Modification of Radioprotective 2-Aminoethanethiol Derivatives by N,N′-Polymethylene Bridging[1]

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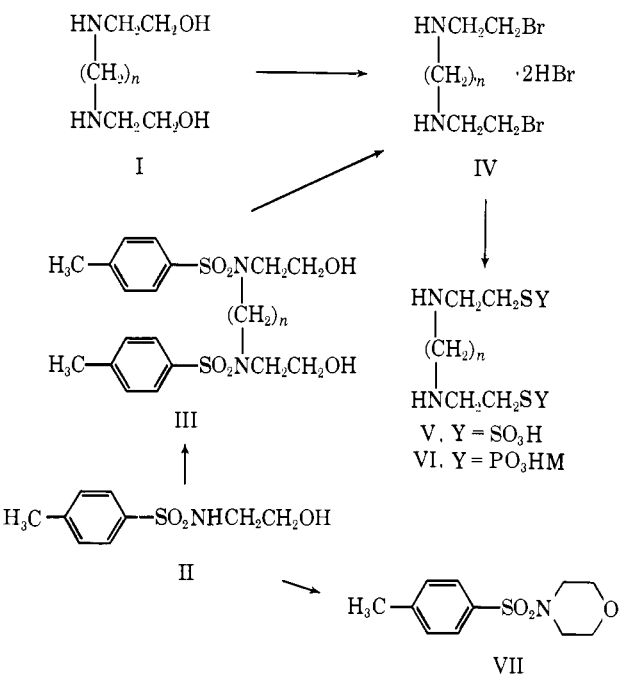
Compounds of the type $HO_3SSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SSO_3H$ (V, n = 2–6) and $MHO_3PSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SPO_3HM$ (VI, n = 2, M = Li; n = 3–6, M = Na) were prepared from the bromo mustards $BrCH_2CH_2NH(CH_2)_nNHCH_2CH_2Br \cdot 2HBr$ (IV, n = 2–6) for evaluation as radioprotective agents. Four of the key intermediates IV (n = 3–6) were prepared *via* a facile route involving treatment of N-(2-hydroxyethyl)-*p*-toluenesulfonamide with α,ω-dibromoalkanes followed by detosylation of the resulting N,N′-polymethylenebis[N-(2-hydroxyethyl)-*p*-toluenesulfonamides] (III, n = 3–6) with concomitant bromodehydroxylation in refluxing 48% HBr. Blocking of the amino groups was essential in the steps leading to IV (n = 4 and 5) in order to preclude competitive cyclization reactions. Treatment of IV with appropriate thioanions afforded V and VI. Two of the phosphorothioates VI (n = 3 and 4) showed good radioprotective activity.

S-2-Aminoethylthiosulfuric acid[2] and the corresponding phosphorothioic acid[3] have been reported to protect mice against lethal doses of radiation. N,N′-Polymethylene bridging of two like units of these compounds was undertaken for evaluation of the effect of such combination on their radioprotective properties.

We considered N,N′-bis(2-bromoethyl)-α,ω-alkanediamine hydrobromides IV (n = 2–6), which may be derived from the corresponding 2,2′-(polymethylenediimino)diethanols I (n = 2–6), to be the intermediates most practicable for general preparations of both V and VI. Group I compounds (n = 2, 3, 6) have been prepared from 2-aminoethanol and α,ω-dihaloalkanes,[4,5] but I (n = 4, 5) cannot be satisfactorily obtained in this manner because of preferential cyclization to pyrrolidine and piperidine derivatives as previously encountered in the synthesis of putrescine and cadaverine mustards,[5] the chloro analogs of IV (n = 4, 5). These mustards were eventually constructed from the diamines by a five-step process in which protecting N-benzyl groups, introduced by benzaldehyde Schiff base formation and hydride reduction before hydroxyethylation, were removed by hydrogenolysis in the final step after chlorodehydroxylation; alternative reversal of the last two steps, not preferred as a route to the mustards, satisfactorily provided I (n = 4, 5) as intermediates.[5]

