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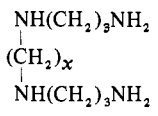
Synthesis and Antitumor Evaluation of the Presumed Cytotoxic Metabolites of Spermine and *N,N'*-Bis(3-aminopropyl)nonane-1,9-diamine†

Mervyn Israel,* Estelle C. Zoll, Naseem Muhammad, and Edward J. Modest

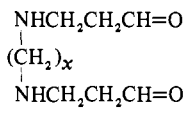
The Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, Massachusetts 02115. *Received July 7, 1972*

4,14-Diazaheptadecanedialdehyde (**2b**), the presumed cytoactive metabolite of the novel experimental antitumor agent *N,N'*-bis(3-aminopropyl)nonane-1,9-diamine (**1b**), was synthesized as its dihydrochloride salt and evaluated for *in vivo* tumor growth inhibition. The same synthetic sequence was also used to prepare 4,9-diazadodecanedialdehyde (**2a**), the presumed metabolite of the ubiquitous polyamine spermine (**1a**). The bisaldehyde products were characterized by microchemical analysis and ir and nmr spectral data and by conversion to their bis-2,4-dinitrophenylhydrazone derivatives. Studies with **2a** and **2b** established that they did not undergo spontaneous decomposition to acrolein under a variety of experimental conditions, including those normally employed in cell culture technique.

N,N'-Bis(3-aminopropyl)nonane-1,9-diamine (**1b**)[2] is an experimental antitumor agent of novel chemical structure. A homolog of the ubiquitous polyamine spermine (**1a**), compound **1b**, when administered in the form of its 4HCl salt, inhibits the growth of a variety of leukemias and solid tumors in mice, rats, and hamsters, as evidenced by increase in mean survival time of tumor-bearing animals and/or reduction in mean tumor size.[2-4] In particular, against the murine C1498 myelogenous leukemia, **1b**·4HCl significantly inhibits tumor growth at the implant site and prevents leukemic infiltration into distant organs.[4] The limiting factor in the use of this agent has been a nephrotoxic reaction seen after long-term daily administration.[3,4]



1a, $x = 4$
b, $x = 9$



2a, $x = 4$
b, $x = 9$

A number of pieces of evidence (*vide infra*) suggested that 4,14-diazaheptadecanedialdehyde (**2b**), a probable cytotoxic metabolite of **1b**, be considered as the agent actually responsible for the *in vivo* antitumor effects exhibited by the polyamine. We should like now to describe the synthesis and antitumor evaluation of **2b** and, also, the preparation of 4,9-diazadodecanedialdehyde ("oxidized spermine," **2a**), which was similarly examined for antitumor activity, as well as for its possible spontaneous degradation to acrolein.

Chemistry. Attempted reduction‡ of *N,N'*-bis(2-cyanoethyl)butane-1,4-diamine[2] to **2a** was, in general, unsuccessful. At best, reduction of the bisnitrile with lithium aluminum triethoxyhydride[6] gave a small yield of **2a** isolated as its bis-2,4-dinitrophenylhydrazone derivative. Attempts to isolate **2a** from this reaction as the free base, as a salt, or as some other carbonyl derivative from which the aldehyde could be easily regenerated led to red rubbery polymeric material. The lack of promise of this route dissuaded us from attempting the reaction with the corresponding biscyanoethylnonanediamine.

The successful synthesis of **2a** and **2b** was accomplished by adaptation and modification of a route to **2a** suggested by Fukami and coworkers.[5] The Japanese investigators in a brief communication[5] claimed the synthesis of an oxalate salt of **2a** *via* a sequence which utilized the acetal-protected aldehyde intermediate, 3-amino-1,1-diethoxypropane (**6**); no physical or chemical evidence was offered in support of the **2a** oxalate assignment and no details of the synthetic sequence have been described. Accordingly, we developed independently laboratory procedures for the sequence shown in Scheme I.§

