Synthesis of the Antileukemic Compound N,N''-[5-[Bis(2-chloroethyl)amino]-1,3-phenylene]bisurea

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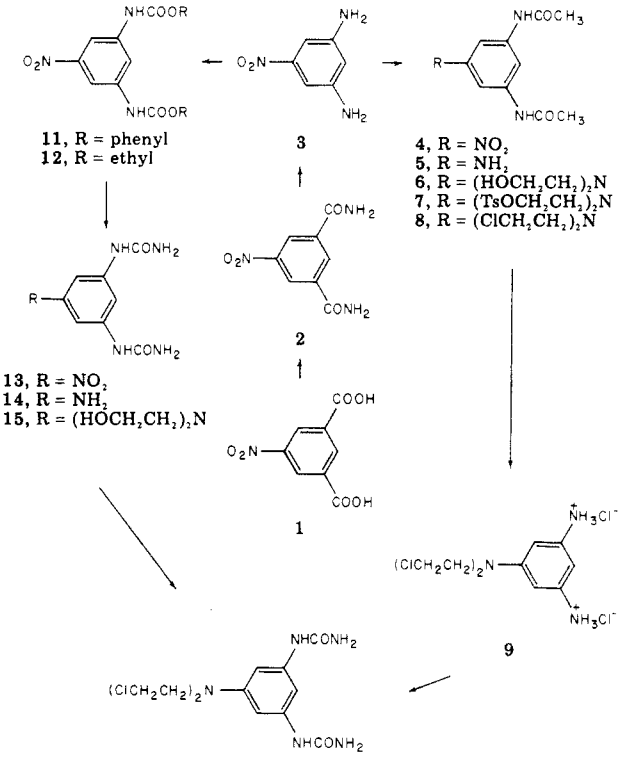
Conversion of 5-nitro-1,3-benzenedicarboxylic acid (1) to the diamide 2 followed by hypochlorite rearrangement to the diamine 3 and subsequent reaction with acetic anhydride gave the bisacetamide 4. Reduction to the amine 5 followed by treatment with ethylene oxide formed the diol 6. The latter was converted to the bistosylate 7, which underwent facile displacement with lithium chloride in acetone to give the mustard 8. Removal of the acetyl groups with hydrochloric acid gave 9, which reacted with potassium cyanate to provide the bisurea 10. In an alternative, but less satisfactory synthesis of 10, the compound (5-nitro-1,3-phenylene)biscarbamic acid diphenyl ester (11), or the corresponding diethyl ester 12, was converted by ammonolysis to 13. The nitrodiurea 13 was next reduced to the amine 14, the hydrochloride of which reacted with ethylene oxide to give the diol 15. Treatment of the latter in dimethylformamide with *N*-chlorosuccinimide in the presence of triphenylphosphine gave 10 in low yield. The nitrogen mustards 8, 9, and 10 showed significant antitumor activities against P388 lymphocytic leukemia in mice.

It has been reported that the aromatic nitrogen mustard 4-[bis(2-chloroethyl)amino]phenylurea[1] was effective in combination therapy with 4-[bis(2-chloroethyl)amino]-fluoroacetylaminobenzene, causing a high proportion of regressions in established tumors.[2] The objective of the present work was to prepare for biological testing a derivative of aniline mustard in which two ureido functions are located in positions meta to the bis(2-chloroethyl)-amino moiety, the potential advantages of this orientation having been discussed previously.[3,4]

Recent syntheses of multifunctional aromatic nitrogen mustards have indicated continuing research in this area.[5,6] Of particular interest were methods described for the synthesis of a mustard from picramic acid,[7] in which either ethylene oxide or 2-chloroethanol was used to introduce the bis(2-hydroxyethyl) moiety onto an amino function (in picramic acid) subject to deactivating influences. Numerous attempts to convert 3,5-dinitroaniline into its *N,N*-bis(2-hydroxyethyl) derivative (a possible intermediate for the present synthesis) under similar conditions were unsuccessful.

