17%) and uracil (37, 18.5 mg, 83%). Compound 36 decomposed when dissolved in $CDCl_3$ to give uracil and an unidentified product.

1-[3-*O*-Acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-(phenylseleno)-β-D-xylofuranosyl]uracil (38). This compound was prepared in 98% yield as a foam from 19 (477 mg) by the procedure described for the preparation of 34: UV (MeOH) λ_{max} 261 nm (ε 11 400); ^1H NMR ($CDCl_3$) δ 0.09 (6 H, s, SiMe), 0.91 (9 H, s, SiBu-*t*), 2.04 (3 H, s, Ac), 3.67-4.00 (3 H, m, H-2′ and CH_2-5′), 4.31 (1 H, m, H-4′), 5.34 (1 H, t, $J_{2',3'} = J_{3',4'} = 5.9$ Hz, H-3′), 5.64 (1 H, dd, $J_{5,6} = 8.3$ Hz, $J_{5,NH} = 1.5$ Hz, H-5), 6.15 (1 H, d, $J_{1',2'} = 5.4$ Hz, H-1′), 7.28-7.35 (3 H, m, Ph), 7.62-7.69 (2 H, m, Ph), 7.74 (1 H, d, H-6), 8.68 (1 H, br, NH); MS *m/z* 483 (M^+ - Bu-*t*), 423 (M^+ - Bu-*t* - AcOH). Anal. Calcd for $C_{23}H_{32}N_2O_6SeSi$: C, 51.21; H, 5.98; N, 5.19. Found: C, 51.03; H, 5.99; N, 5.09.

Selenoxide Elimination of 38 To Form 39 and 40. Compound 38 (102 mg, 0.19 mmol) in CH_2Cl_2 (3 mL) was treated with *m*-CPBA (39 mg, 0.23 mmol) at room temperature for 2 h and worked up by the procedure described for the reaction of 6. A mixture of selenoxides obtained after column chromatography (2% EtOH in $CHCl_3$) was dissolved in THF (5 mL) containing Et_3N (78 μL, 0.57 mmol). The solution was heated at 60 °C for 2 h, evaporated, and chromatographed on a silica gel column (hexane:EtOAc = 2:1). This gave 39 (22 mg, 30%, mp 144-146 °C) and 40 (14 mg, 22%, mp 173-174 °C) as solids, which were analytically pure.

Physical data of 39 are as follows: UV (MeOH) λ_{max} 260 nm (ε 10 400); ^1H NMR ($CDCl_3$) δ 0.08 (6 H, s, SiMe), 0.89 (9 H, s, SiBu-*t*), 2.23 (3 H, s, Ac), 3.87 and 3.93 (2 H, each as dd, $J_{4',5'} = 2.2$ and 1.5 Hz, $J_{gem} = 11.9$ Hz, CH_2-5′), 4.69 (1 H, m, H-4′), 5.67 (1 H, dd, $J_{5,NH} = 1.8$ Hz, $J_{5,6} = 8.1$ Hz, H-5), 5.86 (1 H, m, H-2′), 7.05 (1 H, m, H-1′), 8.01 (1 H, d, H-6), 9.05 (1 H, br, NH); MS *m/z* 325 (M^+ - Bu-*t*). Anal. Calcd for $C_{17}H_{26}N_2O_6Si$: C, 53.40; H, 6.85; N, 7.33. Found: C, 53.40; H, 7.01; N, 7.06.

Physical data of 40 are as follows: UV (MeOH) λ_{max} 224 nm (ε 10 000), 250 nm (ε 9300); ^1H NMR ($CDCl_3$) δ 0.10 (6 H, s, SiMe), 0.91 (9 H, s, SiBu-*t*), 4.62 (2 H, s, CH_2-5′), 5.84 (1 H, dd, $J_{5,6} = 8.3$, $J_{5,NH} = 1.5$ Hz, H-5), 6.32 and 6.43 (2 H, each as d, $J_{2',3'} = 3.4$ Hz, H-2′ and H-3′), 7.51 (1 H, d, H-6), 8.73 (1 H, br, NH); MS *m/z* 265 (M^+ - Bu-*t*). Anal. Calcd for $C_{15}H_{22}N_2O_4Si$: C, 55.89; H, 6.88; N, 8.69. Found: C, 55.64; H, 6.97; N, 8.66.