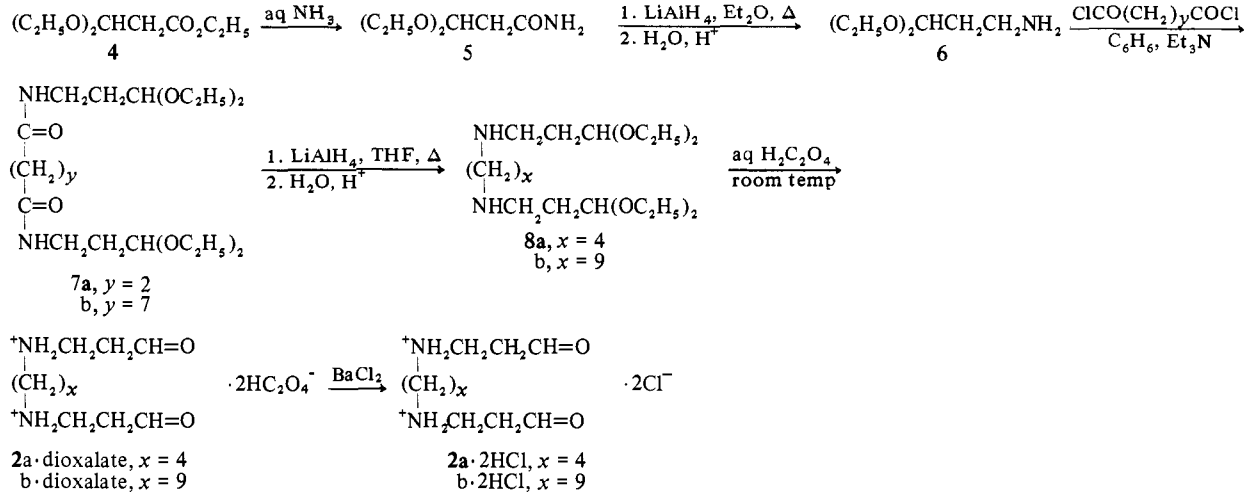
Amination of 3-chloro-1,1-diethoxypropane, as suggested in the patent literature,[7] was not a useful preparative procedure for **6**. In our hands, the pressure reaction always

†This investigation was supported in part by Research Grant C6516 and Research Career Development Award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. A brief account of this work has appeared.[1]

‡Fukami, *et al.*,[5] have since reported a similar inability to obtain **2a** *via* reduction of **3a**. Their communication also reports unsuccessful attempts to prepare **2a** *via* reduction of *N,N'*-bis(2-carboxyethyl)- and *N,N'*-bis(2-carbomethoxyethyl)butane-1,4-diamine and by oxidation of *N,N'*-bis(hydroxypropyl)butane-1,4-diamine.

§We thank Dr. Hiroshi Fukami, Pesticide Research Institute, Kyoto University, and Dr. Hideaki Yamada, Research Institute for Food Science, Kyoto University, for an exchange of correspondence and helpful advice relative to this work and for a comparison sample of the dinitrate salt of **8a**.

Scheme I



gave a dark red viscous product from which less than 2% of **6** could be recovered by preparative vapor-phase chromatography; the bulk of the product was polymeric in nature. In the present investigation, **6** was prepared from ethyl 3,3-diethoxypropionate (**4**) *via* amidation, followed by reduction. Compound **4**[8-10] was prepared in a new way by means of a modified Reformatsky reaction involving triethyl orthoformate and ethyl bromoacetate in the presence of zinc; the procedure was based upon that used by Kupiecki and Coon[11] for the analogous preparation of methylmalonic semialdehyde diethylacetal. The diethoxy ester **4** was converted into the amide **5** by treatment with aqueous NH_3 at room temperature, essentially according to the procedure of McElvaine and Clarke.[9] Reduction of **5** to **6** was achieved with $LiAlH_4$ in Et_2O. For this reduction at least 1.6 molar equiv of reducing agent per mole of **5** must be used. Otherwise, a bright red complex forms during the reaction and only polymer is obtained after hydrolysis. We routinely used 2.0 molar equiv of $LiAlH_4$ to ensure maximum yield in this reduction.

