Methotrexate Analogs. 7. Synthesis of Two Higher Homologs and a Positional Isomer of Methotrexate Diethyl Ester as Potential Antitumor Agents

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Analogs of methotrexate diethyl ester (**1**) were prepared, in which the distances separating the ester functions from each other and from the carboxamide function of the *p*-aminobenzoate moiety were varied *via* the use of methylene groups as "spacers". The diethyl esters **3** and **4**, with D,L-α-aminoadipate and D,L-α-aminopimelate side chains in place of L-glutamate, displayed approximately the same order of activity as compound **1** against bacterial and mammalian cells in culture, and were inhibitors of the enzyme dihydrofolate reductase. When given intraperitoneally to L1210 leukemic mice at a dose of 120 mg./kg. q3d 1,4,7, compound **4** produced a 67% increase in survival and no evidence of toxicity, whereas methotrexate diethyl ester (**1**) gave a 44% increase in survival at a dose of 45 mg./kg. q3d 1,4,7 but was toxic at higher doses. The positional isomer **2** was inactive.

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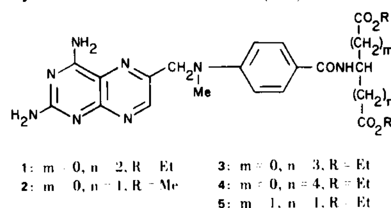
Chemical and biological studies involving side chain altered analogs of the classical folic acid antagonist methotrexate (MTX) have been greatly stimulated by the advent several years ago of a versatile new approach to pteridine synthesis (1). Advantageous use of this method has been made in the preparation of several kinds of analogs, including some with lipophilic side chains devoid of any free carboxyl groups (2,3), others with a variety of monobasic L-amino acid methyl or ethyl esters in place of L-glutamate (4), still others with a 7-methyl substituent in the pteridine ring (5), and finally an interesting compound in which N^{10} is replaced by a sulfur atom (6).

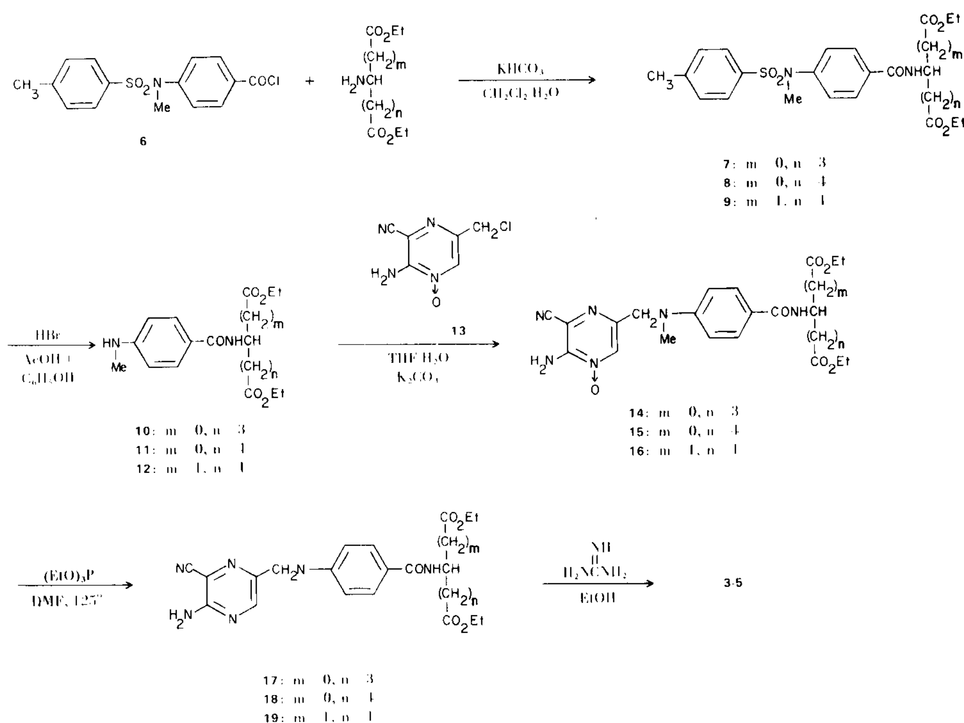
The only compound in our initial study (2,3) with *in vivo* activity against L1210 leukemia in the mouse approaching that of MTX was the diethyl L-glutamate analog **1**. This was of great interest because a dimethyl L-glutamate ester had been prepared and studied some years previously as a potential means of facilitating the passage of MTX across the blood-brain barrier (7). A convenient direct esterification procedure was then developed, which afforded high yields of MTX diesters ranging in alkyl chain length from dimethyl to di-*n*-octyl (8). A similar series of diesters was synthesized independently in another laboratory (9,10).

