Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, MD. The authors are grateful to Dr. Herbert Lazarus, formerly of the Dana-Farber Cancer Institute and now at the University of Miami School of Medicine, and Dr. John M. Idriss of these laboratories for the cell culture determinations and also to Linda B. Anderson for assistance with the in vivo antitumor assays. Generous supplies of bulk daunorubicin hydrochloride for this work were kindly provided by Farmitalia-Carlo Erba, Milan, Italy, through the assistance of Adria Laboratories, Inc., Columbus, OH.

Registry No. 2·HCl, 23541-50-6; 7, 26295-55-6; 8, 65026-79-1; 9, 77270-18-9; 10, 97191-43-0; 11, 97191-44-1; 12, 80787-28-6; 13, 80787-29-7; 14, 97191-45-2; 15, 97191-46-3; 16, 97191-47-4; monosodium malonate, 2922-55-6; monosodium succinate, 2922-54-5; monosodium glutarate, 3343-88-2; monosodium adipate, 18996-34-4; monosodium pimelate, 6142-21-8; monosodium azelate, 17356-30-8; monosodium sebacate, 19455-73-3.

Synthesis and Antimetastatic Properties of Stereoisomeric Tricyclic Bis(dioxopiperazines) in the Lewis Lung Carcinoma Model[1]

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Synthesis of *trans*- and *cis*-tetrahydrodipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9(2*H*,4*H*,8*H*,10*H*)-tetrone analogues **10** and **11** belonging to the bis(dioxopiperazine) class of antitumor agents and their bis(morpholinomethyl) derivatives **12** and **13** are described with use of 2,5-dimethylpyrazine as the starting material. Synthetic studies utilizing 3,6-disubstituted 2,5-dioxopiperazine precursors are included. Evaluation of **10–13** in the Lewis Lung carcinoma model indicated the bis(morpholinomethyl) analogue *cis*-**13** to be antimetastatic, whereas the trans isomer **12** was toxic at a similar dose effecting a decrease in the life span of treated mice. The parent bis(dioxopiperazines) **10** and **11** were ineffective as antitumor or antimetastatic drugs.

Chemical, biochemical, and pharmacological properties of antitumor bis(dioxopiperazines) recently have been reviewed.[2] Cyclic analogues **4–9** of **1** and **2** exhibited stereoselective effects in various tumor models.[3-6] In the solid state a cis "face to face" conformation of the dioxopiperazine rings were observed in antimetastatic racemic **2** and *cis*-**5**.[7,8] However, such a conformation seems not to be essential for activity since tricyclic bis(dioxopiperazine) *trans*-**6** exhibited antimetastatic properties in the B16-F10 melanoma model.[5,9]

Recent reports from China[10] have indicated the bis(morpholinomethyl) derivative of **1** (namely, **3**) to be effective in various human malignancies. Although such compounds are predictably unstable and undergo hydrolysis to the parent dioxopiperazines, morpholinomethyl–N groups may impart antineoplastic properties to a molecule owing to possible alkylating activities not unlike those proposed[11] for certain hydroxymethyl–N metabolites of therapeutically useful drugs. Comparative analysis of morpholinomethyl derivatives **8** and **9** with the respective parent imides **6** and **7** in a postoperative Lewis Lung (LL) carcinoma model revealed morpholinomethyl cis-syn-trans isomer **9** to be more effective as an inhibitor of metastasis than the other three analogues (**6–8**).[12] Additionally, the order of decreasing activity was **9** > **8** > **7** > **6** when assessed in terms of survival or antimetastatic data. The increased activity observed for the morpholinomethyl derivatives over their respective parents was attributed[12] either to increased solubility and drug delivery (prodrug) or to an intrinsic antitumor activity of the morpholinomethyl–N functionality possibly reflecting macromolecular alkylation. In this report we describe the synthesis of analogues **10–13** and biological evaluation of these compounds in the LL carcinoma model using the postoperative protocol. Such studies are of interest since previous investigations of conformationally constrained analogue pairs (**4**, **5** and **6**, **7**) suggested that a "cisoid" relationship of imide functionalities was necessary for antimetastatic activity.[4-6] Clearly, interatomic distances between groups in **10** and **11** are markedly different than those found in **6** and **7**. Consequently, selective antitumor activity observed for morpholinomethyl analogues would provide support for intrinsic antitumor activity of the morpholinomethyl–N functionality.

Chemistry. Our synthetic strategy utilized the analysis shown in Scheme I. Two pathways were explored for the synthesis of piperazines **20–22**. One involved selective reduction of the amide functionalities in 3,6-disubstituted 2,5-dioxopiperazine precursors. Dioxopiperazine diester **23** of undefined stereochemistry was reported[13] to have been formed on cooling a solution of ethyl aminomalonate

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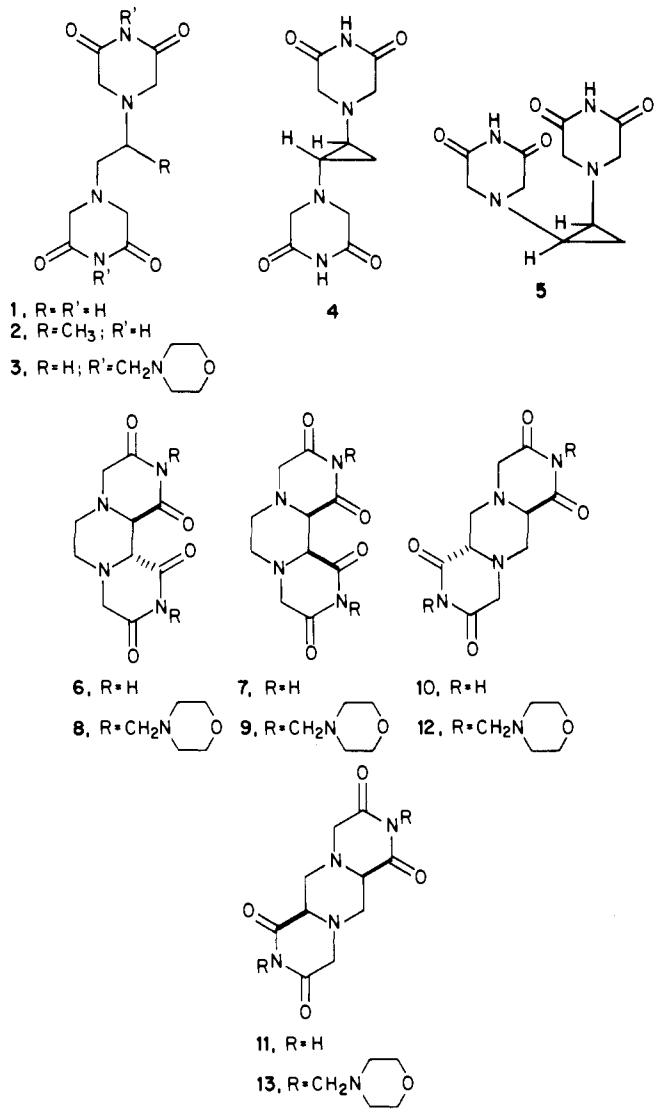
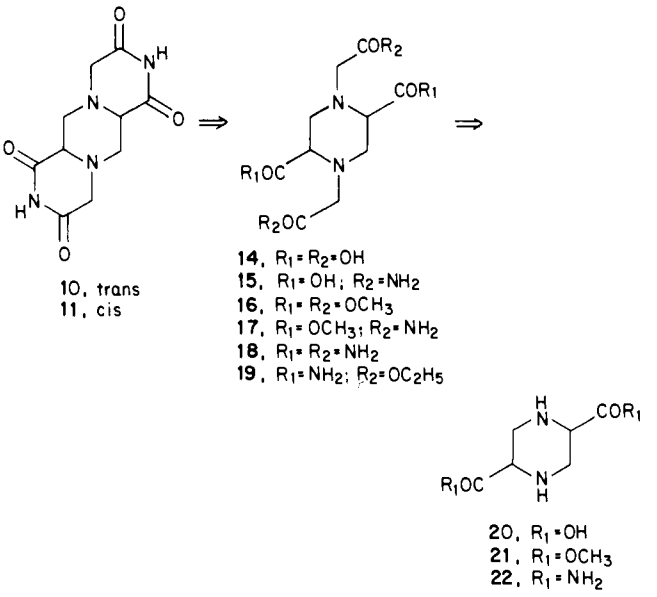