6 (2 equiv) was condensed with the diacid chlorides of succinic and azelaic acids to give the corresponding bisamides **7a** and **7b**. These were reduced by means of $LiAlH_4$ in THF to give the bisacetalamines **8a** and **8b**, which were obtained as viscous pale yellow oils and used without further purification for conversion to the aldehydes. The ir spectra of crude **8a** and **8b** showed no -CONH- absorption and the nmr spectra ($CDCl_3$) showed ethoxy signals (methyl triplet and methylene quartet) superimposed on complex methylene and amine signals and also an acetal methine triplet. Further characterization of the bisacetalamines was provided by the preparation of their dioxalate and dinitrate salts; the naponate (naphthalene-1,5-disulfonate) repository salt form of **8b** was also prepared.

Upon treatment with dilute oxalic acid, the bisacetalamine bases initially formed sparingly soluble oxalate salts, which gradually dissolved with hydrolysis of the acetal protecting function. The resulting bisaldehydes **2a** and **2b** could then be recovered as their oxalate salts. Hydrolysis was shown to be complete at this stage by the presence in the recovered solids of a downfield aldehyde proton signal at δ 9.73 ppm in the nmr (DMSO-d_6) and the absence of discernible ethoxy and acetal methine signals.

However, because of the known nephrotoxicity of oxalate itself, these salts were inappropriate derivatives for *in vivo* investigation. Treatment of the oxalate salts of **2a** and **2b** or, alternatively, of the oxalic acid hydrolysis mixtures with aqueous $BaCl_2$ afforded the bisaldehyde products as their 2HCl salts. The dihydrochloride salts continued to show the aldehyde proton signal in the nmr at δ 9.73 ppm (DMSO-d_6) and, again, ethoxy signals and the acetal methine triplet characteristic of the bisacetalamines (**8**) were absent. In addition, these products clearly showed aldehyde absorption at 5.85 μ in the ir, a signal which had previously been masked by the oxalate in the original hydrolysis samples.

When sampled in D_2O or CD_3OD, compounds **2a** and **2b** dihydrochloride lost the aldehyde proton resonance and, instead, a methine triplet at δ 4.64 ppm appeared. The ease of addition of H_2O and alcohol across the carbonyl groups suggested by this spectral observation actually resulted in a complication in the preparation of **2a**·2HCl for microchemical analysis. To remove the small quantity of Ba oxalate contaminant from the bisaldehyde 2HCl salt samples, advantage was taken of the insolubility of Ba oxalate in EtOH, the bisaldehyde dihydrochloride being recovered from the alcohol solution after separation of the Ba oxalate. With **2a**·2HCl, this purification procedure always resulted in the formation of 2-4% of the diethyl acetal (or ethyl hemiacetal), as evidenced by the appearance of ethoxy signals, particularly the more distinctive methyl triplet, in the nmr. Because of this, it was not possible to obtain analytical data for **2a**·2HCl which met the usual standard of acceptability; for our best sample, the found carbon and nitrogen values differed from theory by about 1%. On the other hand, **2b**·2HCl, which was in general more stable and easier to work with, passed through the alcohol purification step without alteration and satisfactory analytical data were obtained for this material.

The unambiguity of the synthetic route and the spectral and microchemical data clearly support the bisaldehyde structure assignment for the target compounds. The structures of **2a** and **2b** were additionally confirmed by the formation of their corresponding bis-2,4-dinitrophenylhydrazone derivatives. The dihydrochloride salts of **2a** and **2b** are hygroscopic; failure to protect from moisture results in polymerization of the sample, probably *via* aldol condensation, to give pink to red rubbery material with weak uv absorption at 260-264 nm.

The presence of the small quantity of acetal in samples of **2a**·2HCl was considered to have had no significant bearing on results from subsequent biological studies with these materials.