Attention was next given to synthetic pathways involving compound 3. Two routes were explored, the more successful starting with acetylation to 4 in the presence of excess acetic anhydride and then catalytic reduction of the nitro group to give the amine 5. Treatment of the latter in aqueous acetic acid with a fivefold molar excess of ethylene oxide gave the diol 6. Direct replacement of the hydroxyl groups was next attempted using thionyl chloride, which caused extensive degradation and gave no recognizable products. The use of chlorinating agents was therefore abandoned in favor of prior bistosylation of 6 followed by displacement with lithium chloride in acetone, a modification of the synthetic method described by Werner.[8] The same conversion (7 to 8) using the system calcium chloride in 2-ethoxyethanol gave lower yields (26 vs. 63%) and involved a troublesome stability problem during filtration of the crude product. Subsequent hydrolysis of the diacetyl mustard 8 to the diamine dihydrochloride 9 was performed by brief warming in concentrated hydrochloric acid. The fact that 9 was found to be the di- (rather than the mono-) hydrochloride was important in assessing the feasibility of using the potassium cyanate method of urea synthesis in the next step. The precursor diamine 3 had been shown previously by potentiometric titration to have only *one* basic amino function, a fact which was corroborated by failure to obtain the diurea 13 when 3 was dissolved in acetic acid and treated with potassium cyanate.

As predicted, application of the potassium cyanate method to the conversion of 9 to 10 using the aqueous acetic acid as the solvent gave satisfactory results. The overall yield of 10 over nine steps starting with 1 was 7%.



Ts = *p*-toluenesulfonyl

An alternative, but lower yield synthesis of 10 (0.4% from 1) also utilized the intermediate 3. Thus it was found that the latter could be converted to the diurea 13 by ammonolysis of either 11 or 12 in yields of 96 and 24%, respectively. The advantage of using phenoxy as the leaving group during ammonolysis was suggested by the carbamate syntheses described by Minoli *et al.*,[9] upon whose work the present method is based. Subsequent reduction of 13 gave the amine 14, which was conveniently isolated as its hydrochloride and treated with ethylene oxide to give the diol 15. Numerous attempts to employ standard chlorinating agents (e.g., thionyl chloride and phosphorus oxychloride) to the conversion of 15 to 10 were unsuccessful. An attempt to form the bistosylate of 15 for possible displacement with lithium chloride gave extensive degradation and the tarry product showed infrared evidence for dehydration of the urea functions (νCN 4.4 μ).

Table I. Elemental Analysis and Ultraviolet Absorption Data

Compd	Formula	Analyses	λ max, nm[a]	ε × 10⁻³
5	$C_{10}H_{13}N_3O_2$	C, H, N	305	2.3
			235	40.0
6	$C_{14}H_{21}N_3O_4$	C, H, N	320	3.1
			245	40.7
			230	28.7
7	$C_{28}H_{33}N_3O_8S_2$	C, H, N	316	2.9
			245	38.7
8	$C_{14}H_{19}Cl_2N_3O_2$	C, H, N, Cl	214	2.9
			243	40.6
			226	26.4
9	$C_{10}H_{17}Cl_4N_3$	C, H, N, Cl	374	9.3
			281	18.6
			235	22.8
10	$C_{12}H_{17}Cl_2N_5O_2$	C, H, N, Cl	307	2.6
			239	57.1
			219	35.0
11	$C_{20}H_{15}N_3O_6$	C, H, N	350	1.7
			303	3.5
			247	29.3
			233	53.6
12	$C_{12}H_{15}N_3O_6$	C, H, N	297	3.5
			240	27.2
13	$C_8H_9N_5O_4$	C, H, N	300	4.2
			238	31.6
			223	52.2
14	$C_8H_{11}N_5O_2$	C, H, N	290	1.0
			278	1.3
			243	20.8
			228	45.2
15	$C_{12}H_{19}N_5O_4$	C, H, N	310	1.9
			258	10.7
			238	35.5
			221	22.3

[a] Solvent was CH_3OH except for compounds 11 (CH_3CN), 13 (0.1 N HCl), and 15 (0.1 N HCl).