in acetone. Later reports by Hino and Sato[14,15] indicated that ethyl aminomalonate did not dimerize to **21** at elevated temperatures. Our results were in agreement with these later reports; ethyl aminomalonate prepared according to the method of Schipper and Day[16] could not be dimerized under various conditions.

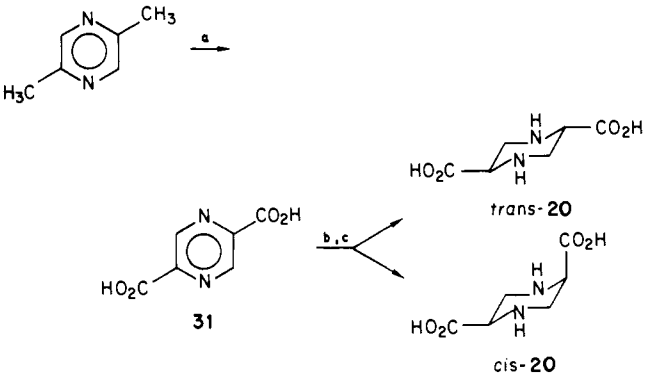
We therefore investigated the dioxopiperazinediol **24** as a possible precursor to the piperazinedicarboxylates **20–22**. Geometric isomers of diol **24** were prepared according to the procedure of Rao and Ravindranath[17] from commercially available serine methyl ester hydrochloride. Hydroxyl protection was necessary for further synthetic work since isomers of **24** are insoluble in most organic solvents. Attempts to prepare the THP ethers failed. Isomers of diacetates **25** were obtained from reaction of isomerically pure **24** with AcCl in HOAc, but such derivatization did not markedly improve solubility characteristics. Geometric assignments for **25** are tentative. Respective ^{13}C and ^{1}H NMR spectra for the pure isomeric esters were nearly identical and thus not useful for defining stereochemistry.

Selective Borch reduction[18] of *cis*-**25** was not successful; reaction of *cis*-**25** with triethyloxonium tetrafluoroborate

Scheme I

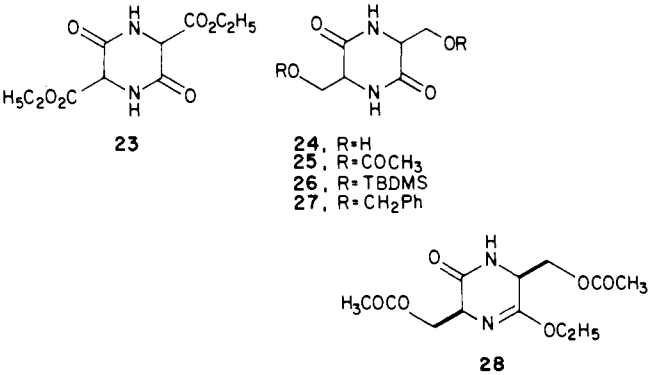


Scheme II[a]



[a] a = SeO_2, pyridine–water, reflux; b = KOH, H_2O, Pd/C, H_2, 40–42 psi, 50–60 °C; c = HCl.

only afforded mono(imino ether) **28** in 25% yield. The desired bis(imino ether) could not be obtained even when using higher reaction temperatures or longer reaction times. Similarly, attempts to obtain the piperazine from *tert*-butyldimethylsilyl ether **26** prepared in 63–64% yield from pure *cis*- or *trans*-**24** was not successful.



Synthesis of benzyl-protected diol **27** had been previously reported by Russian investigators.[19] Our attempts to reproduce their procedure resulted in poor yields of intermediates. Alternatively, Fisher esterification of

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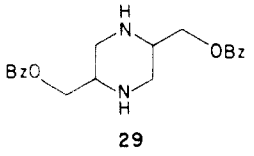
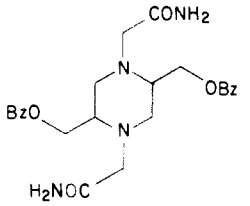
commercially available *O*-benzyl-L-serine followed by chromatography of the resultant methyl ester hydrochloride (Amberlite IRA-45/CH_3OH) and solvent removal yielded an oily eluate which dimerized, affording a cis–trans mixture of **27** in 57% yield upon standing at room temperature for 7 days. Although these isomers could be separated by chromatography (silica gel/$CHCl_3$), for convenience, the mixture was employed in subsequent reactions. The dioxopiperazine geometry could not be assigned from their NMR spectra but could be confirmed following conversion to the corresponding piperazines **29**. $LiAlH_4$ reduction of diastereomeric **27** afforded corresponding piperazines **29**, which upon treatment with iodoacetamide/K_2CO_3 provided diastereomers **30** in 64% yield. Isomers were separated (silica gel/$CHCl_3$) and their structures confirmed by NMR analysis (see Experimental Section).



29



30

Attempted debenzylation of diamide **30** using catalytic hydrogenation (Pd/C),[20] transfer hydrogenation,[21,22] Na/liquid NH_3,[23] or Me_3SiI[24] were unsuccessful owing to competing N-dealkylation.

The second route provided intermediate piperazine-2,5-dicarboxylates **20–22** by reduction of the corresponding pyrazine precursors. Preparation of **20** had been previously reported,[25] but no evidence for the proposed geometry was presented. We prepared **20** from the corresponding pyrazine **31**, which was obtained in 65–71% yield from SeO_2 oxidation of commercially available 2,5-dimethylpyrazine (Scheme II).[26] Catalytic hydrogenation of an alkaline solution of **31** using 10% Pd on carbon afforded an isomeric mixture of **20**, which was separated by fractional crystallization under controlled pH. Gradual acidification of the concentrate initially crystallized *trans*-**20** (pH ~ 5.5–6.5). Further reduction in pH crystallized *cis*-**20**. Geometric configurations were assigned by ^1H NMR analysis.