Among MTX analogs containing side chains other than L-glutamate, the dimethyl L-aspartate ester **2** proved to have only marginal activity against L1210 leukemia in the mouse, whereas monobasic L-amino acid esters were essentially inactive (4). In contrast, all L-glutamate esters thus far tested have shown significant activity in this test system, with increases in median survival approaching in some instances those produced by MTX itself (8). Thus it appeared reasonable to conclude that activity in MTX analogs containing an amino acid ester side chain may require the presence of *two ester groups separated by at least three carbon atoms* (11). To test this hypothesis we needed to obtain the heretofore unknown higher homologs **3** and **4** with a side chain consisting of diethyl α-aminoadipate and diethyl α-aminopimelate, respectively. This paper describes the chemical synthesis of the D,L-form of these compounds as well as the diethyl D,L-β-aminoglutarate ester **5**, the parent acid of which was reported recently by Lee and co-workers (12).



The sequence of steps employed in the synthesis of compounds **3-5** is shown in Chart I. The diethyl esters of D,L-α-aminoadipic, D,L-α-aminopimelic, and D,L-β-amino-

Chart I



glutaric acids were allowed to react with *p*-(*N*-methyl-*N*-tosylamino)benzoyl chloride (**6**) (13) in a two-phase system consisting of methylene chloride and aqueous potassium bicarbonate. Removal of the *N*-tosyl blocking group in the resulting compounds **7-9** was achieved by reaction at room temperature with 30% hydrogen bromide in glacial acetic acid containing phenol. The amines **10-12** obtained in this fashion were then condensed with 2-amino-3-cyano-5-chloromethylpyrazine N^1-oxide (**13**) (14). Coupling reactions were performed at room temperature in aqueous tetrahydrofuran in the presence of potassium carbonate, and deoxygenation of adducts **14-16** to give amino nitriles **17-19** was accomplished with triethyl phosphite in hot *N,N*-dimethylformamide as previously described (2,3). Condensation of **17-19** with guanidine (generated *in situ* from guanidine hydrochloride and a stoichiometric amount of sodium ethoxide) thereupon yielded the desired pteridines **3-5**. These were solids, in contrast to nearly all the intermediates which were thick oils with the exception of compounds **9**, **11**, **18**, and **19**. The final products were purified by column chromatography on silica gel, and their purity was established on the basis of tlc, quantitative ultraviolet absorption spectra, and microchemical analysis. Since the starting materials were D,L-mixtures and no effort was made to achieve optical resolution at any stage of the synthesis, all the products must be assumed to be racemic.

The use of α-aminoadipate, α-aminopimelate, and β-aminoglutarate ester side chains appears to be markedly disadvantageous in this reaction sequence, in accord with our previous observation (3) that yields of final product were lower when the side chain contained diethyl glutamate than when it consisted of a side chain lacking ester functions. Although the exact reason for these low yields is not known, they are perhaps attributable to Claisen and other tar-forming side reactions for which guanidine can act as a catalyst. The use of the mixed anhydride synthesis with 4-amino-4-deoxy-N^{10}-methylpteroic acid (4) offers an alternative synthetic approach which avoids this problem, but it should be noted that even *via* the mixed anhydride synthesis the yield of final product seldom exceeds 40% when the side chain contains an ester group and is in fact often only 10-20%.