Biochemistry and Bioassay. Several excellent current reviews on the metabolism and biological functions of spermine and its biogenetic precursor spermidine [*N*-(3-aminopropyl)butane-1,4-diamine] are available.[12-14] Of significance here are the observations that, despite their widespread occurrence and established interactions with nucleic acids and subcellular components, these polyamines in experimental systems are converted by an enzyme (bovine plasma amine oxidase#)[15] to cytotoxic derivatives which potently inhibit the growth of bacteria,[16-19] chick-embryo fibroblasts,[20] mammalian spermatozoa,[18] and a variety of mammalian cell lines in culture.[2,21-26] The "oxidized spermine" metabolite also inactivates various bacterial, plant, and animal viruses.[5,27-32] Evidence has been presented[25,33,34] in support of 4,9-diazadodecanedialdehyde (**2a**) as the enzymatic product derived from spermine. Many investigators credit this bisaldehyde with the growth-inhibition and viral-inactivation effects cited above; the interaction of the bisaldehyde with DNA[5,25,35-41] has been proposed as the mechanism through which this growth inhibition is expressed.

In addition to the enzyme present in bovine plasma, there exist a number of amine oxidases capable of effecting the oxidative deamination of spermine and spermidine; some of these are known to be present in the kidneys of various animals.[42-44] It is not unreasonable to suggest that the known[45,46] renal toxicity produced in animals by parenterally administered spermine may be the result of significant enzymatic conversion of the polyamine to its cytoactive form at this sensitive organ site.[15]

Compound **1b** contains the polyamine structural feature[23,24,26] required by bovine plasma amine oxidase for oxidative deamination. Like spermine, **1b** inhibits the growth of KB (human epidermoid carcinoma) cells in culture when the medium is supplemented with whole calf serum (containing bovine plasma amine oxidase) but does not inhibit KB cells which have been adapted to horse serum (lacking an amine oxidase with spermine specificity).[24,26] On the basis of the spermine-bovine plasma amine oxidase example, the active metabolite from **1b** may be the bisaldehyde **2b**. The synthesis of **2b** was accomplished in the hope that it would produce the *in vivo* antitumor effects of the tetramine and that its pharmacology would be such that a toxic concentration in the kidney would not occur.

However, against two transplantable mouse leukemias **2b** · 2HCl failed to exhibit tumor-inhibitory activity. The agent was evaluated for its ability to increase the survival time of BDF_1 and DBA_2 mice bearing the L1210 and P1534 lymphatic leukemias, respectively. Details of the assay procedures employed at The Children's Cancer Research Foundation have been described;[47] compounds were administered intraperitoneally in freshly prepared aqueous solutions. No therapeutic advantage was observed for **2b** • 2HCl at nontoxic doses (up to 5 mg/kg/day for 4 days beginning the first day after tumor implantation). The compound was quite toxic, a single administration of 10 mg/kg or more leading to the death of all animals within 2 hr. Compound **2a** • 2HCl, although less toxic than **2b**, gave a similar pattern of results *in vivo*; acute toxicity (LD_{100}) was observed with a single dose of 40 mg/kg of **2a** • 2HCl. Compound **2a** • 2HCl at 10 mg/kg/day for 4 days showed marginal (+25%) increase in mean survival of animals bearing the P1534 leukemia. In view of the lack of antitumor action at nontoxic dosages and the immediate CNS-induced death with a toxic dose of the bisaldehydes, histopathological examination of the tissues and organs of treated animals was not undertaken.

With respect to the nature of the cytoactive agent in the spermine-bovine plasma amine oxidase system, it is generally agreed that spermine undergoes enzymatic conversion into the bisaldehyde **2a**. However, Alarcon[48,49] has suggested that the cytotoxic effects seen in this system are due not to **2a** but rather to acrolein generated from **2a** by its spontaneous decomposition. The acrolein hypothesis has gained recent support from the work of Li and Zeller[50] and of Kimes and Morris.[51]

The availability of the dihydrochloride salt of **2a**, prepared by unambiguous synthesis, provided us with the opportunity to examine this question. Prior to a quantitative determination with respect to time, we wished first to ascertain qualitatively whether acrolein was indeed generated by decomposition of **2a**. For this, advantage was taken of the ease of formation and identification of acrolein 2,4-dinitrophenylhydrazone. Samples of **2a**·2HCl dissolved in 25 ml of the appropriate medium were placed in a small test tube or erlenmeyer flask. Air or nitrogen was bubbled through the magnetically stirred solution and the exit gases were carried directly into 10 ml of a standard test solution of 2,4-dinitrophenylhydrazine.[52] Control runs showed the sensitivity of this system to be better than 0.35 μmol of acrolein.