Piperazine tetraacids **14** were obtained by alkylation of the pure isomers of diacids **20** with use of bromoacetic acid and K_2CO_3 in water. Use of chloroacetamide under similar reaction conditions afforded the diamide diacid *trans*-**15** from *trans*-**20**. *cis*-**15** could not be obtained by this procedure. Attempts to prepare the target bis(dioxopiperazines) **10** and **11** from the tetraacids **14** using NH_3,[27] urea,[28] or formamide[29] resulted in decomposition of **14**. Similarly, refluxing in Ac_2O,[30] heating neat, use of DCC, or heating in acidic solvents (CH_3CO_2H, H_2SO_4, or PPA) did not effect cyclization of *trans*-**15** to **10**. While Fisher esterification of *trans*-**15** afforded tetraester *trans*-**16**, other methods of esterification[31–33] did not produce the desired amide ester *trans*-**17** presumably owing to the insoluble nature of *trans*-**15**. Both cis and trans tetraesters **16** also were obtained in good yields (69–74%) from Fisher esterification of the corresponding tetraacids **14**. Although reaction of tetraesters **16** with liquid NH_3 in a sealed tube produced excellent yields of the corresponding tetraamides **18** and none of the bis(imides), reaction of either isomer of **16** with NH_3 in MeOH/NaOMe afforded small amounts (≤16%) of desired *trans*-**10** in addition to the major product *trans*-**18**. Likewise, although heating **16** with formamide failed to produce the bis(dioxopiperazines) **10** or **11**, reaction with formamide in the presence of NaH/refluxing dimethoxyethane or dioxane afforded **10** as the exclusive product in about 25% yield. Heating either isomer of tetraamide **18** in PPA at 85 °C for 15–30 min afforded small amounts (10–15%) of **10**; decomposition of the reactants was predominant.

In order to further improve the yield of **10** and also to obtain samples of the cis isomer **11**, we investigated use of intermediates **21** and **22**. Diacids **20** upon refluxing in methanolic HCl afforded the corresponding diesters **21**. Attempts to prepare diester diamides **17** by alkylation of **21** with iodoacetamide was unsuccessful. Alternatively, reaction of **21** with liquid NH_3 in a sealed tube afforded excellent yields of diamides **22**. N-Alkylation of **22** using ethyl bromoacetate and K_2CO_3 in Me_2SO afforded the diamide diesters **19**, which upon refluxing in EtOH/NaOEt afforded the target bis(dioxopiperazines) **10** and **11** in 71–89% yield. Conversion to the corresponding bis(morpholinomethyl) derivatives **12** and **13** took place in Me_2SO in the presence of morpholine and formaldehyde. Unlike morpholinomethyl analogues **8** and **9**, these compounds were virtually insoluble in water.

NMR Spectral Analysis. Characterization of isomeric tricycles **10** and **11** was based in part upon analysis of the 500-MHz proton NMR spectrum of precursor piperazine-2,5-dicarboxylic acids *cis*- and *trans*-**20**. The piperazine ring proton resonance signals for *trans*-**20** exhibited an AMX pattern. The axial H_A resonance signal of the ring methylene group appeared as a deceptively simple triplet at δ 3.64. The geminal (14.1 Hz) and axial–equatorial (3.8 Hz) coupling constants were derived by analysis of the H_M equatorial doublet of doublets (δ 4.21) of the ring methylene group. The H_X methine doublet of doublets centered at δ 4.53 exhibited axial–equatorial and diaxial (12.4 Hz) couplings.

For *cis*-**20**, which rapidly interconverts on the NMR time scale, an ABX pattern was observed for the piperazine proton resonance signals wherein calculated constants were δ_A 3.93 and δ_B 4.02 (J_{AX} = 4.4 Hz and J_{BX} = 7.1 Hz) with δ_X (observed) 4.71 and J_{AB} (observed) = 14.5 Hz. Splitting patterns similar to those exhibited by *cis*- and *trans*-**20** were identified, respectively, for cis and trans diesters **21** and diamides **22** and the diester diamide *trans*-**19**. The cis diester diamide **19** provided a first-order spectrum at 90 MHz which was in agreement with the proposed

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Table I. Lewis Lung Carcinoma (LL) Metastasis Study[a]

	survival data				autopsy data[b]				
							av no. of metastasis		
compd	survival days	MST[c]	% ILS[d]	N/T[e]	av body wt, g	av lung wt, mg	<2 mm	>2 mm	M/T[f]
control	20, 23, 24, 25, 26, 28, 28, 28, 29, 32	27.0		0/10	16.3	741	0	49	10/10
10	18, 26, 28, 28, 31	28.0	4	0/5	16.9	853	0	48	4/4
11	17, 19, 28, 29, >40	28.0	4	1/5	19.3	640	0	22	2/3
12	17, 25, 26, 26, >40	26.0	<0	1/5	18.9	700	0	38	3/4
13	19, 32, >40, >40, >40	>40	>48	3/5	19.2	358	0	6	1/4

[a] BDF_1 female (19–21 g) mice; implantation on day 0. Amputation on day 8. Post amputational schedule: 160 mg/kg from day 8, Q2Dx4 (ip). Compounds were administered in suspension (saline). [b] Autopsy data of dying mice and mice killed day 40. [c] Median survival time (days). [d] Increase in life span of 25% or greater indicates activity. [e] Number of 40 days survivors/total mice. [f] Number of mice with metastasis/total.

structure. Relative to tricyclic bis(dioxopiperazines), the spectrum of *trans*-**10** exhibited a characteristic deceptively simple triplet for the H_A axial proton (δ 2.18) of the central ring methylene group not unlike that observed for the equivalent proton resonance signal in piperazine diacid precursor *trans*-**20**. The cis isomer **11** exhibited a spectrum (90 MHz) wherein the axial H_A and equatorial H_B resonance signals of the central ring methylene group appeared as broad multiplets between δ 2.7 and 2.9.

Biological Results and Discussion

The effects of compounds **10–13** in the postoperative LL carcinoma model is summarized in Table I. BDF_1 female mice weighing 19–21 g were used in the study. LL (10^5 cells) was implanted im in the leg on day 0, and the tumor-bearing legs were amputated on day 8. Dying mice and the mice killed on day 40 following implantation were autopsied.

Among the four compounds evaluated (**10–13**), only the *cis*-bis(morpholinomethyl) analogue **13** markedly inhibited the metastasis. This isomer also provided for a significant increase in life span of the treated mice. This stereoselective antimetastatic effect is of particular interest in this series. Unlike the situation in the tetraazaperhydrophenanthrene series (**6–9**), wherein morpholinomethyl derivatives were considerably more water soluble than their parent imides, in the tetraazaanthracene series (**10–13**) all compounds were water insoluble. Since the parent bis(dioxopiperazine) *cis*-**11** did not exhibit any appreciable activity in this assay, it is unlikely that the activity of **13** is due to its metabolic conversion to **11**. Rather, it seems likely that certain bis(morpholinomethyl) derivatives possess intrinsic antitumor activities. No obvious conclusions can be drawn to explain why cis ring junctures in both tricyclic systems provide the most effective morpholinomethyl derivatives in the LL model. Although morpholinomethyl derivatives are predictably unstable to hydrolysis, these data do not support the proposal that the activity of such derivatives is only attributable to the parent imide.[34]