Biological Studies.

Bioassay results for diesters **3** and **4** in three *in vitro* test systems are shown in Table I, which also includes for comparison some data previously reported for the diethyl ester (3,8). It is evident from these results that growth-inhibitory activities against the bacterial organism *S. faecium* and the human cell line employed in this study are strikingly similar for all three compounds and can be correlated with ID_{50} values against purified *L. casei* dihydrofolate reductase. Compound **5**, as expected from the

Table I

Bioassay of *N*-(4-Amino-4-deoxy-N^{10}-methylpteroyl)aminoalkanedicarboxylic Acid Esters *in Vitro*

Compound	50% Inhibition Dose (ID_{50}) *S. faecium* (a) μg./ml.	CCRF-CEM (b) μg./ml.	*L. casei* DHFR (c) moles/liter
1	0.02	0.01 (ID_{60})	7×10^{-7}
3	0.05	0.02	4×10^{-6}
4	0.04	0.02	5×10^{-6}

(a) Assay at a folate concentration of 0.001 μg./ml.; see reference (22). (b) Human lymphoblastic leukemia cells in suspension culture; see reference (23). (c) DHFR = dihydrofolate reductase (kindly supplied by Dr. R. L. Kisliuk, Tufts-New England Medical Center); assay performed as previously described (3).

reported lack of activity of the parent acid (12), was inactive.

The *in vivo* antitumor evaluation of compounds **3** and **4** against L1210 leukemia in mice is summarized in Table II. The diethyl ester **1** is also shown for comparison. At the maximum tolerated doses (45 mg./kg. and 120 mg./kg., respectively) compounds **1** and **3** each produced only a 44% increase in survival of test animals versus untreated controls. Compound **4**, on the other hand, caused 55% and 67% prolongations in lifespan at doses of 60 mg./kg. and 120 mg./kg., respectively. The higher optimal dose for compounds **3** and **4** relative to compound **1** may be attributable to the fact that **3** and **4** were prepared from D,L-α-amino acid esters whereas **1** was prepared from diethyl L-glutamate (3). The D-isomer of methotrexate is known to be less active than the L-isomer against the L1210 tumor (12).

The enhanced survival observed *in vivo* with compound **4** is of interest in view of the fact that its activity is somewhat lower than that of compounds **1** and **3** against bacterial and mammalian cells in culture, and is significantly lower than that of compound **1** against the cell-free enzyme (see Table I). These findings suggest that compound **4** may owe its favorable activity against L1210 leukemia to a combination of secondary pharmacokinetic properties rather than to any simple quantitative difference in interaction with dihydrofolate reductase. Among the pharmacologic phenomena that have to be considered as being potentially responsible for the activity of compound **4** are 1) cleavage of the diester by esterases in the serum or ascites fluid of the mouse; and 2) transport of the diester (or its diacid) across the cell membrane of leukemic versus normal cells. It has already been established that MTX esters such as **1** are cleaved extensively to free MTX by esterases in mouse serum (10,15,16), and it seems reasonable to assume that the diethyl D,L-α-aminoadipate and diethyl D,L-α-aminopimelate analogs **3** and **4** can undergo similar enzymic cleavage *in vivo*. In other words these compound probably represent "latent" derivatives of the corresponding diacids, the synthesis and biological activity of which have been reported for the first time only recently (17). It is also possible, however, that these diesters produce their cytotoxic effect, in part, by a mechanism different from that of the parent diacid (18).

Table II

In Vivo Activity of *N*-(4-Amino-4-deoxy-N^{10}-methylpteroyl)aminoalkanedicarboxylic Acid Esters Against L1210 Leukemia in Mice (a)

Compound	Dose (b) mg./kg.	Mean Survival days	Increase %
Control		9.0	
1	22.5	12.0	+33
	45	13.0	+44
3	60	11.0	+22
	120	13.0	+44
4	60	14.0	+55
	120	15.0	+67

(a) Male BDF_1 mice, injected intraperitoneally (ip) with 10^5 leukemic cells (L1210) on day 0, were randomized into groups of five, and each group received ip injections of drug in 10% Tween 80 suspension on days 1, 4, and 7. The untreated control group consisted of fifteen or more animals. (b) Compound **1** was prepared from diethyl L-glutamate (3), whereas the synthesis given in this paper for compounds **3** and **4** utilizes diethyl D,L-α-aminoadipate and D,L-α-aminopimelate respectively. This difference may be in part responsible for the finding that optimal doses were about three times higher with compounds **3** and **4** than with compound **1**.