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Syntheses and ^1H NMR Conformational Analyses of Diastereomeric 4,4′-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione)s and a Synthetically Related Tricyclic Octahydro-2,2-dimethyl-6-oxo-1,3-dioxolo[4,5-*g*]quinoxaline-5,8-diacetic Acid Ester

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Efficient syntheses for five of the six possible diastereoisomeric 4,4′-(4,5-dihydroxy-1,2-cyclohexanediyl)bis-(2,6-dioxopiperazine)s (5-9) and a synthetically related tricyclic 1,3-dioxolo[4,5-*g*]quinoxaline ring system 45 from their respective (4,5-dihydroxy-1,2-cyclohexanediyl)bis(carbamate)s 11-16 via isopropylidene-protected intermediates are described. Solution conformations of all targets and several synthetic intermediates in DMSO-d_6 were determined using ^1H NMR and NOE methods, and the structure for polyheterocycle 45, obtained during attempted preparation of the sixth possible dioxopiperazine diastereomer 10, was determined with the additional aid of 2D COSY, 2D HETCOR, and ^1H-^{13}C correlation of long-range coupling (COLOC). Taken together, these studies provide evidence for the differences in reaction conditions required for bis(dioxopiperazine) synthesis, a relatively comprehensive analysis of dioxopiperazine and hydroxyl substituent effects on cyclohexane DMSO-d_6 solution conformations, and a preliminary analysis of aqueous solubility differences.

Introduction

The regioisomeric dioxopiperazines 1-3 are found in numerous natural and unnatural organic compounds, and these substances possess a multitude of important biological properties.[1] Unlike compounds found in the 2,3- and 2,5-dioxo series 1 and 2, 2,6-dioxo regioisomers 3 mainly have biological significance as bis(2,6-dioxopiperazine)s (4a), and such analogues have unusually poor and/or unpredictable solubility properties.[1] Nonetheless, dioxopiperazines of this class exhibit synergistic antitumor effects in combination with clinically efficacious antineoplastic drugs, ameliorate the toxicity of cancer chemotherapeutic agents such as the anthracycline antibiotics, and have geometry-dependent anti- and prometastatic activities which are of both theoretical and clinical significance.[1-3]

Optically pure enantiomers[1,4] and bis(morpholinomethyl) Mannich derivatives[1,5,6] (4b) have had limited success when used to improve solubility properties of bis(dioxopiperazine)s, but to our knowledge there has been no systematic structure-property relationship analysis of

(1) For a comprehensive review of dioxopiperazines, see: Witiak, D. T.; Wei, Y. *Progress in Drug Research*; Jucker, E., Ed.; Birkhäuser Verlag: Basel, 1991; Vol. 35, pp 249-363.

(2) See pages 302-341 in ref 1.

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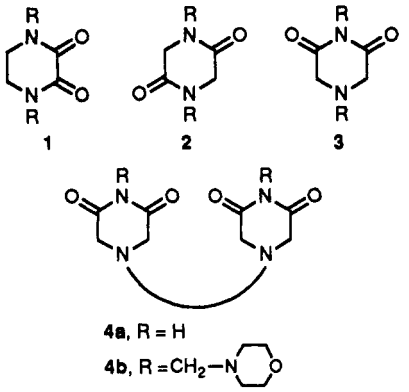
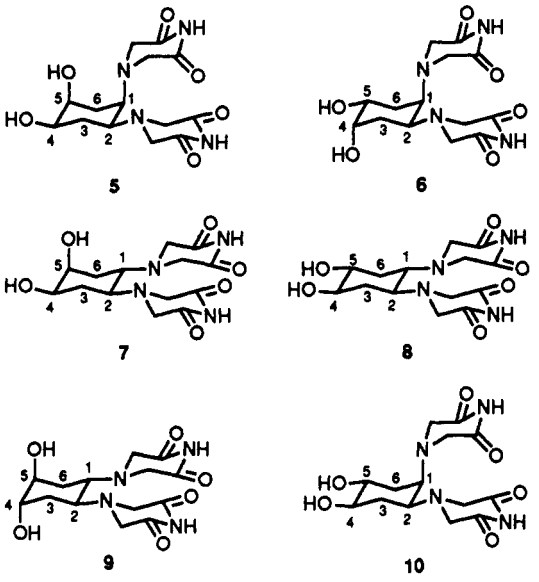
0022-3263/91/1956-5408$02.50/0



the problem. Since hydroxyl groups may increase water solubility via intermolecular hydrogen bonding, we desired samples of the six possible diastereomeric bis(hydroxylated) cyclohexanediylbis(2,6-dioxopiperazine)s **5–10** for purposes of studying conformational influences on these and the described biological properties. The synthetic chemistry summarized leads to five (**5–9**) of the six possible diastereomers whose preferred conformations in DMSO-d_6 were determined using ^{1}H NMR and NOE measurements.