Experimental Section

Melting points were determined in open capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Infrared spectra were recorded with a Beckman Model 4230 spectrophotometer. Nuclear magnetic resonance spectra were recorded with either a Bruker WP-80, HX-90E, 300-MHz or a Nicolet 500-MHz spectrophotometer. Me_4Si ($CDCl_3$, Me_2SO, pyridine-d_5) or TSP (D_2O) were used as internal standards unless otherwise specified. Chemical shifts are reported on the δ scale with peak multiplicities: c, complex; d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; s, singlet; and t, triplet. Mass spectra were recorded with a Du Pont Model 21-491 mass spectrometer with a Model 21-094 data system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

***cis*-3,6-Bis[(acetyloxy)methyl]piperazine-2,5-dione (25)** was prepared by using methodology similar to the methodology used for the prepartion of the (chloroacetyl)oxy analogue.[17] A mixture of *cis*-**24** (1.0 g, 5.75 mmol), AcCl (1.8 mL, 25.1 mmol), and glacial HOAc (2.5 mL) was stirred at room temperature for 5 h. An additional 2.5 mL of HOAc was added at the end of 1.5 h. The mixture was diluted with Me_2CO, filtered, and recrystallized from MeOH, yielding 1.15 g (77.7%) of white crystals: mp 227–228 °C; IR (KBr) 1755 (ester), 1685 (amide) cm^{-1}; NMR (Me_2SO-d_6, 90 MHz) δ 2.04 (s, 6 H, $COCH_3$), 4.21 (br s, 6 H, OCH_2CH), 8.42 (s, 2 H, CONH). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

***trans*-3,6-Bis[(acetyloxy)methyl]piperazine-2,5-dione (25)** was prepared by using methodology similar to that employed for the preparation of the (chloroacetyl)oxy analogue.[17] A mixture of *trans*-**24** (1.0 g, 5.75 mmol), AcCl (1.8 mL, 25.1 mmol), HOAc (5 mL), and 10 drops of H_2SO_4 was stirred for 2 days at room temperature with occasional warming. The mixture was diluted with H_2O, filtered, and recrystallized from MeOH, yielding 0.53 g (35.8%) of white crystals: mp 237–238 °C; IR (KBr) 1740 (ester), 1680 (amide) cm^{-1}; NMR (Me_2SO-d_6, 90 MHz) δ 2.02 (s, 6 H, $COCH_3$), 4.22 (s, br, 6 H, OCH_2CH), 8.37 (s, 2 H, CONH). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

***cis*-3,6-Bis[(acetyloxy)methyl]-5-ethoxy-3,6-dihydro-2-(1*H*)-pyrazinone (28)**. To a suspension of *cis*-**25** (0.5 g, 1.94 mmol) in methylene chloride (10 mL) under nitrogen was added 4 mL of a 1 M solution of triethyloxonium tetrafluoroborate ($Et_3O^+BF_4^-$) in CH_2Cl_2 and the mixture stirred at room temperature. A second 4-mL portion of the $Et_3O^+BF_4^-$ solution was added after 6 h. The mixture was stirred at room temperature for 24 h and then carefully poured onto saturated aqueous $NaHCO_3$ solution (30 mL). The organic layer was separated, washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was recrystallized from petroleum ether, affording 0.13 g (25%) of a white solid: mp 118–120 °C; IR (KBr) 1740 (ester), 1700 (N=C), 1675 (amide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.27 (t, 3 H, OCH_2CH_3), 2.05 (s, 3 H, $COCH_3$), 2.12 (s, 3 H, $COCH_3$), 4.03–4.50 (c, 8 H, OCH_2CH plus OCH_2CH_3), 6.60 (s, 1 H, CONH); MS (70 eV), m/e 286 (M^+).

***cis*-3,6-Bis[[(*tert*-butyldimethylsilyl)oxy]methyl]-piperazine-2,5-dione (26)**. A mixture of *cis*-**24** (1.0 g, 5.75 mmol), *t*-$BuMe_2SiCl$ (2.08 g, 13.8 mmol), and imidazole (1.96 g, 28.7 mmol) in DMF (15 mL) was stirred at room temperature for 10 h. The reaction mixture was diluted with H_2O and the solid extracted with $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. The solid obtained was recrystallized from EtOAc, yielding 1.47 g (63.5%) of white crystals: mp 188–190 °C; IR (KBr) 1680 (amide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 0.11 [s, 12 H, $Si(CH_3)_2$], 0.92 [s, 18 H, Si(*t*-Bu)], 3.7–4.17 (m, 6 H, OCH_2CH), 6.30 (s, br, 2 H, CONH). Anal. ($C_{18}H_{38}N_2O_4Si_2$) C, H, N.

***trans*-3,6-Bis[[(*tert*-butyldimethylsilyl)oxy]methyl]-piperazine-2,5-dione (26)**. A mixture of *trans*-**24** (1.0 g, 5.75 mmol), *t*-$BuMe_2SiCl$ (2.08 g, 13.8 mmol), and imidazole (1.96 g, 28.7 mmol) in DMF (5 mL was stirred at room temperature for 22 h. The reaction mixture was diluted with H_2O, and the solid was filtered and recrystallized from MeOH to yield 1.47 g (63.6%) of white crystals: mp 226–228 °C; IR (KBr) 1680 and 1690 (amide)

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cm^{-1}; NMR (pyridine-d_5, 90 MHz) δ 0.12 [s, 12 H, $Si(CH_3)_2$], 0.91 [s, 18 H, Si(*t*-Bu)], 4.1–4.47 (m, 6 H, OCH_2CH). Anal. ($C_{18}H_{38}N_2O_4Si_2$) C, H, N.

***cis*- and *trans*-3,6-Bis[(phenylmethoxy)methyl]-2,5-piperazinedione (27).** To a suspension of *O*-benzyl-L-serine (12 g, 0.06 mol) in MeOH (100 mL) was passed HCl gas until the solution became clear. After refluxing for 6 h, the solvent was concentrated under reduced pressure. The residual *O*-benzyl-L-serine methyl ester hydrochloride was dissolved in MeOH (75 mL) and passed through a column (3 × 46 cm) of weak base type ion exchange resin (Amberlite IRA-45, 16–50 mesh) which had been washed with 5% aqueous $NaHCO_3$ solution (500 mL) followed by H_2O (600 mL) and MeOH (400 mL). The effluent and MeOH wash (500 mL) were concentrated to an oil, which was kept at room temperature for 7 days. The resulting semisolid was triturated with EtOAc and filtered to yield 6.17 g (56.7%) of *cis*- and *trans*-**27**. The isomers were separated on a silica gel column by elution with $CHCl_3$. The trans isomer eluted first and was recyrstallized from MeOH, affording white crystals: mp 205–207 °C; IR (KBr) 1675 (amide) cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 3.71 (dd, 2 H, OCH_2CH, J_{AB} = 9.47 Hz, J_{AX} = 7.14 Hz), 3.80 (dd, 2 H, OCH_2CH, J_{AB} = 9.47 Hz, J_{BX} = 3.34 Hz), 4.18–4.22 (m, 2 H, OCH_2CH), 4.53 (d, 2 H, OCH_2Ph, J_{AB} = 11.82 Hz), 4.56 (d, 2 H, OCH_2Ph, J_{AB} = 11.82 Hz), 6.17 (s, 2 H, CONH), 7.27–7.38 (m, 10 H, Ph). Anal. ($C_{20}H_{22}N_2O_4$) C, H, N. Continued elution with $CHCl_3$ yielded the cis isomer as white crystals: mp (MeOH) 173–174 °C; IR (KBr) 1685 (amide) cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 3.67 (dd, 2 H, OCH_2CH, J_{AM} = 9.24 Hz, J_{AX} = 8.28 Hz), 3.85 (dd, 2 H, OCH_2CH, J_{AM} = 9.24 Hz, J_{MX} = 3.28 Hz), 4.18–4.22 (m, 2 H, OCH_2CH), 4.44 (d, 2 H, OCH_2Ph, J_{AB} = 11.69 Hz), 4.46 (d, 2 H, OCH_2Ph, J_{AB} = 11.69 Hz), 6.33 (s, 2 H, CONH), 7.22–7.37 (m, 10 H, Ph). Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