Our approach to the preparation of the bromo mustards IV (n = 4, 5) involved protection of the amino groups by tosylation, which permitted *in situ* combination of steps and made the isolation of I (n = 4, 5) unnecessary. The reaction sequence that evolved from this investigation consisted of two steps: (1) alkylation of N-(2-hydroxyethyl)-*p*-toluenesulfonamide (II), which was prepared *in situ*, with α,ω-dibromoalkanes in dimethylformamide and potassium carbonate as base; and (2) detosylation of the resulting N,N′-polymethylenebis[N-(2-hydroxyethyl)-*p*-toluenesulfonamides] (III, n = 4, 5) with concomitant bromodehydroxylation in refluxing 48% hydrobromic acid (see Scheme I). The facility and convenience of this method prompted its application to the preparation of IV (n = 3, 6), even though preclusion of competing cyclization reactions was not required in these instances. The intermediates III (n = 3, 5) were isolated as oils and used without further purification, since their infrared absorption spectra compared favorably with those of pure, crystalline homologs III (n = 4, 6). The attempted preparation of IV (n = 2) by this method failed in the first step. Alkylation of II with 1,2-dibromoethane under the conditions described gave the cyclized product 4-(*p*-tolylsulfonyl)-morpholine (VII), which was at first mistaken for the expected III (n = 2) because of melting point coincidence (see Experimental Section). The identity of VII became apparent when morpholine hydrobromide (VIII) was isolated following an attempted conversion of the alkylated product to IV (n = 2) by the action of refluxing hydrobromic acid. Pmr spectroscopy aided in

SCHEME I



(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2028.

(2) (a) B. Holmberg and B. Sörbo, *Nature*, **183**, 832 (1959); (b) A. Kaluszyner, P. Czerniak, and E. D. Bergmann, *Radiation Res.*, **14**, 23 (1961).

(3) (a) B. Hansen and B. Sörbo, *Acta Radiol.*, **56**, 191 (1961); (b) S. Åkerfeldt, *Acta Radiol., Therapy, Phys. Biol.*, **1**, 465 (1963); *Chem. Abstr.*, **60**, 13534 (1964).

(4) G. Faust and W. Fiedler, *J. Prakt. Chem.*, [4] **21**, 113 (1963).

(5) W. W. Lee, B. J. Berridge, Jr., L. O. Ross, and L. Goodman, *J. Med. Chem.*, **6**, 567 (1963).

the identification of VIII; both VII and VIII were identical with authentic samples. The required IV (n = 2) was, however, obtained in two steps from 2-aminoethanol *via* the intermediate I (n = 2) according to recently reported procedures.[4]

Reaction of IV (n = 2–6) with sodium thiosulfate in hot aqueous solution afforded N,N′-polymethylenebis(S-2-aminoethylthiosulfuric acids) (V, n = 2–6); V (n = 4–6) crystallized readily from the cooled reaction solutions, and V (n = 2, 3) precipitated with addition of ethanol. Treatment of IV (n = 3–6) with trisodium phosphorothioate in water–dimethylformamide in essentially the manner described by Åkerfeldt for preparation of sodium hydrogen S-2-aminoethyl phosphorothioate[6a] afforded crystalline disodium salts VI (n = 3–6, M = Na) of N,N′-polymethylenebis(S-2-aminoethylphosphorothioic acids) as polyhydrates that required equilibration in a 58% relative humidity hygrostat in order to establish constant hydrate composition. Similar handling of the disodium salt VI (n = 2, M = Na) was not possible because of deliquescence. The dilithium salt VI (n = 2, M = Li), prepared from IV (n = 2) and trilithium phosphorothioate,[6c] was obtained as a crystalline tetrahydrate following equilibration at 58% relative humidity. Analysis of unequilibrated VI hydrates was unreliable on account of the variation of hydration with changing humidity, and attempts to obtain anhydrous samples of VI (n = 4, 5, M = Na) gave products that showed evidence of decomposition under the drying conditions required. Attempted conversions of the disodium salts V (n = 4, 5, M = Na) to free acids by suspension in acetic acid followed by precipitation from water with ethanol[6b] resulted in isolation of products whose analyses were unsatisfactory and, in the case of VI (n = 4, M = H), worsened after attempted further purification.

