_2)_3$], 3.1–3.7 (m, 3, H_1, H_2, H_6 of ring), 3.8–4.3 (m, 2, FCH_2CH_2), 4.1–4.8 (m, 2, FCH_2CH_2), 4.5–4.8 (m, 2, OH; exchanged with D_2O), 8.1–8.4 (m, 1, NH; exchanged with D_2O); TLC (9:1 $CHCl_3$–MeOH, silica gel, UV) single spot, R_f 0.55; ninhydrin negative. Anal. ($C_9H_{16}FN_3O_4$) C, H, N.

N′-(2-Fluoroethyl)-N′-(cis-2-hydroxycyclohexyl)-N-nitrosourea (6c). The reaction of *cis*-2-aminocyclohexanol (6.3 g, 55 mmol; dry ice, 12 g) with **3** (17 g, 66 mmol) as in the preparation of **6a** was complete after 2.5 h. Concentration gave an oil, which was washed several times with hexane (450 mL) to remove 2-nitrophenol and excess **3**. The resulting solid was dried (6.1 g) and washed with hexane (50 mL) containing a few drops of ethanol, leaving 4.7 g, mp 55.5–58 °C. Filtrates deposited three crops of **6c**, two of which were free of a UV-absorbing impurity, **2**, according to TLC (R_f 0.6): 0.6 g, mp 50–54 °C; 0.3 g, mp 58–60

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°C. These homogeneous samples were combined (5.6 g), dissolved in methanol (60 mL), treated with Norit, and concentrated to a pale-yellow oil, which was triturated in hexane until solid: 4.7 g, mp 56.5–58 °C. The original hexane washings deposited crystals (2.7 g, mp 59–61 °C), which were also free of UV-absorbing impurities: total yield 58%; IR (CCl_4 soln) 3635 (OH), 3425 (NH), 2985 (sh), 2940, 2910 (sh), 2860 (CH), 1730 (C=O), 1520 (CNH), 1490 (N=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.0–2.0 [m, 8, $(CH_2)_4$], 3.6–4.1 (m, 1, CHOH), 3.8–4.4 (m, 2, FCH_2CH_2), 4.1–4.8 (m, 2, FCH_2CH_2), 4.8–5.0 (d, 1, OH; exchanged with D_2O), 7.7–8.0 (d, 1, NH; exchanged with D_2O), similar to *cis*-2-hydroxy-CCNU[25] in 1.0–2.0 region, supporting cis configuration;[7] TLC (99:1 $CHCl_3$–MeOH, silica gel, UV) single spot, R_f 0.29. Anal. ($C_9H_{16}FN_3O_3$) C, H, N.

2-Deoxy-2-[[[(2-fluoroethyl)nitrosoamino]carbonyl]amino]-D-glucose (6d). The reaction of an ethanolic suspension of 2-amino-2-deoxy-D-glucose[31] (8.8 g, 49 mmol) as the carbonate (from dry ice, 11 g) and **3** (15 g, 59 mmol), unlike typical examples, was not complete in 2–3 h; the mixture was stirred intermittently at room temperature and at 35–40 °C (total of 16 h) during 2 days and evaporated to dryness. The tan residue was triturated in ether and dissolved in warm methanol (700 mL); the solution was treated with Norit, concentrated to near dryness, and diluted with ether (150 mL) while being cooled. The solid (9.7 g) was recrystallized from ethanol (~410 mL): yield 4.4 g (30%); mp 149 °C dec; IR (KBr) 3650–3100 (OH, NH), 3000–2850 (CH), 1690 (C=O), 1550 (CNH), 1495 (N=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.0–3.9 (m, 6, H_{2-5}, 2 H_6), ~4.1 (m, 2, CH_2N), 4.44 (t, 1, 6-OH), ~4.4 (m, 2, CH_2F), 4.8–5.1 (m, 2, 3-OH, 4-OH), 5.14 (d, 1, H_1), 6.62 (d, 1, 1-OH), 7.8 (d, 1, NH); TLC (4:1 $CHCl_3$–MeOH, silica gel, UV) single spot, R_f 0.44, with some streaking. HPLC assay [mobile phase 97:3 0.1 M AcOH (pH 4.0)–MeOH][33] showed an anomeric mixture with the slower eluting fraction, probably α, predominating; earliest reading indicated a ratio of ~14:86; and equilibrium (54:46) was reached in 60–90 min. Anal. ($C_9H_{16}FN_3O_7$) C, H, N.

2,4,5-Trichlorophenyl (2-Fluoroethyl)carbamate (4). A general procedure[16] much like that for **2** was followed for the conversion of hexane-extracted, vacuum-distilled 2,4,5-trichlorophenyl chloroformate (49.0 g, 0.189 mol; Aldrich) to **4**. The dried ($MgSO_4$) ether extract was evaporated to dryness and the residue stirred in hexane (700 mL) for 25 min: yield of **4** 44.7 g (85%); mp 127.5–129.5 °C [analytical sample, mp 126–128 °C (from EtOH–hexane)[34]]; TLC (99:1 $CHCl_3$–MeOH, silica gel, UV) one major spot, R_f 0.81, and a slower moving, very minor spot. Anal. ($C_9H_7Cl_3FNO_2$) C, H, N.