A low yield (1.3%) of **10** was obtained using a direct replacement procedure[10,11] in which a dimethylformamide solution of **15** was treated with *N*-chlorosuccinimide in the presence of triphenylphosphine.

Biological Results. Compounds **8**, **9**, and **10** showed activity against P388 lymphocytic leukemia in mice when screened by the Drug Research and Development Branch, National Cancer Institute.[12] The ratios of survival times for treated to control tumor-bearing animals (%T/C) were 196, 215, and 194 at dose levels (mg/kg) of 225, 30, and 120, respectively. Compounds showing percent T/C values greater than 125 are considered to be active in this test system. Survival data further indicated that the compounds were nontoxic at the stated dose levels. Additional screening experiments involving other tumor systems are planned.

Experimental Section

Melting points were obtained on a Thomas-Hoover Unimelt using open capillary tubes and are uncorrected. Petroleum ether refers to that fraction boiling from 30 to 60°. Analyses indicated in Table I only by the symbols of the elements were within ±0.4% of the theoretical values. The ir spectra were obtained on a Perkin-Elmer Model 137 spectrophotometer and the uv spectra by G. B. Smith and staff using Cary Models 11 and 118 spectrophotometers. Varian Associates A-60A and JEOL (USA) C-60HL instruments were used by A. W. Douglas and staff for recording NMR spectra. In each case where the preparation of a new compound is described it was found to have ir (Nujol), NMR (Me_2SO-d_6, except for compound 11 where acetone-d_6 was used), and uv spectra (see Table I) which were in accord with expectation. The authors are grateful to R. N. Boos (and J. P. Gilbert) and associates for microanalyses. We thank J. L. Smith for mass spectrometry and M. M. Ponpipom for helpful advice. Assistance in the preparation of intermediates was provided by J. J. Seman.

The preparative procedures described for the known compounds 1–4 are modifications of those found in the literature. Improvements in yields and ease of manipulation were necessitated by the quantities required for experimentation.

5-Nitro-1,3-benzenedicarboxamide (2). To a mixture prepared from 190 g (0.90 mol) of 5-nitro-1,3-benzenedicarboxylic acid (1), 134 ml (1.85 mol) of thionyl chloride, and 480 ml of chlorobenzene was added 14.4 ml of dimethylformamide.[13] This was stirred and heated cautiously to 90–95° (1.5 h), maintained in this range for 30 min, and allowed to cool. Following vacuum evaporation (water aspirator) the residual oil was poured into 1.5 l. of concentrated aqueous ammonia. The gelatinous solid was air-evaporated and then stirred with 10 l. of boiling water. Filtration while hot, followed by washing with ethanol and then with ether and drying gave 156.3 g (83%) of pure **2**, which decomposed when heated above 300°, in accord with the literature.[14]

5-Nitro-1,3-phenylenediamine (3). Aqueous NaOCl (1 l., 1.52 M) (from NaOH and Cl_2) having an excess basicity of 0.06 equiv/l. was combined with a solution of 117 g of NaOH in 1630 ml of water and cooled to 0°. To this was added 156.3 g (0.75 mol) of **2** while maintaining the temperature near 0°. Stirring was continued for 30 min whereupon the reaction mixture was heated cautiously to 95°. An exothermic rise of 10° was noted in the vicinity of 70°. The reaction mixture was maintained at 95° for 1 h, after which it was cooled to give 109.4 g of impure product. This was dissolved in 2.2 l. of 1 N HCl (60°), treated with Darco KB, and filtered through Celite 545 (Fisher), and the solid precipitated by the addition of 1 l. of 3 N aqueous ammonia. The red powder was washed with water and dried to give 105.4 g (92%) of pure **3**: mp 143–144° (lit.[15] mp 142–143°); potentiometric titration in HOAc using $HClO_4$ as the titrant, equiv wt found, 153.7, 152.4; calcd for one basic amino function, equiv wt 153.1.