Antiradiation screening of V (n = 2–6) and VI (n = 2–6) in mice was carried out at the Walter Reed Army Institute of Research, Washington, D. C., according to a previously described procedure;[7] screening results are recorded in Table I.[8] These results show that the radioprotective activity of S-2-aminoethylthiosulfuric acid is all but negated by simple N,N′-polymethylene linkage; the slight activity shown by V (n = 5) suggests that a long methylene bridge might be requisite for appreciable activity. On the other hand, the same modifications of S-2-aminoethylphosphorothioic acid allow good retention of activity if the connecting chain is limited to three and four methylene groups.

Table I

Radioprotective Activities of N,N′-Polymethylene-Bridged Aminoethanethiol Derivatives

n	M	x	Approx acute LD_{50}, mg/kg	Dose range, mg/kg	Vehicle of administration	Antiradiation rating[a]
			$HO_3SSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SSO_3H$ (IV)			
2			150–350	150–350	MC-Tw[b]	0
3			150–350	150–350	Saline	0
4			50–150	50–150	MC-Tw	0
5			50–150	50–150	MC-Tw	+
6			50–150	<50	MC-Tw	0
			$MHO_3PSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SPO_3HM \cdot xH_2O$ (V)			
2	Li	4	>750	150–350	PO_4 buffer, pH 7	0
3	Na	8	350–750	150–350	PO_4 buffer, pH 7	+++
4	Na	8	350–750	150–350	PO_4 buffer, pH 7	+++
5	Na	7	50–150	<50	PO_4 buffer, pH 7	+
6	Na	8	<50	<50	PO_4 buffer, pH 7	+

[a] Scale: none (0), slight (+), fair (++), good (+++); $H_2NCH_2CH_2SH$ is rated +++; $H_2NCH_2CH_2SSO_3H$, +++; $H_2NCH_2CH_2SPO_3H_2$, +++. [b] Compound suspended in physiological saline solution containing 0.2% methylcellulose and 0.4% Tween 80.

(6) (a) S. Åkerfeldt, *Acta Chem. Scand.*, **13**, 1479 (1959); (b) S. Åkerfeldt, *ibid.*, **14**, 1980 (1960); (c) S. Åkerfeldt, *ibid.*, **16**, 1897 (1962).

(7) L. Field, A. Ferretti, R. Crenshaw, and T. Owen, *J. Med. Chem.*, **7**, 39 (1964).

(8) The authors are indebted to Drs. D. P. Jacobus and T. R. Sweeney for the antiradiation test data.

(9) Unless otherwise noted, melting points were determined with a Mel-Temp apparatus.

Experimental Section[9]

N,N′-Polymethylenebis[N-(2-hydroxyethyl)-*p*-toluenesulfonamides] (III, n = 3–6).—A mechanically stirred mixture of 2-aminoethanol (1.00 mole), anhydrous K_2CO_3 (1.00 mole), and dimethylformamide (375 ml) was treated dropwise with a solution of *p*-toluenesulfonyl chloride (1.00 mole) in dimethylformamide (375 ml), the reaction temperature being maintained at less than 60° by moderate external cooling. The mixture was then heated to 115–125°, and more K_2CO_3 (2.00 moles) was added followed by dropwise addition of the appropriate α,ω-dibromoalkane (0.50 mole). Heating at about 120° with vigorous stirring was continued for 2–3 hr. The mixture was then cooled to room temperature and poured into water (7.5 l.); III (n = 4, 6) separated readily as crystalline solids, but III (n = 3, 5) precipitated as oils. The oils were extracted with benzene, and removal of the solvent from the water-washed and dried ($MgSO_4$) benzene solutions left the crude products as pale yellow oils in yields of 41 and 64%, respectively, which were used as such in the next step. The solids were recrystallized once from ethanol prior to use in the next step. The yield of III (n = 4), mp 97–98°, was 40%. The analytical sample, mp 100–101°, was obtained by further recrystallization from ethanol.

Anal. Calcd for $C_{22}H_{32}N_2O_6S_2$: C, 54.52; H, 6.65; N, 5.78. Found: C, 54.35; H, 6.70; N, 5.61.

Similarly, the yield of III (n = 6), mp 98–102°, was 33%; the analytical sample had mp 107–108°.

Anal. Calcd for $C_{24}H_{36}N_2O_6S_2$: C, 56.22; H, 7.08; N, 5.46. Found: C, 56.43; H, 6.96; N, 5.17.