***cis*- and *trans*-2,5-Bis[(phenylmethoxy)methyl]piperazine (29).** A mixture of *cis*- and *trans*-**27** (0.5 g, 1.4 mmol) and LAH (0.21 g, 5.6 mmol) in dry THF (50 mL) was refluxed under N_2 for 6 h. The mixture was cooled to room temperature and the excess LAH carefully decomposed with $Na_2SO_4 \cdot 10H_2O$. The mixture was filtered, the salts were extracted twice with hot THF, and the combined extracts were concentrated under reduced pressure, yielding a semisolid mixture of *cis*- and *trans*-**29** (0.43 g), which was not further purified but used as such for the preparation of *cis*- and *trans*-**30**.

***cis*- and *trans*-2,5-Bis[(phenylmethoxy)methyl]-1,4-piperazinediacetamide (30).** A mixture of crude *cis*- and *trans*-**29** (4.42 g, ~0.01 mol), K_2CO_3 (3.74 g, 0.027 mol), and iodoacetamide (5.52 g, 0.03 mol) in absolute EtOH (50 mL) was stirred at room temperature for 24 h. The mixture was diluted with H_2O (50 mL) and extracted three times with $CHCl_3$ (50 mL). The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated under reduced pressure to afford 5.19 g of crude *cis*- and *trans*-**30** which were purified by chromatography (silica gel/$CHCl_3$). The trans isomer eluted first (1.17 g, 19.63%): mp (MeOH) 200–201 °C; IR (KBr) 1640 (amide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.41 [deceptively simple triplet (dd), 2 H, axial H of ring CH_2], 2.57–3.60 (c, 12 H, other ring H plus NCH_2CO and OCH_2), 4.44 (s, 4 H, OCH_2Ph), 5.43 (br s, 2 H, $CONH_2$), 7.08 (br s, 2 H, $CONH_2$), 7.31 (s, 10 H, Ph). Anal. ($C_{24}H_{32}N_4O_4$) C, H, N. Continued elution afforded an intermediate fraction (0.91 g, 15.27%, cis–trans mixture) followed by pure cis isomer (1.73 g, 29.02%): mp (EtOAc) 160–162 °C; IR (KBr) 1685 (amide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.56–3.59 (c, 14 H, ring H plus NCH_2CO and OCH_2), 4.47 (s, 4 H, OCH_2Ph), 5.54 (br s, 2 H, $CONH_2$), 7.01 (br s, 2 H, $CONH_2$), 7.31 (s, 10 H, Ph). Anal. ($C_{24}H_{32}N_4O_4$) C, H, N.

Pyrazine-2,5-dicarboxylic acid (31) was prepared according to the procedure of Schut et al.[26] A mixture of 2,5-dimethylpyrazine (25.0 g 0.23 mol), pyridine (500 mL), SeO_2 (125 g, 1.13 mol), and H_2O (50 mL) was refluxed for 12–13 h in a 1-L round-bottom flask equipped with a mechanical stirrer and a reflux condenser. The reaction mixture was cooled to room temperature and the precipitate A filtered and washed four times with hot pyridine–H_2O (10:1). The combined filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 2 N NH_4OH solution (50 mL) and concentrated under reduced pressure. This process was once repeated and the resulting brown colored residue was dissolved in 2 N NH_4OH solution (180 mL) to which was added concentrated HCl solution (150 mL) to give a precipitate B. Precipitates A and B were combined and washed three times with 2 N HCl solution (70 mL) followed by ice-cold H_2O. The solid was placed in a 2-cm diameter column and centered so that the eluate would enter a second 7-cm-diameter column containing decolorising carbon (Norit A, 250 g, packed in H_2O). The top column was eluted with 2 N NH_4OH solution until such time that a neutralized sample of the eluent obtained from the charcoal column showed no strong color with $FeSO_4$. To 400-mL portions of eluent (generally totalling >3 L) was added 100 mL of concentrated HCl solution to yield a white precipitate of **31**. The precipitate was filtered, washed with 2 N HCl solution followed by ice-cold H_2O, and dried at ca. 75 °C under reduced pressure to yield 27.65 g (71.1%) of a white solid: mp 271–272 °C dec (lit.[26] mp varied between 255 and 260 °C in evacuated capillary tubes); IR (KBr) 1725 (carboxylic acid) cm^{-1}; NMR (20% K_2CO_3/D_2O, 80 MHz) δ 9.11 (s, ring protons).

***cis*- and *trans*-Piperazine-2,5-dicarboxylic Acid (20).** To a suspension of **31** (3.0 g, 0.02 mol) in H_2O (90 mL) was added KOH (3.0 g, 0.05 mol). The mixture was warmed and to the resulting solution was added 10% Pd–C (1.0 g). This mixture was hydrogenated (Parr shaker) under 40–42 psi at 50–60 °C for 12 h. The catalyst was filtered and the filtrate concentrated to about 30 mL under reduced pressure. Dropwise addition of concentrated HCl solution (pH 5.5–6.5) to the cooled concentrate (ice bath) afforded 1.19 g (38.5%) of white crystals: mp >280 °C (*trans*-**20**); IR (KBr) 1635 (carboxylic acid) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 500 MHz) δ 3.64 [deceptively simple triplet (dd), 2 H, axial H of ring CH_2], 4.21 (dd, 2 H, equatorial H of ring CH_2, J_{gem} = 14.1 Hz, J_{ae} = 3.8 Hz), 4.53 (dd, 2 H, ring CH, J_{aa} = 12.4 Hz, J_{ae} = 3.8 Hz). Anal. ($C_6H_{10}N_2O_4$) C, H, N. Continued addition of concentrated HCl solution (pH 5.0–5.5) afforded 1.09 g (35.3%) of solid cis–trans mixture. Further acidification (pH 4.0–5.0) yielded 0.77 g (24.9%) of white crystals (*cis*-**20**): mp >280 °C; IR (KBr) 1652 (carboxylic acid) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 500 MHz) δ 3.93 (dd, 2 H, H of ring CH_2, J_{AB} = 14.5 Hz, J_{AX} = 4.4 Hz), 4.02 (dd, 2 H, H of ring CH_2, J_{AB} = 14.5 Hz, J_{BX} = 7.1 Hz), 4.71 (q, 2 H, ring CH). Anal. ($C_6H_{10}N_2O_4$) C, H, N.