DL-3-Amino-2,6-piperidinedione Hydrobromide.[35] Hydrogen bromide was bubbled into a stirring suspension of benzyl DL-(2,6-dioxo-3-piperidinyl)carbamate[36] (30.2 g, 0.115 mol) in nitromethane (168 mL) for several hours, the disappearance of starting material being monitored by TLC (95:5 $CHCl_3$–MeOH, silica gel, UV). The product was washed with ether: yield 22.0 g (92%); mp 273–276 °C (mp of analytical sample 274–277 °C).[37]

N-(2,6-Dioxo-3-piperidinyl)-N'-(2-fluoroethyl)urea (5). To a stirring solution of *N,N*-diisopropylethylamine (13.9 g, 108 mmol; Aldrich) in ethanol (100 mL) was added first DL-3-amino-2,6-piperidinedione hydrobromide (20.7 g, 98.9 mmol) and ethanol (200 mL) and then **4** (28.4 g, 99.2 mmol) and more ethanol (450 mL). Near complete solution occurred before precipitation began. The mixture was stirred overnight and the precipitate (16.9 g) recrystallized from ethanol (dissolved in 500 mL, solution filtered and concentrated to 300 mL), giving a slightly purplish first crop (14.3 g, mp 163.5–166.5 °C). Concentration of the reaction mixture filtrate in vacuo yielded, when diluted with ethanol (100 mL), crude additional **5**, which was recrystallized from acetonitrile: 0.9 g, mp 162–164 °C. These first crops were essentially free of 2,4,5-trichlorophenol according to TLC (9:1 $CHCl_3$–MeOH, silica gel, UV; product spot, R_f 0.40) and represented a 71% yield; other crops were less pure. An analytical sample was obtained from a pilot run in which the reaction mixture was warmed at 40 °C for 45 min and stirred overnight: mp 161–163 °C (from EtOH); IR (KBr) 3360 (amide NH), 3170, 3090 (imide NH), 1700, 1655, 1610 (C=O), 1580, 1530 (CNH) cm^{-1} (a spectrum very similar to that of the 2-chloroethyl analogue).[5] Anal. ($C_8H_{12}FN_3O_3$) C, H, N.

N'-(2,6-Dioxo-3-piperidinyl)-N-(2-fluoroethyl)-N-nitrosourea (6e, PFNU). An excess of dinitrogen trioxide (>>14.1 g, Matheson) was bubbled into a stirring, filtered solution of **5** (17.2 g, 80.6 mmol) in acetic acid (185 mL). As nitrosation proceeded, the reaction mixture temperature, initially at 25 °C and rising to ~28 °C, was lowered by an ice bath to as low as 8 °C. After 30 min, the mixture was diluted with cold water (1.4 L) and excess nitrosating agent removed in a stream of nitrogen. The crude, light-yellow product was collected, dried some in vacuo, dissolved in boiling ethanol (4.5 L), and allowed to crystallize from the filtered solution, which stood overnight and was chilled: yield 12.8 g (66%), mp 163–164 °C dec; IR (KBr) 3340 (amide NH), 3200, 3100 (imide NH), 1730, 1700, 1685, 1670 (C=O), 1545 (CNH), 1508 (N=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.8–2.6 (m, 2, CH_2CH_2CO), 2.3–3.1 (m, 2, CH_2CH_2CO), 3.9–4.4 (m, 2, FCH_2CH_2), 4.1–4.8 (m, 2, FCH_2CH_2), 4.7–4.95 (m, 18 CH), 8.9–9.15 (d, 1, amide NH), 10.8 (s, 1, imide NH); TLC (3:1 $CHCl_3$–Me_2CO, silica gel, UV, Griess reagent) one spot, R_f 0.47; essentially insoluble in water. Anal. ($C_8H_{11}FN_4O_4$) C, H, N.

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Registry No. **1**, 406-34-8; **1**·HCl, 460-08-2; **2**, 91390-32-8; **3**, 91390-33-9; **4**, 91390-34-0; (±)-**5**, 91390-35-1; **6a**, 79955-35-4; **6b**, 91390-36-2; **6c**, 91390-37-3; α-**6d**, 91390-38-4; β-**6d**, 91390-40-8; (±)-**6e**, 91390-39-5; *o*-$NaOC_6H_4NO_2$, 824-39-5; $ClC(O)O$-*o*-$C_6H_4NO_2$, 50353-00-9; NO_2CH_3, 75-52-5; $NH_2CH_2CH_2OH$, 141-43-5; glutaraldehyde, 111-30-8; (1α,2β,3α)-2-nitro-1,3-cyclohexanediol, 38150-01-5; (1α,2β,3a)-2-amino-1,3-cyclohexanediol, 38332-12-6; *cis*-2-aminocyclohexanol, 931-15-7; 2-amino-2-deoxy-D-glucose, 3416-24-8; 2,4,5-trichlorophenyl chloroformate, 16947-69-6; benzyl DL-(2,6-dioxo-3-piperidinyl)carbamate, 6398-07-8; DL-3-amino-2,6-piperidinedione hydrobromide, 2686-86-4.

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