EXPERIMENTAL (19)

Diethyl D,L-α-Aminoadipate and Diethyl D,L-α-Aminopimelate.

D,L-α-Aminopimelic acid (Sigma Chemical Co., St. Louis, Mis-

souri) (5.0 g., 0.029 mole) was added to absolute ethanol (100 ml.) which had previously been saturated with dry gaseous hydrogen chloride (10 g.). The mixture was allowed to reflux for 4 hours, and was then concentrated to a volume of 60-70 ml. on the rotary evaporator. Water (100 ml.) was added, the *p*H was adjusted to 9.0 with potassium bicarbonate, and the product was extracted into ether (7 x 200 ml.). Drying over magnesium sulfate and solvent evaporation yielded 5.5 g. (83%) of diethyl D,L-α-aminopimelate as a pale yellow mobile oil showing the requisite ester carbonyl band in the infrared at 1750 cm^{-1}. A similar procedure led to esterification of D,L-α-aminoadipic acid (Sigma Chemical Co.) in 43% yield. The diesters were used immediately in order to avoid diketopiperazine or polyamide formation.

Diethyl D,L-β-Aminoglutarate.

Diethyl glutaconate (Aldrich Chemical Co., Milwaukee, Wisconsin) (25 g., 0.14 mole) was added to absolute ethanol (120 ml.) which had previously been saturated at room temperature with dry ammonia gas. Passage of ammonia through the solution was continued at 52° (bath temperature) for 36 hours, whereupon the reaction mixture was cooled and concentrated to dryness on the rotary evaporator at room temperature. The oily residue was converted into a crystalline hydrochloride salt by dissolving it in ether (100 ml.), saturating the solution with gaseous hydrogen chloride at 0°, and storing in the cold for several hours until precipitation occurred; yield 24 g. (76%), m.p. 83-84° (lit. (20) 83.5-84.5°). The hydrochloride salt was sufficiently pure to be used directly in the next step.

Diethyl β-*N*-[*p*-(*N*-Methyl-*N*-tosylamino)benzoyl]aminoglutarate (**9**) (21).

Diethyl β-aminoglutarate hydrochloride (20) (24 g., 0.1 mole) was added to a solution of *p*-(*N*-methyl-*N*-tosyl)aminobenzoyl chloride (**6**) (13) (32 g., 0.1 mole) in dichloromethane (500 ml.), and the slurry was added gradually to a solution of potassium bicarbonate (20 g., 0.2 mole) in water (300 ml.). After vigorous overnight agitation at room temperature, the organic layer was separated, washed with 1 *N* hydrochloric acid, rinsed with water, dried, and evaporated to a semi-solid (46 g., 95% yield). Recrystallization from ethanol with the aid of decolorizing carbon gave small colorless needles, m.p. 107-108°.

Anal. Calcd. for $C_{24}H_{30}N_2O_7S$: C, 58.76; H, 6.16; N, 5.71; S, 6.54. Found: C, 59.00; H, 6.39; N, 5.66; S, 6.40.

Diethyl α-*N*-[*p*-(*N*-Methylamino)benzoyl]aminopimelate (**11**).