***trans*-2,5-Dicarboxy-1,4-piperazinediacetic Acid (14).** A mixture of *trans*-**20** (1.0 g, 5.7 mmol), bromoacetic acid (1.99 g, 14.3 mmol), and K_2CO_3 (5.13 g, 37.1 mmol) in H_2O (25 mL) was stirred at room temperature for 24 h. The reaction mixture was cooled (ice bath) and acidified (concentrated HCl solution) to pH 1–2, stored at 4 °C overnight, and filtered. The solid was washed with cold H_2O followed by Me_2CO and dried to afford 1.45 g (87.6%) of a white solid (*trans*-**14**): mp >280 °C (slow decomposition >230 °C); IR (KBr) 1655 (carboxylic acid) cm^{-1}; NMR (20% K_2CO_3/D_2O, 500 MHz) δ 2.26 [deceptively simple triplet (dd), 2 H, axial H of ring CH_2], 2.76 (d, 2 H, NCH_2CO, J_{AX} = 15.4 Hz), 3.04–3.09 (m, 4 H, other ring H), 3.31 (d, 2 H, NCH_2CO, J_{AX} = 15.4 Hz). Anal. ($C_{10}H_{14}N_2O_8 \cdot 4H_2O$) C, H, N.

***cis*-2,5-Dicarboxy-1,4-piperazinediacetic acid (14)** was prepared from *cis*-**20** according to conditions identical with those described for the preparation of *trans*-**14**, affording 1.09 g (66.1%) of a white solid: mp 200–220 °C (slow decomposition); IR (KBr) 1740 and 1720 (carboxylic acid) cm^{-1}; NMR (20% K_2CO_3/D_2O, 500 MHz) δ 2.61 (dd, 2 H, equatorial H of ring CH_2, J = 11.3 and 3.5 Hz), 2.97 (d, 2 H, NCH_2CO, J = 15.7 Hz), 3.16–3.18 (m, 2 H, ring CH), 3.23–3.25 (m, 2 H, axial H of ring CH_2), 3.28 (d, 2 H, NCH_2CO, J = 15.8 Hz). This NMR spectrum changes with lower K_2CO_3 concentration. Anal. ($C_{10}H_{14}N_2O_8$) C, H, N.

***trans*-1,4-Bis(2-amino-2-oxoethyl)-2,5-piperazinedicarboxylic Acid (15).** A mixture of *trans*-**20** (1.0 g, 5.7 mmol), chloroacetamide (1.28 g, 13.7 mmol), and K_2CO_3 (3.48 g, 25.2 mmol) in H_2O (25 mL) was stirred at room temperature for 35 h. The reaction mixture was cooled (ice bath), acidified (concentrated HCl solution) to pH 1–2, stored at 4 °C overnight, and filtered. The solid was washed with cold H_2O followed by Me_2CO and dried to afford 1.08 g (65.8%) of a white solid: mp 247–248 °C dec; IR (KBr) 3440 and 3310 (NH), 1705 (carboxylic acid), 1650 (amide) cm^{-1}; NMR (20% K_2CO_3/D_2O, 80 MHz) δ 2.39 [deceptively simple triplet (dd), 2 H, axial H of ring CH_2], 2.86 (d, 2 H, NCH_2CO, J_{AB} = 16.2 Hz), 3.0–3.2 (m, 4 H, other ring H), 3.36 (d, 2 H, NCH_2CO, J_{AB} = 16.2 Hz). Anal. ($C_{10}H_{16}N_4O_6$) C, H, N.

Dimethyl *trans*-2,5-Bis(methoxycarbonyl)-1,4-piperazinediacetate (16). HCl gas was bubbled (ca. 5 min) into a stirred suspension of *trans*-**14** (4.0 g, 13.8 mmol) in MeOH (200 mL) and the mixture refluxed for 5 h. The solvent was removed under reduced pressure and the residual solid dissolved in 10% aqueous Na_2CO_3 solution (50 mL) and extracted with four 50-mL portions of $CHCl_3$. The combined extract was washed with H_2O and dried ($MgSO_4$) and the solvent removed under reduced pressure to yield 3.55 g (74.4%) of a white solid (*trans*-**16**): mp (MeOH) 152–153 °C; IR (KBr) 1740 (ester) cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 3.07 (dd, 2 H, equatorial H of ring CH_2, J_{gem} = 11.3 Hz, J_{ae} = 4.5 Hz), 3.44 (d, 2 H, NCH_2CO, J_{AB} = 17.1 Hz), 3.42–3.48 (m, 2 H, ring H), 3.60–3.62 (m, 2 H, ring H), 3.62 (d, 2 H, NCH_2CO, J_{AB} = 17.1 Hz), 3.69 (s, 6 H, CO_2CH_3), 3.75 (s, 6 H, CO_2CH_3). Anal. ($C_{14}H_{22}N_2O_8$) C, H, N.

Dimethyl *cis*-2,5-Bis(methoxycarbonyl)-1,4-piperazinediacetate (16). HCl gas was bubbled through a stirred suspension of *cis*-**14** (0.5 g, 1.72 mmol) in MeOH (50 mL) until all solids dissolved. The solution was refluxed for 6 h, the solvent removed under reduced pressure, and the residual solid dissolved in 10% aqueous Na_2CO_3 solution (25 mL). The aqueous solution was extracted with four 25-mL portions of $CHCl_3$ and the combined extract was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure to afford 0.41 g (69.4%) of *cis*-**16** as a viscous oil: IR (neat) 1745 (ester) cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 3.01 (dd, 2 H, equatorial H of ring CH_2, J_{AM} = 11.1 Hz, J_{MX} = 3.6 Hz), 3.30 (dd, 2 H, axial H of ring CH_2, J_{AM} = 11.1 Hz, J_{AX} = 6.6 Hz), 3.53 (s, 4 H, NCH_2CO), 3.67–3.70 (m, 2 H, other ring H), 3.70 (s, 6 H, CO_2CH_3), 3.72 (s, 6 H, CO_2CH_3). Anal. ($C_{14}H_{22}N_2O_8 \cdot 0.8H_2O$) C, H, N.

***trans*-1,4-Bis(2-amino-2-oxoethyl)-2,5-piperazinedicarboxamide (18).** NH_3 was condensed (5–10 mL) into a pressure bottle containing *trans*-**16** (0.2 g, 0.58 mmol) in MeOH (3 mL) held at −78 °C (Me_2CO, dry ice). The bottle was stoppered and let stand at room temperature for 2 days. NH_3 was released slowly after cooling the bottle (ice bath). The solid was filtered, washed with MeOH, and dried to afford 0.14 g (85.4%) of a white solid (*trans*-**18**): mp >260 °C (discoloration >230 °C); IR (KBr) 3420 and 3300 (NH), 1660 and 1630 (amide) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 80 MHz) δ 3.1–3.4 (m, 2 H), 3.5–4.0 (m, 6 H), 4.1–4.3 (m, 2 H). Anal. ($C_{10}H_{18}N_6O_4$) C, H, N.