N,N′-Bis(2-bromoethyl)-α,ω-alkanediamine Dihydrobromides (IV, n = 3–6).—The following description of the preparation of IV (n = 4) is typical of the procedure used for converting III to IV. The results and actual scale used are listed in Table II. A stirred mixture of III (n = 4) (150 g, 0.308 mole) and 48% HBr (1500 ml) was heated to boiling under a distillation apparatus (Claisen head). Complete solution occurred before boiling commenced. The solution was distilled until 750 ml of distillate had been removed, and the extent of heating was then diminished to cause simple refluxing. After the solution had refluxed continuously for 40 hr, it was again heated until distillation occurred and 90 ml of distillate was removed. The heating was again lessened to cause reflux without distillation, and the solution was refluxed for 2 hr. This process of alternately distilling 90-ml portions and then refluxing for 2 hr was repeated four more times until the total volume of distillate collected amounted to 1200 ml. The dark residual liquid was then cooled and diluted with water (500 ml). The resultant solution was treated with Norit and filtered through a Celite mat. The clear red filtrate was evaporated to dryness under reduced pressure with the aid of several added portions of ethanol. The remaining red solid was triturated thoroughly with a large volume of acetone; and the acetone-insoluble, virtually white crude product was collected and washed on the funnel with acetone. Recrystallization from methanol followed by drying *in vacuo* at room temperature afforded pure IV (n = 4).

N,N′-Polymethylenebis(S-2-aminoethylthiosulfuric Acids) (V, n = 2–6).—Summarized results of the following preparations are listed in Table III.

A. V (n = 2).—A solution of IV (n = 2)[4] (15.0 g, 34.4 mmoles) and sodium thiosulfate pentahydrate (17.1 g, 68.8 mmoles) in water (38 ml) was heated at 80° for 30 min. The solution was chilled in an ice bath and, with vigorous magnetic stirring, ethanol (150 ml) was gradually added. The colorless syrup

TABLE II

N,N'-BIS(2-BROMOETHYL)-α,ω-ALKANEDIAMINE DIHYDROBROMIDES (IV)

$BrCH_2CH_2NH(CH_2)_nNHCH_2CH_2Br \cdot 2HBr$

n	Molar scale	Yield, %	Mp, °C dec	Formula	% carbon Calcd	% carbon Found	% hydrogen Calcd	% hydrogen Found	% bromine Calcd	% bromine Found
3	0.445	29	255–258	$C_7H_{16}Br_2N_2 \cdot 2HBr$	18.68	18.89	4.03	4.18	71.05	71.0
4	0.308	75	262–264	$C_8H_{18}Br_2N_2 \cdot 2HBr$	20.71	21.02	4.35	3.92	68.89	68.1
5	0.268	45	241–243	$C_9H_{20}Br_2N_2 \cdot 2HBr$	22.61	22.78	4.64	4.77	66.75	66.8
6	0.332	26	249–251	$C_{10}H_{22}Br_2N_2 \cdot 2HBr$	24.41	24.55	4.92	4.76	64.98	64.5

TABLE III

N,N'-POLYMETHYLENEBIS(S-2-AMINOETHYLTHIOSULFURIC ACIDS) (V)

$HO_3SSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SSO_3H$

n	Scale (mmoles of 11)	Yield, %	Mp, °C dec	Formula	% carbon Calcd	% carbon Found	% hydrogen Calcd	% hydrogen Found	% nitrogen Calcd	% nitrogen Found	% sulfur Calcd	% sulfur Found
2	34.4	65	*a*	$C_6H_{16}N_2O_6S_4 \cdot 2H_2O$	19.13	19.44	5.35	5.18	7.44	7.43	34.06	34.5
3	1.6	91	185–187	$C_7H_{18}N_2O_6S_4$	23.71	23.84	5.12	5.20	7.90	7.73	36.18	35.9
4	44.2	74	214–217	$C_8H_{20}N_2O_6S_4$	26.07	25.96	5.47	5.26	7.60	7.45	34.80	34.8
5	20.9	74	214–216	$C_9H_{22}N_2O_6S_4$	28.26	28.27	5.80	5.89	7.32	7.03	33.53	33.0
6	10.2	70	194–197	$C_{10}H_{24}N_2O_6S_4$	30.28	30.51	6.10	6.05	7.06	6.96	32.34	32.2

[a] Indefinite from 110°.