A mixture of diethyl α-aminopimelate (3.74 g., 0.0161 mole), *p*-(*N*-methyl-*N*-tosylamino)benzoyl chloride (4.66 g., 0.0144 mole), potassium bicarbonate (11.6 g., 0.116 mole), dichloromethane (80 ml.), and water (80 ml.) was stirred vigorously at room temperature for 3 days. The crude coupling product **8** was isolated in the same manner as compound **9** and then kept at room temperature for 4 hours in a mixture of 30% hydrogen bromide in glacial acetic acid (32 ml.) and phenol (3.2 g.). Work-up of the product exactly as described in the preparation of compound **12** (see below) led to spontaneous crystallization during evaporation of the dichloromethane solution. Recrystallization from ethyl acetate afforded a beige powder (3.23 g., 61.5% yield). Analytically pure **11** was obtained after a second recrystallization from ethyl acetate, m.p. 94-95°.

Anal. Calcd. for $C_{19}H_{28}N_2O_5$: C, 62.61; H, 7.74; N, 7.68. Found: C, 62.75; H, 7.74; N, 7.62.

Diethyl β-*N*-(*p*-*N*-Methylaminobenzoyl)aminoglutarate (**12**).

bromide in glacial acetic acid (200 ml.), and phenol (8.5 g.) was stirred at room temperature for 4 hours, and then diluted with ether (2 lit.). The heterogeneous mixture was shaken thoroughly and the layers were allowed to separate. After decantation of the ether layer, the oily residue was dissolved in water which was basified carefully with potassium carbonate (caution: vigorous carbon dioxide evolution!) and extracted several times with dichloromethane. Drying and solvent evaporation left a yellow oil (30.5 g., 98% yield) which was used without additional purification in the next step. For microanalysis a portion of the oil was redissolved in dichloromethane, the solution was decolorized with charcoal, and dry hydrogen chloride was passed through with cooling until a crystalline *hydrochloride salt* was formed, m.p. 145-147° dec.

Anal. Calcd. for $C_{17}H_{24}N_2O_5 \cdot HCl$: C, 54.76; H, 6.76; Cl, 9.51; N, 7.51. Found: C, 54.87; H, 6.84; Cl, 9.49; N, 7.51.

Diethyl α-*N*-[*p*-[*N*-(2-Amino-3-cyanopyrazin-5-ylmethyl)-*N*-methylamino]benzoyl]aminoadipate (**17**).

A stirred solution of diethyl α-aminoadipate (7.96 g., 0.0391 mole) and *p*-(*N*-methyl-*N*-tosylamino)benzoyl chloride (11.3 g., 0.0350 mole) in dichloromethane (200 ml.) was treated dropwise with a solution of potassium bicarbonate (28 g., 0.28 mole) in water (200 ml.). After 18 hours of vigorous stirring at room temperature, the organic layer was separated, washed with 1 *N* hydrochloric acid (2 x 200 ml.), rinsed with water, dried, and evaporated. The oily residue, consisting of crude compound **7**, was taken up directly in 30% hydrogen bromide in glacial acetic acid (80 ml.), phenol (8 g.) was added, and the solution was stirred at room temperature for 4 hours. Ether (320 ml.) was then added and the two-phase mixture was agitated vigorously for several minutes. The ether layer was decanted and the remaining oil was taken up in dichloromethane (325 ml.). Extraction with 1 *N* potassium bicarbonate (2 x 325 ml.), drying, and solvent evaporation gave compound **10** as an amber-colored oil (5.91 g., 48% crude yield). This oil and the *N*-oxide **13** (14) (3.12 g., 0.0169 mole) were dissolved directly in tetrahydrofuran (50 ml.) and a solution of potassium carbonate (3.53 g., 0.0255 mole) in water (50 ml.) was added. After 2.5 hours of vigorous agitation at room temperature, the mixture was diluted with water (200 ml.) and extracted with chloroform (3 x 200 ml.). The combined chloroform extracts were washed with water, dried, and evaporated to a viscous oil (**14**, 5.84 g.) which was heated for 45 minutes at 125° (internal temperature) in a mixture of triethyl phosphite (25 ml.) and *N*,*N*-dimethylformamide (20 ml.). Evaporation under reduced pressure and recrystallization of the solid residue from a 30:70 mixture of ethyl acetate and benzene gave compound **17** as a yellow solid (3.21 g., 19% overall yield based on compound **6**, 54% yield based on compound **13**). The first crop consisted of analytically pure pale yellow crystals, m.p. 133-134°.