Results and Discussion

Syntheses. Known diastereomeric dihydroxycyclohexanediyl bis(carbamate)s **11–16**,[7] protected as their isopropylidene ketals **17–22**, serve as precursor to the intermediate isomeric diamines **23–28**, five of which (**23–27**) are ultimately convertible to targets **5–9** via tetraesters **29–33** and bis(dioxopiperazine)s **35–39** (Scheme I and Chart I). Whereas conversion of diol **11** to the corresponding acetonide **17** using acetone–perchloric acid[8] or trimethylsilyl enol ether[9] was unsuccessful, all six protected diastereomers **17–22** are readily prepared by employing 2-methoxypropene (2-MP) in TsOH–acetone rather than in DMF.[10,11] Acetone solvent is easily removed in vacuo, and the individual diastereomeric derivatives are obtained in 81–95% yields with relatively minor modifications in such experimental details as the (a) molar ratios of 2-MP to starting diol (2:1 for **11–13** and 4:1 for **14–16**), (b) reaction temperatures (0 °C using **11–14** and **16** and room temperature with **15**), and (c) reaction times (0.1 h with **15**, 0.5 h with **11–13**, and 3 h with **14** and **16**). For the synthesis of acetonides **17** and **18** it is convenient to use a mixture of diols **11** and **12** since separation of the products, but not the reactants, on silica gel ($CHCl_3$–acetone, 15:1) is facile.

Scheme I

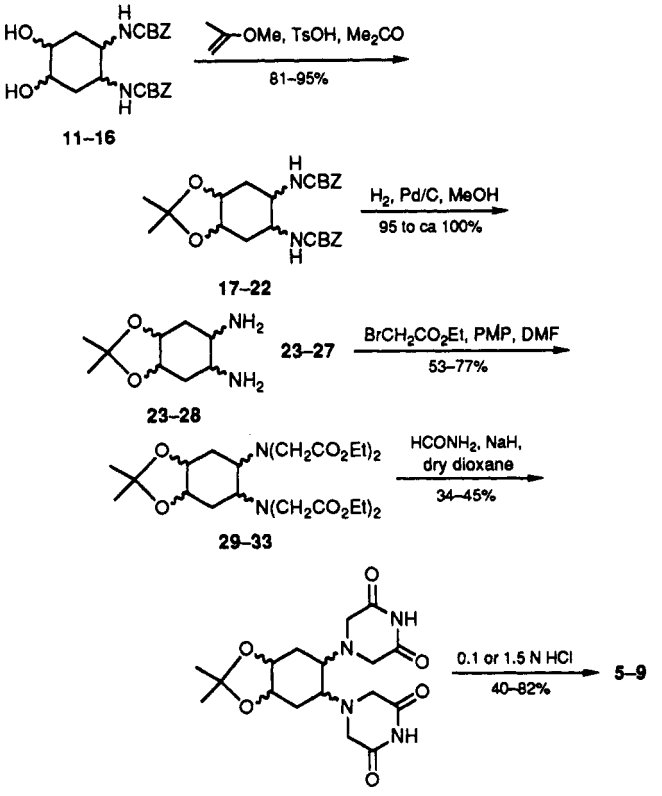
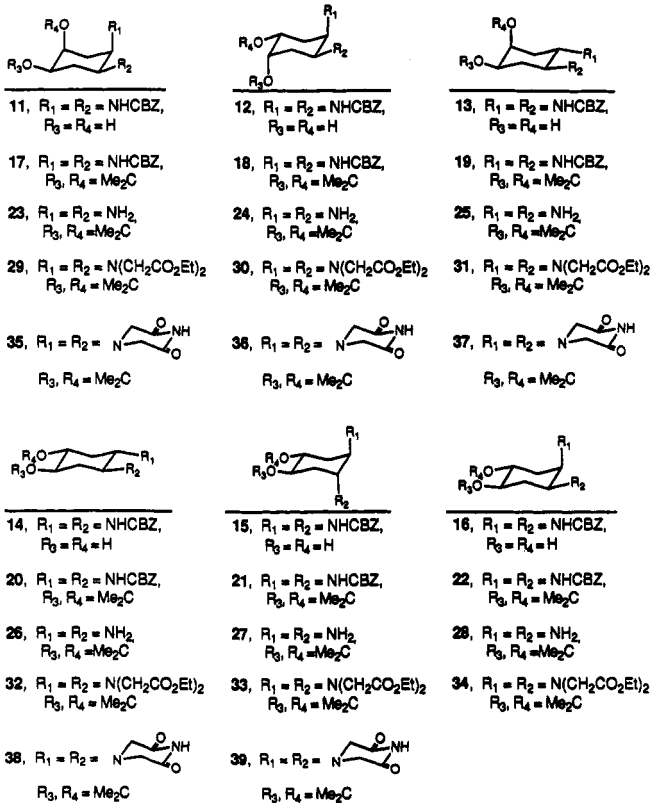