***N,N'*-(5-Nitro-1,3-phenylene)bisacetamide (4).** To 425 g (4.2 mol) of stirred acetic anhydride was added in one portion 10.0 g (0.065 mol) of **3**. The solid dissolved immediately and shortly thereafter a yellow precipitate appeared. After 10 min the reaction mixture was cooled and filtered, and the product was washed with ether, providing 15.2 g (98%) of **4**. Melting point determination showed no visible change on heating to 300°, in agreement with the literature.[16]

***N,N'*-(5-Amino-1,3-phenylene)bisacetamide (5).** A slurry was prepared from 11.9 g (0.05 mol) of **4**, 5.0 g of PtO_2 catalyst, and 1 l. of ethanol. This was placed in a sealed glass reaction vessel and shaken for 20 h in the presence of hydrogen gas at approximately 40 psi (uptake, 97%). The catalyst was removed and the volume reduced to 180 ml under vacuum (bath temperature below 30°), causing the product to separate as a powder which was washed with ether to provide 8.35 g (81%) of **5**: mp 210–212°.

***N,N'*-[5-[Bis(2-hydroxyethyl)amino]-1,3-phenylene]bisacetamide (6).** A mixture of glacial acetic acid (48 ml) and water (25 ml) was used to dissolve 12.6 g (0.061 mol) of **5**. The temperature was lowered to 3° and 14.6 ml (12.9 g, 0.30 mol) of ethylene oxide was added in one portion. After being allowed to stand overnight at room temperature the volatile materials were removed and the residual oil was evaporated with three 80-ml portions of 50% benzene–ethanol. The resultant solid was triturated with a solution prepared by mixing 50 ml of ethanol and 100 ml of ether to give 15.1 g (84%) of **6**: mp 162–165°. Crystallization from ethanol gave the analytical sample: mp 163–165°.

***N,N'*-[5-[Bis[2-[4-(methylphenyl)sulfonyloxy]ethyl]amino]-1,3-phenylene]bisacetamide (7).** A solution was prepared under nitrogen from 11.6 g (0.04 mol) of **6** and 80 ml of pyridine. The temperature was lowered to −8° and 16 g (0.084 mol) of *p*-toluenesulfonyl chloride was added all at once. The reaction mixture was kept at 5° overnight and poured into 200 ml of cold H_2O. The oily product was extracted into $CHCl_3$ (1 l.) and was washed successively with 1 M H_2SO_4, H_2O, and saturated $NaHCO_3$. The $CHCl_3$ layer was dried (Na_2SO_4) and concentrated to dryness. The crude solid (22 g) was chromatographed twice in acetone solution (7 ml/g) over silica gel (280 g). The solid obtained each time on concentration was washed with petroleum ether to give 10.1 g (52%) of **7**: mp 77–84°. The infrared spectrum showed the tosylate doublet at 8.4 and 8.5 μ.

***N,N'*-[5-[Bis(2-chloroethyl)amino]-1,3-phenylene]bisacetamide (8).** **7** (6 g, 0.01 mol) was combined under nitrogen

with 60 ml of acetone and 1.7 g (0.04 mol) of lithium chloride. The mixture was heated at 80° for 2 hr in a glass-lined sealed reaction vessel with agitation. The cooled container was opened under nitrogen, the reaction mixture concentrated to dryness, and the solid (8.5 g) partitioned between 150-ml portions of ethyl acetate and water. The water layer was back-extracted with two 50-ml portions of ethyl acetate and the combined ethyl acetate layers were dried ($MgSO_4$), decolorized (Darco G-60), and chromatographed over 50 g of silica gel 60 (E. Merck). Elution with 700 ml of ethyl acetate and concentration gave 2.9 g of crude product, mp 112–120°, which was dissolved in 19.6 ml of acetone and precipitated by the addition of 19.6 ml of petroleum ether. This provided 2.1 g (63%) of **8**: mp 148–149°.