TABLE IV

N,N'-POLYMETHYLENEBIS(S-2-AMINOETHYL SODIUM HYDROGEN PHOSPHOROTHIOATES)

$NaHO_3PSCH_2CH_2NH(CH_2)_nNHCH_2CH_2SPO_3HNa$ (VI)

n	Yield, %	Formula	% carbon Calcd	% carbon Found	% hydrogen Calcd	% hydrogen Found	% nitrogen Calcd	% nitrogen Found	% phosphorus Calcd	% phosphorus Found
3	88	$C_7H_{18}N_2Na_2O_6P_2S_2 \cdot 8H_2O$	15.50	15.15	6.32	6.33	5.17	5.03	11.42	11.0
4	86	$C_8H_{20}N_2Na_2O_6P_2S_2 \cdot 8H_2O$	17.27	17.37	6.52	6.59	5.04	5.03	11.53[a]	11.4[a]
5	89	$C_9H_{22}N_2Na_2O_6P_2S_2 \cdot 7H_2O$	19.57	19.65	6.57	6.38	5.08	5.06	11.21	11.2
6	86	$C_{10}H_{24}N_2Na_2O_6P_2S_2 \cdot 8H_2O$	20.55	20.56	6.89	6.64	4.79	4.70	10.60	10.7

[a] % Sulfur.

that separated crystallized within a few minutes. Two recrystallizations from water followed by drying *in vacuo* at room temperature afforded pure V(n = 2) as a dihydrate.

B. V (n = **3**).—A solution of IV (n = 3) (0.71 g, 1.6 mmoles) and sodium thiosulfate pentahydrate (0.79 g, 3.2 mmoles) in water (1.6 ml) was heated at 90–95° for 1 hr. Ethanol (16 ml) was added to the cooled solution, and the colorless syrup that separated soon crystallized. Reprecipitation from water (2 ml) solution by addition of ethanol (20 ml) afforded pure V (n = 3) (dried *in vacuo* at room temperature).

C. V (n = **4–6**).—A solution of IV (n = 5) (10.0 g, 20.9 mmoles) and sodium thiosulfate pentahydrate (10.4 g, 41.8 mmoles) in water (20 ml) was refluxed for 1 hr. The crystalline precipitate that separated when the mixture was chilled was collected and recrystallized from water to yield pure V (n = 5) (dried *in vacuo* at room temperature).

Trilithium phosphorothioate hexahydrate was prepared by the procedure of Åkerfeldt,[6c] who obtained a 5.5 hydrate but did not clearly describe the conditions used to attain constant weight. The prepared sample was found to undergo slight weight changes with respect to changes in ambient relative humidity, but analysis immediately after equilibration at constant 58% relative humidity[10] indicated a hexahydrate.

Anal. Calcd for $Li_3PSO_3 \cdot 6H_2O$: P, 12.91; S, 13.36. Found: P, 12.8; S, 13.6.

N,N'-Ethylenebis(S-2-aminoethyl Lithium Hydrogen Phosphorothioate) Tetrahydrate (VI, n = 2, M = Li).—Trilithium phosphorothioate hexahydrate (9.60 g, 40.0 mmoles) was dissolved in water (80 ml), and N,N-dimethylacetamide (20 ml) was added. Solid IV (n = 2) (9.15 g, 21.0 mmoles) was added to the magnetically stirred solution followed by more N,N-dimethylacetamide (20 ml). After the solution had been stirred at room temperature for 1.5 hr, ethanol (250 ml) was added causing separation of a white gum. The supernatant was removed by decantation, and the gum was stirred with two 100-ml portions of ethanol. The now virtually solid crude product remaining after decantation of the ethanol was dissolved in water (60 ml), and the solution was treated with Norit and filtered through Celite. The colorless filtrate was added dropwise to magnetically stirred ethanol (600 ml). The white solid that separated was collected, washed successively with ethanol, methanol, and ether, and suction dried under nitrogen pressure. Equilibration in a 58% relative humidity hygrostat[10] afforded VI (n = 2, M = Li) in 80% yield (6.75 g).