Chart I



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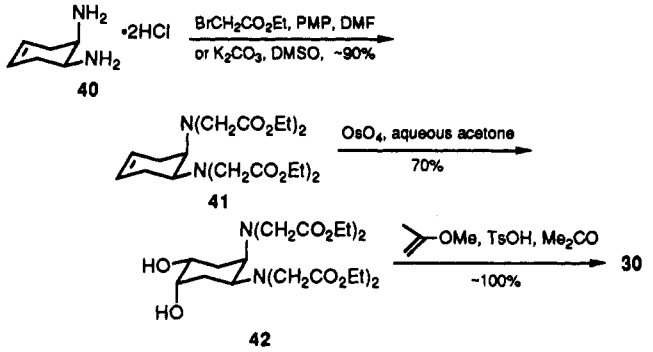
Scheme II



Diastereomeric diamines **23–28** are obtained in virtually quantitative yield upon catalytic (10% Pd/C) hydrogenation (20 psi; MeOH) of the respective bis(carbamate)s **17–22** (Scheme I). However, undesired tricyclic acetone-derived imidazolidines[12] are generated from cis bis(carbamate) diastereomers **17**, **18**, and **22** during solvent removal under reduced pressure and at room temperature. Imidazolidine[12] formation is precluded when the reagent grade MeOH is replaced by HPLC-grade MeOH, which contains <0.001% acetone. All diamines are used without additional purification, and five (**23–27**) of the six undergo tetrakis(N-alkylation) at room temperature (24 h) and in 53–77% yield with ethyl bromoacetate in DMF[13] containing 1,2,2,6,6-pentamethylpiperidine (PMP). Purification of the five tetraesters **29–33** is facilitated by chromatography over silica gel using hexane–ethyl acetate (2:1) as eluant, but owing to considerable tailing diastereomer **31** requires use of a large diameter–short length column and the addition of 1 drop of triethylamine to each 3 mL of eluant.

Since tetraester **30** is obtained from diamine **24** in only 53% yield (Scheme I, Chart I) an alternate synthetic sequence (Scheme II) was developed. Catalytic osmylation[14] of tetraester **41**, prepared from known diamine **40**,[7] takes place from the least hindered side of the double bond.[15] The resulting diol **42**, generated in 70% yield, seems to be uncontaminated with the isomer resulting from addition to the opposite side of the double bond, but further purification is accomplished by chromatography over silica gel using $CHCl_3$–acetone (3:1) as eluant. Conversion to acetonide **30** in approximately 100% yield using 2-MP in TsOH–acetone confirms the stereochemical assignment for intermediate **42**.

Desired intermediate tetraester **34** (Chart I) is not obtained under conditions employed for the production of the other five tetraester diastereomers **29–33**. Rather, diamine **28** reacts with 3 mol of ethyl α-bromoacetate and subsequently undergoes intramolecular cyclization to produce either tricyclic trans-anti-cis lactam **45** or the regioisomeric trans-anti-cis lactam **46** likely via intermediates **43** or **44**, respectively (Scheme III). Structure **45** was established for the tricyclic lactam with the aid of ^{13}C and ^1H NMR, 2D COSY, ^1H–^{13}C 2D HETCOR, ^1H–^{13}C correlation of long-range coupling (COLOC), and NOE