5-[Bis(2-chloroethyl)amino]-1,3-phenylenediamine Dihydrochloride (9). A solution under nitrogen of 1.1 g (3.3 mmol) of **8** in 34 ml of concentrated HCl was immersed in an oil bath at 120° for 3 min. The cooled reaction mixture was diluted with 70 ml of H_2O, neutralized with solid $NaHCO_3$, and extracted with four 75-ml portions of ether. The solution of the diamine in ether was dried (Na_2SO_4) and treated with 1.6 ml of methanolic HCl to give a solid. This was dissolved in 22 ml of methanol and precipitated with 240 ml of ether to give 895 mg (84%) of **9**. NMR spectra of samples prepared in this manner showed minor peaks due to residual acetyl-containing impurities. For analysis, the entire procedure was repeated on a sample of the product, giving a 54% recovery of pure **9**, which decomposed without melting when heated above 200°: mass spectrum (70 eV) m/e 247 (M − 2HCl).

***N,N''*-[5-[Bis(2-chloroethyl)amino]-1,3-phenylene]bisurea (10).** A solution of 0.32 g (1 mmol) of **9** in a mixture of acetic acid (2.5 ml) and water (4.1 ml) was covered with nitrogen and cooled to 10° in an ice bath, and to it was added over a 2-min period a solution of potassium cyanate (0.32 g, 4 mmol) in 3.8 ml of water. The reaction mixture was stored overnight in the cold, and the solid was washed with water and with ether. The product (260 mg) was crystallized from acetone to give 170 mg (51%) of **10**: mp softens 190–195°. Differential thermal analysis showed an endotherm at 203° followed immediately by exothermal decomposition. The analytical sample was vacuum dried at 120°.

(5-Nitro-1,3-phenylene)biscarbamic Acid Diphenyl Ester (11). To a mixture of 30.6 g (0.2 mol) of **3**, 76.7 g (0.9 mol) of sodium bicarbonate, and 620 ml of 1,2-dimethoxyethane was added 76.7 ml (94.4 g, 0.6 mol) of phenyl chloroformate over an 8-min period. This was stirred for 1 h and filtered, and the filtrate was poured into 3 l. of petroleum ether. The precipitated solid amounted to 75.7 g (97%) of **11**: mp 208–210°.

(5-Nitro-1,3-phenylene)biscarbamic Acid Diethyl Ester (12). Compound **3** (14.4 g, 0.094 mol) was dissolved in 860 ml of absolute ethanol and 35.5 g (0.43 mol) of sodium bicarbonate added to give a slurry. To this was added 27.0 ml (30.7 g, 0.28 mol) of ethyl chloroformate. The reaction mixture was heated under reflux for 1 hr and filtered and the volume reduced to about 250 ml. Addition of excess water to the cooled mixture caused the product to separate. Crystallization from ethanol–water (Darco G-60) provided 26.2 g (94%) of **12**: mp 144.5–146.0°.

Ammonolysis of **12** to the diurea **13** proceeded only at elevated temperatures giving a maximum yield of 24%. The preferred route is via low-temperature ammonolysis of **11** as described in the following preparation.

***N,N''*-(5-Nitro-1,3-phenylene)bisurea (13).** To 600 ml of stirred liquid ammonia was added a solution of **11** (24.2 g, 0.062 mol) in 300 ml of 1,2-dimethoxyethane over a 20-min period. Evaporation of the ammonia gave a yellow solid which was collected by filtration and washed with 1,2-dimethoxyethane and with ether. The yield of **13** was 14.1 g (96%). For analysis, a portion (2 g) was dissolved in concentrated hydrochloric acid (120 ml) and filtered, and the filtrate was diluted with water (480 ml). Yellow needles (1.8 g) were recovered and washed with water and ethanol. When heated the compound decomposed above 315°.