Anal. Calcd. for $C_{24}H_{30}N_6O_5$: C, 59.73; H, 6.26; N, 17.41. Found: C, 59.61; H, 6.11; N, 17.39.

Diethyl α-*N*-[*p*-[*N*-(2-Amino-3-cyanopyrazin-5-ylmethyl)-*N*-methylamino]benzoyl]aminopimelate (**18**).

To a well-stirred solution of the diester **11** (7.99 g., 0.0219 mole) and the *N*-oxide **13** (14) (4.04 g., 0.0219 mole) in tetrahydrofuran (65 ml.) was added dropwise a solution of potassium carbonate (4.53 g., 0.0328 mole) in water (65 ml.). After 2.5 hours at room temperature, the mixture was diluted with water (200 ml.) and extracted with chloroform (3 x 200 ml.). The combined organic layers were washed with water, dried, and evaporated to a viscous oil (compound **15**) which was heated

dimethylformamide (20 ml.) at 125° (internal temperature) for 45 minutes. Evaporation under reduced pressure and recrystallization of the viscous residue from 1:1 ethyl acetate-benzene gave compound **18** (3.81 g., 35% overall yield based on **11** and **13**) as a pale yellow powder. Analytically pure crystals were obtained after a second recrystallization from 1:1 ethyl acetate-benzene, m.p. 116-118°.

Anal. Calcd. for $C_{25}H_{32}N_6O_5$: C, 60.46; H, 6.49; N, 16.92. Found: C, 60.37; H, 6.50; N, 16.76.

Diethyl α-*N*-(4-Amino-4-deoxy-N^{10}-methylpteroyl)aminoadipate (**3**).

Metallic sodium (0.173 g., 0.00752 g.-atom) was dissolved in absolute ethanol (70 ml.), guanidine hydrochloride (0.700 g., 0.00730 mole) was added, and the sodium chloride precipitate which formed was filtered off after 15 minutes. The amino nitrile **17** (3.14 g., 0.00650 mole) was added to the filtrate, and the mixture was stirred under reflux for 15 minutes and then cooled at once to room temperature. The precipitated solid was filtered, washed with ether, and purified by column chromatography on silica gel (30 g., Baker 3405, 60-200 mesh). Fractions were collected and pooled on the basis of visual examination of yellow bands moving down the column and thin-layer chromatographic analysis of portions of the eluate on silica gel (Eastman 6060, 9:1 chloroform-ethanol). Elution of the column with chloroform alone afforded a fast-moving component (*ca.* 0.3 g.) with the same tlc mobility as the starting material (R_f = 0.84). Elution with 5% methanol in chloroform yielded a fraction (0.39 g.) which was tlc-homogeneous (R_f = 0.55), and also some impure material from which it was possible to isolate another 0.03 g. of product after a second column chromatography; total recovery 0.42 g. (12% yield). The twice-chromatographed sample was analytically pure, m.p. 130-134°; λ max (nm) (95% ethanol): 220 (ε 22,216), 262 (25,473), 299 (27,414), 374 (8,002).

Anal. Calcd. for $C_{25}H_{32}N_8O_5$: C, 57.24; H, 6.14; N, 21.36. Found: C, 57.05; H, 6.03; N, 21.32.

Diethyl α-*N*-(4-Amino-4-deoxy-N^{10}-methylpteroyl)aminopimelate (**4**).