Scheme III

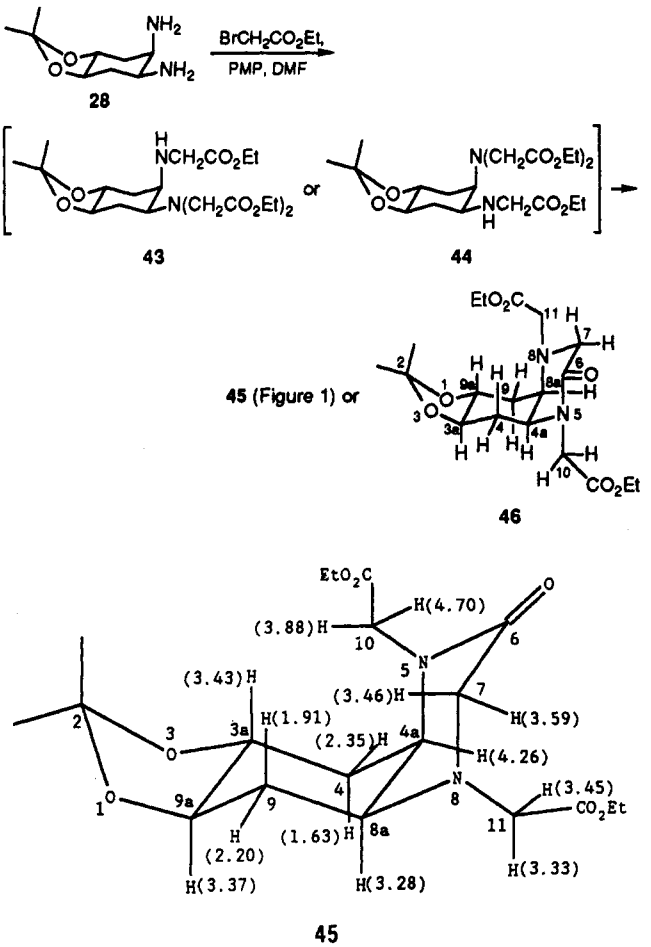


Figure 1. Chemical shifts in ppm taken at 500 MHz for the protons found in (3aα,4aβ,8aβ,9aβ)-diethyl octahydro-2,2-dimethyl-6-oxo-1,3-dioxolo[4,5-*g*]quinoxaline-5,8-diacetic acid ester (**45**). Coupling constants are found in the Experimental Section. The relatively large chemical shift differences for the H_{10} protons likely is due to the anisotopic effect of the lactam carbonyl function; proton $H_{10\alpha}$ lies close to the carbonyl group and inside the deshielded conical area. Molecular modeling (Alchemy II) provides evidence for several important proton spacial interaction differences between diastereomers **45** and **46**. First, the axial $^aH_7$ proton in **45** is in close proximity to the $^aH_9$ protons (2.17 Å), but the distance between the similarly orientated $^aH_7$ and $^aH_4$ protons in **46** is far apart (3.95 Å). Thus, irradiation of the axial $^aH_9$ proton (1.91 ppm) produces a large positive enhancement (nearly 17.3%) for the H_{7B} resonance signal and a small negative enhancement (−1.38%) for the H_{7A} signal. Similarly, when the equatorial $^aH_9$ proton is irradiated, a positive NOE for the H_{7A} resonance signal is observed. Second, 1,3-diaxial proton interactions between $^aH_{3a}$–$^aH_{9a}$ and $^aH_{3a}$–$^aH_4$ are present in lactam **45**, but not in isomer **46**. Rather in lactam **46** 1,3-diaxial proton interactions are to be expected between $^aH_{4a}$–$^aH_{3a}$ and $^aH_{4a}$–$^aH_9$. Positive enhancements of the $^aH_{9a}$ and $^aH_4$ proton resonance signals are observed following irradiation of the $^aH_{3a}$ proton (3.28 ppm). Alternatively, irradiation of the $^aH_4$ proton (1.63 ppm) results in enhancements of the $^aH_{3a}$ and $^aH_{9a}$ proton resonance signals. However, no enhancements of the $^aH_{3a}$ or $^aH_9$ resonance signals are observed following the irradiation of $^aH_{4a}$, and such enhancements are required if the correct structure is **46**. Third, in the presumed thermodynamically preferred conformation the H_{10A} proton is in close proximity to the axial $^aH_{3a}$ (2.89Å) proton in compound **45**, but the axial $^aH_{3a}$ proton in **46** occupies a position too far from any H_{10} proton (3.98Å) to observe a NOE effect. Irradiation of the H_{10A} proton (3.88 ppm) yields an enhancement of the $^aH_{3a}$ resonance signal.

studies. The NOE investigations confirm that conformationally constrained lactam **45** is the isolated diastereomer. Owing to rapid generation of lactam **45** from triester **43** at room temperature, isolation of the pure tris(alkylated)

(12) The imidazolidine generated from diastereomer **18** is characterized: ^1H NMR (250 MHz, $CDCl_3$) δ 4.45 (s, 2 H, methines), 3.64 (m, 2 H, methines), 3.23 (br s, 2 H, NH), 2.29 (m, 2 H, methylenes), 1.25 (s, 3 H, methyls), 1.44 (m, 5 H, 3 methyls and 2 methylenes), 1.31 (s, 6 H, methyls); CI/MS, exact mass calcd for $C_{12}H_{22}N_2O_2$ $(M + H)^+$ 227.1754, found 227.1760.