Anal. Calcd for $C_6H_{16}Li_2N_2O_6P_2S_2 \cdot 4H_2O$: C, 16.99; H, 5.71; N, 6.61; P, 14.61; S, 15.12. Found: C, 17.26; H, 5.72; N, 6.42; P, 14.99; S, 15.27.

N,N'-Polymethylenebis(S-2-aminoethyl Sodium Hydrogen Phosphorothioates) (VI, n = 3–6, M = Na).—To a stirred partial solution of trisodium phosphorothioate (9.00 g, 50.0 mmoles) in water (50 ml) was added the appropriate IV (25.0 mmoles). After complete solution had occurred (20–30 min), dimethylformamide (25 ml) was added. Stirring at room temperature was continued for 1 hr while the product partially separated. The mixture was then chilled in an ice–water bath while more dimethylformamide (50 ml) was added dropwise to cause complete precipitation of the product. After the resultant mixture had stood overnight in a freezer, the crude product was collected with the aid of ethanol and was washed thoroughly with ethanol. The suction-dried material was dissolved in water (about 60 ml) at room temperature, and the solution was treated with Norit and filtered through Celite. Ethanol (about 350 ml) was added dropwise to the chilled (ice–water bath) colorless filtrate with vigorous magnetic stirring. The hydrated white crystalline precipitate that formed was collected, washed with ethanol and ether, and suction dried on the funnel. Equilibration at 58% relative humidity[10] afforded the products listed in Table IV.

4-(p-Tolylsulfonyl)morpholine (VII). A. From II.—Procedures A and B were designed for the preparation of III (n = 2), but the products so obtained were later identified as VII; the reported melting point (144°) of III (n = 2), prepared by the sodium ethoxide promoted reaction of N,N'-ethylenebis-p-toluenesulfonamide with ethylene oxide,[11] coincides with that of VII. A stirred mixture of II[12] (6.88 g, 32.0 mmoles), anhydrous

(10) "Handbook of Chemistry," N. A. Lange, Ed., 9th ed., Handbook Publishers, Inc., Sandusky, Ohio, 1956, p 1420; I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," 3rd ed, The Macmillan Co., New York, N. Y., 1952, pp 138–143.

(11) D. H. Peacock and Y. S. Gwan, *J. Chem. Soc.*, 1468 (1937).

(12) K. H. Slotta and R. Behnisch, *J. Prakt. Chem.*, **135**, 225 (1932).

K_2CO_3 (8.85 g, 64.0 mmoles), and dimethylformamide (20 ml) was heated to 115°, and 1,2-dibromoethane (3.00 g, 16.0 mmoles) was added dropwise. The resultant mixture was heated at 115–120° for 5 hr, allowed to cool somewhat, and poured into water (100 ml). The precipitate that formed was collected, washed with water, and dried *in vacuo*. Recrystallization of this material (0.91 g, mp 132–134°) from ethanol gave 0.67 g (17%) of VII as white needles, mp 144–146°.

B. From 2-Aminoethanol.—A solution of *p*-toluenesulfonyl chloride (860 g, 4.52 moles) in dimethylformamide (800 ml) was added from a dropping funnel to a mechanically stirred mixture of 2-aminoethanol (276 g, 4.52 moles), anhydrous K_2CO_3 (624 g, 4.52 moles), and dimethylformamide (1.6 l.). Throughout the addition period the temperature of the reaction mixture was maintained at 30–35°, moderate external cooling being necessary. Fifteen minutes after the addition had been completed, the continuously stirred mixture was gradually heated to 115–120°. More K_2CO_3 (1.26 kg, 9.03 moles) was added followed by the dropwise addition during about 45 min of 1,2-dibromoethane (424 g, 2.26 moles). The resultant mixture was stirred and heated at 115–120° for 5 hr, allowed to cool, and poured into water (25 l.). The aqueous mixture was allowed to stand overnight at room temperature, and the white crystalline precipitate that separated was collected and washed thoroughly with water. The product, dried *in vacuo* (P_2O_5), was obtained in 15% crude yield (81.0 g) and melted at 139–141°. Recrystallization from ethanol raised the melting point to 144–146°. Further recrystallization from benzene–ligroin (bp 30–60°) gave a sample of VII with mp 146–147°.