Alarcon has reported[49] that a mixture of 6 μmol of **1a** and unfractionated calf serum at pH 6.8–7.0 and 37° gave rise to 1.30 μmol of acrolein after 4 hr and 3.00 μmol (25% of theory) after 22 hr. We found no acrolein formation from 6 μmol of **2a**·2HCl under conditions duplicating those described[49] for **1a**. Also, we failed to detect any acrolein from 6 μmol of **2a**·2HCl up to 72 hr at temperatures of 25, 30, or 37° when assayed in the following systems: distilled H_2O; 0.1 *M* phosphate buffer (pH 6.8, 7.0, or 7.1); Eagle's minimal essential medium[53] (20 ml) diluted to 25 ml with pH 7.0 phosphate buffer; a mixture of Eagle's minimal essential medium (20 ml), unfractionated calf serum (2 ml), and pH 7.0 buffer (3 ml). This last system is identical with the medium employed in the *in vitro* assay of spermine and related polyamines.[2,24,26] A larger sample of **2a**·2HCl (50 mg, 183 μmol) in distilled H_2O or pH 7.0 phosphate buffer similarly gave no acrolein up to 72 hr; based upon the level of detection of acrolein in this system, less than 0.01% of the unsaturated aldehyde, if any, was produced in this experiment. Similar experiments with **2b**·2HCl failed to produce detectable amounts of acrolein. Samples of **1a**·4HCl and **1b**·4HCl in the Eagle's medium-calf serum-pH 7.0 buffer system were observed to give acrolein by this test procedure.

On the basis of our studies with **2a**·2HCl and **2b**·2HCl, we conclude that acrolein is not produced from **2a** by spontaneous generation, by unimolecular decomposition at neutral pH up to 37°, by the polyamine oxidizing enzyme, or by interaction of the bisaldehyde with components of the cell culture medium. These studies do not rule out the possibility of alternative mechanisms for the formation of acrolein from spermine, such as a concerted enzymatic oxidation in which binding of the polyamine to the enzyme would be followed immediately by oxidation and then elimination of acrolein, although such a mechanism is inconsistent with the observations of Bachrach and the Tabors.[25,33,34] The mechanism of formation of acrolein from spermine and the significance of acrolein in the growth-

#Amine: O_2 oxidoreductase (deaminating); EC 1.5.3.3.

inhibitory effects produced by polyamines require further investigation.

It should be noted, however, that the bisaldehydes **2a**·2HCl and **2b**·2HCl inhibited the growth of KB (human epidermoid carcinoma) cells *in vitro*, both in the presence and absence of bovine plasma amine oxidase (*i.e.*, with calf *vs.* horse serum supplement). Significant growth inhibition was observed with both bisaldehydes at and below the ID_{50} of spermine (1.67×10^{-5} mmol/ml) in this system in the presence of calf serum;[24,26] the bisaldehydes were not titrated to a final ID_{50} end point. These assay experiments provide additional support for the structure assignments of the two bisaldehydes and, further, they prove the direct cytotoxicity of these two compounds in this system without the need for generation of acrolein as the cytotoxic agent.

Experimental Section**

Melting points were taken by the capillary method on a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are uncorrected. Nmr spectra were obtained by means of a Varian Associates A-60 spectrometer with TMS as an internal standard, except for D_2O solutions to which sodium 3-(trimethylsilyl)propanesulfonate was added as standard. Ir spectra were recorded on a Perkin-Elmer Model 137B spectrophotometer.