Metallic sodium (0.139 g., 0.00602 g.-atom) was dissolved in absolute ethanol (60 ml.) and guanidine hydrochloride (0.575 g., 0.0062 mole) was added. After 15 minutes of stirring, the sodium chloride precipitate was filtered off, the amino nitrile **18** (14) (2.65 g., 0.00533 mole) was added, and the mixture was stirred under reflux for 30 minutes and placed in the refrigerator. A trace of solid which precipitated on cooling was removed by filtration and the filtrate was concentrated to dryness on the rotary evaporator. The gummy residue was triturated several times with chloroform, the insoluble portion was filtered off, and the filtrate was evaporated to a glassy yellow solid which was purified further by column chromatography on silica gel (70 g., Baker 3405, 60-200 mesh) as described in the preceding experiment. Elution of the column with 5% methanol in chloroform gave several fast-moving components which were not characterized (total *ca.* 0.4 g.), and a slower-moving fraction (0.26 g., 9% yield) which was tlc-homogeneous (R_f = 0.60). Elution with 7.5% and finally 30% methanol in chloroform yielded two other components (total *ca.* 0.5 g.) which moved very slowly on tlc and contained none of the desired product. The fraction with R_f = 0.60 was analytically pure, m.p. 131-134°; λ max (nm) (95% ethanol): 220 (ε 20,406), 262 (25,832), 299 (27,380), 375 (8,050).

Anal. Calcd. for $C_{26}H_{34}N_8O_5$: C, 57.98; H, 6.36; N, 20.80. Found: C, 57.91; H, 6.32; N, 20.83.

Diethyl β-*N*-(4-Amino-4-deoxy-N^{10}-methylpteroyl)aminoglutarate (**5**).

A. A solution of the diester **12** (60 g., 0.178 mole) and the *N*-oxide **13** (14) (33 g., 0.178 mole) in tetrahydrofuran (700 ml.) was treated dropwise with a solution of potassium carbonate (26 g., 0.188 mole) in water (700 ml.). After being stirred vigorously at room temperature for 2 hours, the reaction mixture was diluted with water (2 l.) and the product was extracted with several portions of dichloromethane. The combined organic layers were washed with water, dried, and evaporated to an oil (compound **16**) which was treated directly with triethyl phosphite (350 ml.) at 120-130° (internal temperature) for 2 hours. The reaction mixture was concentrated to dryness under reduced pressure and the oily residue was purified by column chromatography on silica gel (Baker 3405, 60-200 mesh) with 10% ethyl acetate in benzene as the eluent. The chromatographed product (23 g., 28% yield) was an oil which could not be induced to crystallize but was tlc-homogeneous and showed the requisite infrared spectral properties for amino nitrile **19**. This material was dissolved directly in absolute ethanol (500 ml.), guanidine carbonate (5.0 g., 0.056 equivalent) was added, and the mixture was stirred under reflux for 3 hours. After being cooled slightly, the mixture was treated with decolorizing carbon and filtered while still warm, and the filtrate was reduced to a small volume and applied to a column of silicic acid (300 g., Bio-Sil A, 100-200 mesh). The column was eluted first with chloroform and then 2% ethanol in chloroform in order to remove the fast-moving components. Elution with 5% ethanol in chloroform then gave a fraction (3.3 g., 13% yield based on compound **19**) which was tlc-homogeneous (Gelman ITLC Type SG, 5% ethanol in chloroform).

B. When amino nitrile **19** (4.68 g., 0.01 mole) was treated with guanidine in the manner described for compounds **3** and **4** the yield of **5** was 0.48 g. (9%). The analytical sample was obtained from a column chromatographic center cut, m.p. 180-184° dec.; λ max (nm) (95% ethanol): 220 (ε 22,438), 262 (25,858), 296 (26,709), 375 (7,763).

Anal. Calcd. for $C_{24}H_{30}N_8O_5$: C, 56.46; H, 5.92; N, 21.95. Found: C, 56.01; H, 5.87; N, 22.19.

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(19) Melting points (uncorrected) were determined in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) at a heating rate of approximately 2°/minute. Infrared spectra were recorded on a Perkin-Elmer Model 137B double beam recording spectrophotometer and quantitative ultraviolet spectra were determined on Cary Model 11 and Model 15 instruments. Nmr spectra were taken by means of a Varian A60 spectrometer with tetramethylsilane as the reference. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

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(21) All compounds containing the α-aminoadipic, α-aminopimelic, or β-aminoglutaric ester side chain are understood to be mixtures of the D- and L-enantiomers even though they are not so designated.

(22) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.*, **76**, 413 (1958).

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