C. From Morpholine.—A freshly prepared solution of *p*-toluenesulfonyl chloride (2.86 g, 15.0 mmoles) in dimethylformamide (5 ml) was added dropwise to a magnetically stirred solution of morpholine (2.61 g, 30.0 mmoles) in the same solvent (5 ml) at such a rate that the reaction temperature did not exceed 40°. The mixture was stirred 1 hr longer at room temperature and was then diluted with water (80 ml). The white precipitate that formed was collected, washed with water, and dried *in vacuo* at room temperature [yield 2.74 g (76%), mp 144–145°]. Recrystallization from benzene–ligroin raised the melting point to 147–148° (lit.[13] mp 147°). Melting points of mixtures of this authentic VII with the products described above (A and B) were not depressed, and their infrared spectra were identical.

Detosylation of VII.—When VII (14.1 g) [erroneously identified at the time as III ($n = 2$) on the basis of melting point] was subjected to treatment with boiling 48% HBr (150 ml initially) in the manner described above for conversion of III ($n = 3$–6) to IV ($n = 3$–6), the only product isolated was morpholine hydrobromide (4.21 g, 12% yield), mp 210–212° (recrystallized from ethanol). No effort was made to isolate any products that might have resulted from ether cleavage.

Anal. Calcd for $C_4H_9NO \cdot HBr$: C, 28.60; H, 6.00; Br, 47.57; N, 8.34. Found: C, 28.90; H, 5.88; Br, 47.9; N, 8.28.

An authentic sample of **morpholine hydrobromide**, prepared from the free base and ethanolic HBr solution, is identical with the above-described sample with respect to melting point, mixture melting point, tlc, and infrared spectra.

(13) S. Siegel, *Ber.*, **34**, 2900 (1901).

Nucleosides. XXXIII. N^4-Acylated 5-Fluorocytosines and a Direct Synthesis of 5-Fluoro-2′-deoxycytidine[1]

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A series of N^4-acylated 5-fluorocytosines was prepared as starting material for nucleoside synthesis and for chemotherapeutic screening. A direct synthesis of 5-fluoro-2′-deoxycytidine (FCDR, V) and its α-anomer (VII) from the monomercury salt of N^4-toluoyl-5-fluorocytosine (II) was achieved whereby N^4-toluoyl-5-fluoro-2′-deoxycytidine (VIII) was isolated as an intermediate. Compounds II and VIII are converted into 5-fluorouracil (FU) and 5-fluoro-2′-deoxyuridine (FUDR, IX), respectively, by treatment with 0.5 *N* HCl at 37°. The labilization of the exocyclic amino group by aroylation suggested utility of II and VIII as releasers of FU and FUDR (IX) in biological systems. The acylated 5-fluorocytosines are relatively nontoxic compounds exhibiting some activity against systemic *Candida albicans* infections in mice. The nucleoside (VIII) is a potent and toxic agent against experimental tumors in mice. The chemotherapeutic data indicate that *in vivo* the acylated 5-fluorocytosines act as releasers of FC (I) and not of FU, while the nucleoside (VIII) acts as releaser of FCDR (V) and/or FUDR (IX).

5-Fluoro-2′-deoxycytidine (FCDR)[2] was first obtained from 5-fluoro-2′-deoxyuridine (FUDR) by a thiation procedure[3] and its biological and chemotherapeutic properties have been reviewed.[3b] It may be added that, as an inhibitor of the incorporation of formate into DNA thymine in a suspension of Ehrlich ascites carcinoma cells, FCDR was found to be the only fluorinated pyrimidine among 35 screened which was more potent than FUDR.[4a] FCDR showed a relatively high chemotherapeutic index against mouse leukemia B82.[4b] In comparative studies with FUDR, FCDR exhibited a different spectrum of activity against a wide

(1) A preliminary account of this work was presented before the Medicinal Chemistry Section at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept 1963, p 18-O. This investigation was supported in part (to Sloan-Kettering Institute) by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-08748).

(2) The designations for compounds used in this paper (*i.e.*, FU, FC, FCDR, and FUDR) conform to widely prevalent usage in the chemical and biological literature.

(3) (a) J. J. Fox, I. Wempen, and R. Duschinsky, Abstracts (supplement) of the 4th International Congress of Biochemistry, Vienna, 1958, p 6; (b) I. Wempen, R. Duschinsky, L. Kaplan and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).

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