Ethyl 3,3-Diethoxypropionate (4). To a rapidly stirred suspension of 300 g of Zn powder in 75 ml of dry C_6H_6 at reflux was added dropwise, over a 45-min period, a solution of 67 ml (100 g, 0.6 mol) of freshly distilled ethyl α-bromoacetate and 120 ml (105 g, 0.71 mol) of triethyl orthoformate in 225 ml of dry C_6H_6. Near the end of the 45-min period, an additional 75 g of Zn powder was added and, after complete addition, reflux was continued for 6 hr. The reaction mixture was cooled to room temperature and the liquid was decanted into a mixture of 600 ml of Et_2O and 300 g of cracked ice. The flask was rinsed with 2 × 100 ml portions of Et_2O and the combined Et_2O–H_2O mixture was acidified with excess glacial AcOH until all salts were in solution. The Et_2O layer was washed with cold H_2O (450 ml) and then with cold 5% $NaHCO_3$ (450 ml) and dried. The Et_2O was evaporated on a rotary evaporator and the residue was distilled under reduced pressure to give 45.6 g (40%) of **4**, bp 53–54° (1.5 mm), 84° (12 mm) [lit.[54] bp 50–54° (1.75 mm), 91–98° (15 mm)[10]].

3,3-Diethoxypropionamide (5). A suspension of 45.6 g (0.24 mol) of **4** in 900 ml of concentrated NH_4OH was stirred at room temperature until it became homogeneous (*ca.* 10–12 hr). The solution was extracted with 5 × 300 ml portions of $CHCl_3$ and the combined extracts were washed with 100 ml of cold saturated NaCl and then 50 ml of cold H_2O. The $CHCl_3$ solution was dried and the solvent was evaporated. The remaining colorless oil was triturated under cold heptane to give 22.5 g (58%) of **5** as colorless needles, mp 54° (lit.[9] mp 54.5–54.8°).

3-Amino-1,1-diethoxypropane (6). A suspension of 19 g (0.5 mol) of $LiAlH_4$ and 400 ml of Et_2O was stirred for 20 min and then a solution of 40 g (0.25 mol) of **5** in 200 ml of Et_2O was added dropwise so as to maintain gentle reflux. After complete addition, the reaction mixture was maintained at reflux for 5 hr and cooled. The excess $LiAlH_4$ was destroyed by the cautious addition of 8% NaOH; some Et_2O was added to the reaction mixture during this procedure to replace lost volume. With destruction of the excess hydride, the reaction mixture turned from a fine gray suspension to two clear colorless liquid phases above a white precipitate. The inorganic salts were separated by filtration and washed with cold H_2O. The Et_2O layer was separated from the aqueous layer, washed with a small volume of cold H_2O, and dried. The aqueous layer, combined with all the H_2O washes, was extracted with 4 × 50 ml portions of CH_2Cl_2 and the combined CH_2Cl_2 extracts were dried. The dried Et_2O and CH_2Cl_2 solutions were separately evaporated under vacuum and the combined residues were distilled under reduced pressure to give 24 g (66%) of **6**: bp 60–62° (4 mm) [lit.[7] bp 68–70° (20 mm)]; nmr ($CDCl_3$) δ 1.23 [6 H, t, J = 14 Hz, $(OCH_2CH_3)_2$], 1.33 (2 H, s, NH_2), 1.73–1.91 (2 H, m, CH_2CH_2N), 2.83 (2 H, t, J = 16 Hz, CH_2NH_2), 3.47–3.79 [(4 H, m, $(OCH_2CH_3)_2$], and 4.64 ppm (1 H, T, J = 12 Hz, acetal methine).