***cis*-1,4-Bis(2-amino-2-oxoethyl)-2,5-piperazinedicarboxamide (18).** NH_3 was passed (5–10 mL) into a pressure bottle containing *cis*-**16** (0.5 g, 1.44 mmol) in MeOH (2 mL) held at −78 °C (Me_2CO, dry ice) and the reaction mixture worked up as in the preparation of *trans*-**18**, affording 0.38 g (92.7%) of a white solid (*cis*-**18**): mp 233–235 °C dec; IR (KBr) 3420 and 3300 (NH), 1660 (amide) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 80 MHz) δ 3.35–3.90 (c, 4 H, ring CH_2), 3.75 (s, 4 H, NCH_2CO), 4.09–4.26 (m, 2 H, ring CH). Anal. ($C_{10}H_{18}N_6O_4$) C, H, N.

Dimethyl *trans*-Piperazine-2,5-dicarboxylate (21). A suspension of *trans*-**20** (1.0 g, 5.7 mmol) in saturated HCl–MeOH (100 mL) was refluxed with stirring for 24 h. The solvent was removed under reduced pressure and the residual solid dissolved in 20% Na_2CO_3 solution (35 mL). The solution was extracted with seven 30-mL portions of $CHCl_3$, and the combined extracts were washed with cold H_2O, dried ($MgSO_4$), and evaporated under reduced pressure to yield 0.55 g (47.7%) of a white solid. A small portion was recrystallized from Me_2CO–hexane, affording colorless needles: mp 116–118 °C; IR (KBr) 3280 and 3200 (NH), 1730 (ester) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.06 (s, 2 H, NH), 2.80 (dd, 2 H, axial H of ring CH_2, J_{gem} = 12.5 Hz, J_{aa} = 10.0 Hz), 3.15–3.54 (m, 4 H, other ring H), 3.74 (s, 6 H, CO_2CH_3). Anal. ($C_8H_{14}N_2O_4$) C, H, N.

Acidification (concentrated HCl solution) of the cooled (ice bath) aqueous mother liquor afforded 0.29 g of crystalline *trans*-**20**, which was recycled.

Dimethyl *cis*-Piperazine-2,5-dicarboxylate (21). HCl gas was bubbled into a stirred suspension of *cis*-**20** (1.0 g, 5.7 mmol) in MeOH (50 mL) until all solids dissolved. The solution was refluxed with stirring for 12 h during which time it became turbid. The mixtures was stirred at room temperature overnight and the solids filtered. The solids were neutralized (20% Na_2CO_3 solution) and extracted ($CHCl_3$) to afford 56 mg (4.87%) of a white solid (*trans*-**21**) identical in all respects with the material prepared from *trans*-**20**.

The filtrate was evaporated under reduced pressure. The residual solid was dissolved in 20% Na_2CO_3 solution (35 mL) and extracted with six 30-mL portions of $CHCl_3$. The combined extracts were washed with cold H_2O, dried ($MgSO_4$), and evaporated under reduced pressure to yield a colorless oil, which crystallized in vacuo over several hours, affording 0.79 g (69.1%) of *cis*-**21**: mp 65–67 °C; IR (KBr) 3360 and 3340 (NH), 1740 and 1720 (ester) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.16 (s, 2 H, NH), 2.97–3.38 (m, 4 H, ring CH_2), 3.51 (q, 2 H, ring CH), 3.74 (s, 6 H, CO_2CH_3). Anal. ($C_8H_{14}N_2O_4$) C, H, N.

***trans*-Piperazine-2,5-dicarboxamide (22).** NH_3 was passed (5–10 mL) into a pressure bottle containing *trans*-**21** (0.82 g, 4.07 mmol) in MeOH (6 mL) held at −78 °C (Me_2CO, dry ice). The bottle was stoppered and let stand at room temperature for 2 days. NH_3 was released slowly after cooling (ice bath). The solid was filtered, washed with MeOH, and dried to afford 0.67 g (95.4%) of a white solid: mp >260 °C (slow discoloration >230 °C); IR (KBr) 3290 and 3170 (NH), 1655 (amide) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 80 MHz) δ 3.60 (dd, 2 H, axial H of ring CH_2, J_{gem} = 13.9 Hz, J_{aa} = 12.5 Hz), 4.15 (dd, 2 H, equatorial H of ring CH_2, J_{gem} = 13.9 Hz, J_{ae} = 3.7 Hz), 4.53 (dd, 2 H, ring CH, J_{aa} = 12.5 Hz, J_{ae} = 3.7 Hz). Anal. ($C_6H_{12}N_4O_2$) C, H, N.

***cis*-Piperazine-2,5-dicarboxamide (22).** NH_3 was passed (5–10 mL) into a pressure bottle containing *cis*-**21** (0.75 g, 3.70 mmol) in MeOH (5 mL) held at −78 °C (Me_2CO, dry ice) and the reaction mixture worked up as in the preparation of *trans*-**22**, affording 0.52 g (81.5%) of a white solid: mp 201–203 °C dec. The filtrate was evaporated under reduced pressure and the residual solid washed with MeOH and dried to yield an additional 44 mg of white solid, providing a combined yield of 88.4%; IR (KBr) 3450 and 3300 (NH), 1695 and 1665 (amide) cm^{-1}; NMR (D_2O, 90 MHz) δ 2.68–3.08 (m, 4 H, ring CH_2), 3.29 (q, 2 H, ring CH), with 4.61 (s, HOD). Anal. ($C_6H_{12}N_4O_2$) C, H, N.

Diethyl *trans*-2,5-Dicarbamoyl-1,4-piperazinediacetate (19). To a suspension of *trans*-**22** (0.2 g, 1.16 mmol) and anhydrous K_2CO_3 (0.32 g, 2.32 mmol) in 2 mL of Me_2SO was added dropwise ethyl bromoacetate (0.28 mL, 2.55 mmol) and the mixture stirred at room temperature for ca. 24 h. The reaction mixture was diluted with cold H_2O. The white solids were filtered and washed several times with cold H_2O and once with Me_2CO and dried, affording 0.336 g (84.2%) of *trans*-**19**: mp >260 °C (slow discoloration >200 °C); IR (KBr) 3340 and 3200 (NH), 1730 (ester), 1665 (amide) cm^{-1}; NMR (15% CF_3CO_2H/D_2O, 500 MHz) δ 1.19 (t, 6 H, $CO_2CH_2CH_3$), 3.38 [deceptively simple triplet (dd), 2 H, axial H of ring CH_2], 3.67 (q, 4 H, $CO_2CH_2CH_3$), 3.82–3.86 (m, 4 H, equatorial H of ring CH_2 plus NCH_2CO), 3.97 (d, 2 H, NCH_2CO, J_{AB} = 17.5 Hz), 4.28 (dd, 2 H, ring CH, J_{aa} = 10.6 Hz, J_{ae} = 2.7 Hz). Anal. ($C_{14}H_{24}N_4O_6$) C, H, N.