***N,N''*-(5-Amino-1,3-phenylene)bisurea (14).** Compound **13** (28.3 g, 0.12 mol) was dissolved in 570 ml of glacial acetic acid and was hydrogenated with 5 g of 5% Pd/C at room temperature and 40 psi. Hydrogen uptake was 97% complete after 1 h. The catalyst was removed and the filtrate was treated with 1.5 l. of petroleum ether, giving an oil which solidified on scratching. Crystallization from aqueous ammonia gave 16.7 g (67%) of crude **14**. A sample for analysis was prepared by basifying a solution of the product in 0.1 N HCl with aqueous ammonia to give a 50% recovery of pure **14**: mp 214–215° dec.

***N,N''*-[5-[Bis(2-hydroxyethyl)amino]-1,3-phenylene]bisurea (15).** The hydrochloride of **14** (77 g, 0.31 mol), prepared by cooling the acidic aqueous solution of **14** instead of basifying as described in the previous preparation, was dissolved in 770 ml of 50% aqueous acetic acid and cooled to 0–5°. To this was added in one portion 205 ml (4.1 mol) of ethylene oxide. The following day the excess ethylene oxide was removed using a stream of nitrogen, after which the solution was treated with Darco KB, filtered, and concentrated to dryness, giving 66 g (71%) of crude **15**: mp 177–178° dec. Crystallization from a 10:1 mixture of ethanol–water gave the analytical sample: mp 186–187° dec.

Preparation of 10 from 15. A solution of **15** (1.13 g, 3.8 mmol) and *N*-chlorosuccinimide (2.0 g, 15 mmol) in 50 ml of dimethylformamide was covered with nitrogen and cooled to 5°. To this was added 3.9 g (15 mmol) of triphenylphosphine and the reaction mixture was heated at 50° for 2 h, cooled, diluted with 10 ml each of methanol and butanol, and then concentrated to dryness. Preparative scale thin-layer chromatography [20 × 20 cm Brinkmann (E. Merck) silica gel F_{254}, 2 mm thickness] using acetone as the developing solvent (R_f 0.2; uv light) provided 16.2 mg (1.3%) of **10**, identified by thin-layer chromatography and comparison of infrared spectra with samples of **10** prepared from **9**.

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References and Notes

(1) M. H. Benn, A. M. Creighton, L. N. Owen, and G. R. White, *J. Chem. Soc.*, 2365 (1961).
(2) J. F. Danielli, *Ann. Rep. Br. Emp. Cancer Camp.*, **34**, 398 (1956).
(3) G. B. Afanaseva, L. V. Chechulina, and O. N. Gzovskaya, *Khim.-Farm. Zh.*, **7**, 13 (1973).
(4) M. A. Thorn, G. H. Denny, and R. D. Babson, *J. Org. Chem.*, **40**, 1556 (1975).
(5) D. I. Burduliene, V. A. Bieksa, and J. A. Degutis, *Liet. TSR Mokslu Akad. Darb., Ser. B*, 55 (1973).
(6) M. N. Vasil'eva and A. Ya. Berlin, *Zh. Obshch. Khim.*, **32**, 3088 (1962).
(7) Y. M. Abou-Zeid, A. A. Abou-Duf, and M. El-Kerdawy, *J. Pharm. Sci. U.A.R.*, **5**, 133 (1964).
(8) W. Werner, *J. Prakt. Chem.*, **314**, 577 (1972).
(9) G. Minoli, E. Marazzi-Uberti, and S. Casadio, *J. Med. Chem.*, **15**, 998 (1972).
(10) S. Hanessian, M. M. Ponpipom, and P. Lavalle, *Carbohyd. Res.*, **24**, 45 (1972).
(11) A. K. Bose and B. La, *Tetrahedron Lett.*, 3937 (1973).
(12) (a) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972); (b) Instruction Booklet 14, "Screening Data Summary Interpretation", Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, Md., 1972.
(13) H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).
(14) G. M. Bennett and R. L. Wain, *J. Chem. Soc.*, 1108 (1936).
(15) German Patent 1132140 (1962).
(16) B. Flürscheim, *J. Prakt. Chem.*, **71**, 497 (1905).