N,N'-Bis(3,3-diethoxypropyl)azelaic Acid Diamide (7b). A solution of 4.5 g (0.02 mol) of azelaoyl chloride in 50 ml of dry C_6H_6 was added to a solution of 5.88 g (0.04 mol) of **6** and 4.04 g (0.04 mol) of Et_3N in 75 ml of dry C_6H_6 and the resulting mixture was heated at reflux overnight. The Et_3N • HCl precipitate was separated and the C_6H_6 filtrate was washed with a small volume of 5% $NaHCO_3$ and then with cold H_2O and dried. The solvent was removed by evapo ··· n *in vacuo* and the residue was scratched to induce crystalliz ···. The solid was washed with heptane and recrystallized from ···)-heptane to give 7.96 g (90%) of white solid: mp 73–74°; nmr ···Cl_3) δ 1.29 [12 H, t, J = 14 Hz, $(-OCH_2CH_3)_4$]. 1.35–2.40 [18 H, multiple peaks, $(-CH_2-)_7$ and $(-CH_2CH(OEt)_2)_2$]. 3.2–3.95 [12 H, multiple peaks, $(-OCH_2CH_3)_4$ and $(-CH_2NH-)_2$]. 4.63 (2 H, t, J = 10 Hz, 2 acetal methine), and 6.24 ppm (2 H, broad, 2 amide NH). *Anal.* ($C_{23}H_{46}N_2O_6$) C, H, N.

N,N'-Bis(3,3-diethoxypropyl)nonane-1,9-diamine (8b). A solution of 6.7 g (0.015 mol) of **7b** in 50 ml of THF was added dropwise to a suspension of 2.85 g of $LiAlH_4$ in 150 ml of THF at a rate so as to maintain gentle reflux. The reaction was then continued at reflux overnight, after which time it was cooled and the excess hydride was destroyed with 8% NaOH. The inorganic salts were removed by filtration and washed with H_2O. The THF layer was separated from the aqueous layer and evaporated *in vacuo*. The residue was dissolved in a small volume of CH_2Cl_2. The combined aqueous phase and washings were extracted with 3 × 25 ml portions of CH_2Cl_2, the extracts being combined with the CH_2Cl_2 solution from above. Evaporation of the dried and charcoal-clarified CH_2Cl_2 solution gave a pale yellow, somewhat viscous oil which was used without further purification for the subsequent hydrolysis step: 5.6 g (90%); nmr ($CDCl_3$) δ 0.90–2.0 [32 H, multiple peaks containing a strong t centered at δ 1.20, $(OCH_2CH_3)_4$, $(-NH-)_2$, $-(CH_2)_7-$, $(-CH_2-)_2$], 2.50–2.70 [8 H, m, $(-CH_2NHCH_2-)_2$], 3.43–3.67 [8 H, m, $(OCH_2CH_3)_4$], and 4.60 ppm (2 H, t, J = 12 Hz, 2 acetal methine).

The **8b** dinitrate was prepared by the dropwise addition of 1.5 ml of concentrated HNO_3 to a solution of 3 g of crude **8b** dissolved in 30 ml of 1:1 absolute EtOH–Et_2O cooled in a Dry Ice–Me_2CO bath. The dinitrate salt melted at 111–112° with decomposition after crystallization from EtOH–Et_2O. *Anal.* ($C_{23}H_{50}N_2O_4 \cdot 2HNO_3$) C, H, N.

The **8b** dioxalate was prepared by dropwise addition of saturated oxalic acid to a solution of crude **8b** in a minimal volume of H_2O. The dioxalate salt, mp 207° dec, was crystallized from aqueous EtOH. *Anal.* ($C_{23}H_{50}N_2O_4 \cdot 2C_2H_2O_4$) C, H, N.

The **8b** naponate was prepared from crude **8b** and naphthalene-1,5-disulfonic acid and crystallized from hot H_2O, mp 155–158°. *Anal.* ($C_{23}H_{50}N_2O_4 \cdot C_{10}H_8O_6S_2$) C, H, N, S.