Diethyl *cis*-2,5-Dicarbamoyl-1,4-piperazinediacetate (19). To a suspension of *cis*-**22** (0.5 g, 2.91 mmol) and anhydrous K_2CO_3 (0.8 g, 5.81 mmol) in Me_2SO (5 mL) was added ethyl bromoacetate (0.71 mL, 6.4 mmol) dropwise. The mixture was stirred at room temperature for ca. 24 h and filtered. The filtrate was diluted with EtOAc, filtered, and concentrated at 50–60 °C under reduced pressure. The resulting oily residue, which contained traces of inorganic material, was crystallized from MeOH–EtOAc–hexane following filtration of the initially precipitated inorganic substances to afford 0.40 g (40%) of transparent crystals: mp 130–132 °C; IR (KBr) 3410 and 3180 (NH), 1750 and 1730 (ester), 1690 and 1665 (amide) cm^{-1}; NMR (D_2O, 90 MHz) δ 1.11 (t, 6 H, $CO_2CH_2CH_3$), 2.92 (d, 4 H, ring CH_2, J = 4.8 Hz), 3.29 (t, 2 H, ring CH, J = 4.8 Hz), 3.34 (s, 4 H, NCH_2CO), 4.06 (q, 4 H, $CO_2CH_2CH_3$), with 4.61 (s, HOD). Anal. ($C_{14}H_{24}N_4O_6$) C, H, N.

***trans*-Tetrahydrodipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9(2*H*,4*H*,8*H*,10*H*)-tetrone (10).** Sodium metal (31 mg, 1.35 mmol) was added to 5 mL of absolute EtOH and the mixture stirred under N_2. To the solution was added diamide diester *trans*-**19** (0.2 g, 0.58 mmol). After the solution was refluxed under N_2 for 5 h, the solvent was removed under reduced pressure and the residual solid dissolved in cold H_2O (5 mL) and acidified to pH 6–7 (concentrated HCl solution). The crystallized solid was stored at 4 °C overnight, filtered, washed with cold H_2O followed by Me_2CO, and dried to afford 0.104 g (71.23%) of a white solid: mp >280 °C (slow distortion >270 °C); IR (KBr) 3200 and 3100 (NH), 1730 and 1700 (imide) cm^{-1}; NMR (Me_2SO-d_6, 90 MHz) δ 2.18 [deceptively simple triplet (dd), 2 H, axial H of central ring

CH_2], 2.97-3.75 (m, 8 H, other ring H), 11.22 (s, br, 2 H, imide); MS (70 eV), *m/e* 252 (M^+). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

***cis*-Tetrahydrodipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9-(2*H*,4*H*,8*H*,10*H*)-tetrone (11).** Na metal (46 mg, 2 mmol) was added to 7 mL of absolute EtOH and the mixture stirred under N_2. Diamide diester *cis*-**19** (344 mg, 1 mmol) was added to the resulting solution and the mixture refluxed under N_2 for 6 h. The solvent was evaporated under reduced pressure, and the residual solid dissolved in cold H_2O (5 mL) and acidified (concentrated HCl solution) to pH 5 (approximate). The crystallized solid was stored at 4 °C overnight, filtered, washed with cold H_2O followed by Me_2CO, and dried to afford 226 mg (89.68%) of a white solid: mp >260 °C (slow decomposition >200 °C); IR (KBr) 3250 and 3105 (NH), 1730 and 1690 (imide) cm^{-1}; NMR (Me_2SO-d_6, 90 MHz) δ 2.7-2.9 (m, br, 4 H, central ring CH_2), 3.3-3.5 (m, 6 H, other ring H), 3.55 (s, 2 H, imide); MS (70 eV), *m/e* 252 (M^+). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

***trans*-Tetrahydro-2,8-bis(4-morpholinylmethyl)dipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9(2*H*,4*H*,8*H*,10*H*)-tetrone (12).** To a suspension of *trans*-**10** (315 mg, 1.25 mmol) in Me_2SO (5 mL) was added morpholine (0.38 mL, 4.37 mmol) and HCHO (0.37 mL of a 37% solution, 5.0 mmol). The mixture was stirred at 55-65 °C for 5 h and then at room temperature overnight. Me_2SO was removed by distillation under reduced pressure and the residual solid triturated with EtOH, filtered, washed (EtOH), and dried to afford 488 mg (86.83') of a white solid which underwent slow decomposition above 225 °C: IR (KBr) 1735 and 1685 (imide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.35 [deceptively simple triplet (dd), 2 H, axial H of central ring CH_2], 2.54-2.65 (m, 8 H, NCH_2 of morpholine), 3.05-3.91 (c, 16 H, other ring H), 4.78 (s, 4 H, NCH_2N). Anal. ($C_{20}H_{30}O_6N_6$) C, H, N.

***cis*-Tetrahydro-2,8-bis(4-morpholinylmethyl)dipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9(2*H*,4*H*,8*H*,10*H*)-tetrone (13).** To a solution of *cis*-11 (100 mg, 0.39 mmol) in Me_2SO (2 mL) was added morpholine (0.12 mL, 1.39 mmol) and HCHO (0.12 mL of a 37% solution, 1.59 mmol). The solution was stirred at 55-65 °C for 5 h and at room temperature overnight. Me_2SO was removed by distillation under reduced pressure and the residual oil crystallized from Et_2O-Me_2CO, affording 153 mg (85.95%) of a white solid: mp 179-181 °C dec; IR (KBr) 1735 and 1680 (imide) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 2.50-2.62 (m, 8 H, NCH_2 of morpholine), 2.83-3.89 (c, 18 H, ring H), 4.79 (s, 4 H, NCH_2N). Anal. ($C_{20}H_{30}O_6N_6$) C, H, N.

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Antiparasitic Agents. 6.[1] Synthesis and Anthelmintic Activities of Novel Isothiocyanatophenyl-1,2,4-oxadiazoles

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The syntheses and anthelmintic activities of 31 3- and 5-(isothiocyanatophenyl)-1,2,4-oxadiazoles are reported. In the primary anthelmintic screen, 3-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (**39**) showed 100% nematocidal activity and 3-(2-furanyl)-5-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (**63**), 3-(2-furanyl)-5-(2-chloro-4-isothiocyanatophenyl)-1,2,4-oxadiazole (**64**), and 3-(2-furanyl)-5-(4-chloro-3-isothiocyanatophenyl)-1,2,4-oxadiazole (**66**) showed 100% taeniacidal activity when administered orally to mice. The two most active members of this series, **39** and **63**, were active against the gastrointestinal nematodes of sheep at 100 mg/kg. In addition, **39** was also found to be active against hookworms in dogs at a single, oral dose of 200 mg/kg.

During the early stages of our anthelmintic development program, 1,2,4-oxadiazole Ia emerged as a potential lead.[2] This finding was shortly followed by the rediscovery of the antiparasitic oxadiazoles Ib[3] and Ic[4] (Figure 1). Several other 1,2,4-oxadiazoles had been reported to possess antiparasitic activity.[5-8] We decided to focus our investigation on isothiocyanatophenyl-substituted 1,2,4-oxadiazoles. Our earlier work on heterocyclic isothiocyanates had yielded compounds II and III equivalent to thiabendazole in anthelmintic activity[1,9] (Figure 2). While the main thrust of this study was directed toward the evaluation of 3- and 5-(isothiocyanatophenyl)-1,2,4-oxadiazoles[10] (Table III), numerous nitro intermediates[11] and various 3- and 5-substituted 1,2,4-oxadiazoles were also screened for an-

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