4,14-Diazaheptadecanedialdehyde (2b). To a suspension of 3.0 g (0.0071 mol) of crude **8b** in 50 ml of H_2O was added with stirring 10 ml of a solution of 6.45 g (0.071 mol) of oxalic acid in 150 ml of H_2O. After 15 min the rest of the oxalic acid solution was added; a quantity of sparingly soluble **8b** dioxalate precipitated at this time and stirring was continued until no more material returned to solution (usually 3–5 hr). The reaction solution was filtered to remove a small quantity of insoluble material. Reduction of the filtrate to half-volume, followed by overnight refrigeration, gave a white precipitate of **2b** oxalate; the nmr spectrum of this material in DMSO-d_6 showed an aldehyde signal at δ 9.73 ppm.

In later experiments, the oxalate salt was not isolated. The filtrate from above was treated directly with a solution of 15 g of $BaCl_2 \cdot 2H_2O$ in 500 ml of water with stirring. After 0.5 hr, the Ba oxalate precipitate was separated and the filtrate was lyophilized. The residue was treated with 300 ml of cold (−20°) EtOH; the suspension was stirred for 10 min and filtered to remove additional Ba oxalate. The cold filtrate was treated with charcoal and refiltered. The volume was reduced to ~5 ml on a rotary evaporator at 0–10°, Et_2O was added, and the solution was scratched with cooling to induce crystallization. The resulting solid, **2b**·2HCl, was dried over P_2O_5 *in vacuo* at room temperature: 1.4 g (58%); mp dec above 350°; ir (KCl) λ 5.85 μ; nmr (DMSO-d_6) δ 9.73 ppm. *Anal.* ($C_{15}H_{30}N_2O_2 \cdot 2HCl$) C, H, Cl, N.

2b bis(2,4-dinitrophenylhydrazone) disulfate monohydrate was crystallized from glacial AcOH, mp 195–198° dec. *Anal.* ($C_{27}H_{38}N_{10}O_8 \cdot 2H_2SO_4 \cdot H_2O$) C, H, N, S.

N,N'-Bis(3,3-diethoxypropyl)succinic acid diamide (7a) was prepared according to the method described for **7b**. Succinyl chloride (3.08 g) and 6.44 g of **6** gave 6.6 g (90%) of product after crystallization from $CHCl_3$–petroleum ether (bp 60–90°): mp 83–85° (lit.[5] mp 84–85°); nmr ($CDCl_3$) δ 1.25 (12 H, t, J = 14 Hz), 1.71–1.89 (4 H, m), 2.53 (4 H, s), 3.15–3.87 (12 H, multiple peaks), 4.59

**Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.; found values are within ±0.4% of theory, except where otherwise noted.

(2 H, t, J = 10 Hz), and 6.62 ppm (2 H, broad).

N,N'-Bis(3,3-diethoxypropyl)butane-1,4-diamine (8a). Reduction of 7a to 8a, as described for 8b, gave a pale yellow oil (80–85%): nmr ($CDCl_3$) δ 1.25 (12 H, t, J = 14 Hz), 1.44–2.10 (10 H, multiple peaks), 2.61–2.81 (8 H, m), 3.25–3.86 (8 H, multiple peaks), and 4.64 ppm (2 H, t, J = 10 Hz).

The 8a dinitrate was obtained in 69% yield after crystallization from EtOH-Et_2O, mp 145–146° (lit.[5] mp 144–145°).

The 8a dioxalate had mp dec above 300°. *Anal.* ($C_{18}H_{40}N_2O_4 \cdot 2C_2H_2O_4$) C, H, N.

4,9-Diazadodecanedialdehyde (2a). Hydrolysis of 0.8 g (0.0022 mol) of 8a, according to the procedure used to prepare 2b, gave 0.39 g (62%) of 2a·2HCl: mp 270–272° dec; ir (KCl) λ 5.85 μ; nmr (DMSO-d_6) δ 9.71 ppm. *Anal.* ($C_{10}H_{20}N_2O_2 \cdot 2HCl$) H. C was found 0.78 high and N was 0.98 low (see Discussion).

2a bis(2,4-dinitrophenylhydrazone) disulfate monohydrate was crystallized from glacial AcOH, mp 175–180° dec. *Anal.* ($C_{22}H_{28}N_{10}O_8 \cdot 2H_2SO_4 \cdot H_2O$) C, H, N, S.

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