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Table I. ^{1}H NMR Resonance Signal Assignments for the Target Bis(2,6-dioxopiperazine) Diastereomers 5–9 determined in DMSO-d_6 and at 500 MHz

compd	NH	OH	NCH_2	H_1	H_2	$^eH_3$	$^aH_3$	H_4	H_5	$^eH_6$	$^aH_6$
5	11.01	4.62	3.57	2.76		1.90[b]	1.52	3.49		1.90[b]	1.52
6	11.11	4.45	3.51, 3.49	2.91		1.72[b]		3.71		1.72[b]	
7	10.94, 10.92	4.45, 4.39	3.40[b]	3.01	2.82	1.54[b]		3.37[b]	3.73[b]	1.68	1.34
8	10.92	4.65	3.44, 3.39	2.86[b]		1.75[b]	1.14[b]	3.14[b]		1.75[b]	1.14[b]
9	10.94	4.72	3.44, 3.40	3.00[b]		1.63[b]	1.55[b]	3.62		1.63[b]	1.55[b]

[a] Superscript e = equatorial; superscript a = axial. [b] Center of multiplet (ppm).

species was unsuccessful. Cyclization also takes place when the product is stored at −25 °C.

The ^{13}C NMR (125 MHz) spectrum (Experimental Section) for lactam **45** (Figure 1) is in agreement with the assigned structure. Assignments for the central cyclohexanediyl ring protons were established by analysis of ^{1}H NMR and 2D COSY spectra (Figure 1). COLOC study reveals coupling between $^eH_4$–C_9 (78.4 ppm), $^eH_4$–C_{8a} (57.3 ppm), and H_{4a}–C_9 (23.2 ppm) and confirms the ^{1}H chemical shift assignments in the central cyclohexanediyl ring for lactam **45**. Also, chemical shift assignments for methylene protons H_7, H_{10}, and H_{11} are consistent with the COLOC study wherein long-range coupling correlations between the protons with signals at 4.70 (H_{10X}) and 3.88 (H_{10A}) ppm and the carbonyl carbon C_6 (168.5 ppm) are readily observed. Long-range J_{C-H} correlation is also observed between the H_{8a} proton (3.28 ppm) and the C_7 carbon (51.6 ppm) as well as the H_7 proton (3.53 ppm) and the C_{8a} carbon. Furthermore, NOE studies (500 MHz) support the ^{1}H resonance signal assignments that are consistent with structure **45**, but not diastereomer **46**. A summary of the NOE results is found in the caption for Figure 1.

To circumvent degradation of either the dioxolane (during acid workup)[16] or dioxopiperazine (in aqueous alkaline solution)[17] rings during formamide cyclization and reaction workup the reaction vessels containing products **35–39** are immersed in a water bath held at room temperature, and the contents are stirred vigorously during dropwise addition of a solution of TsOH in dioxane. The addition is continued until the pH of the reaction mixtures reach 4.1, a pH near to the isoelectric point of all bis(dioxopiperazine)s.[6,16] Sufficient MeOH is added to provide a clear solution. Stepwise concentration of the dioxane/MeOH solvent and fractional crystallization followed by filtration produces the desired targets, but this procedure needs to be individualized for each diastereomer (see the Experimental Section). The lowest yield (40%) obtained for bis(dioxopiperazine) **6** likely is a function of its greater water solubility [29.3 mg/mL at 25 °C vs 1–2 mg/mL for the other four diastereomers (**5**, **7**, **8**, and **9**)].

DMSO-d_6 Solution Conformation Studies. Conformational analysis of the five diastereomeric dihydroxycyclohexanediylbis(2,6-dioxopiperazine)s **5–9** was carried out using ^{1}H NMR and NOE methods. The ^{1}H NMR resonance signal assignments determined at 500 MHz are found in Table I. In all cases the D_2O exchangeable imide proton resonance signals appear between 10.9 and 11.2 ppm, and the hydroxyl proton signals between 4.4 and 4.7 ppm. Resonance signals for methylene protons are between 3.3 and 3.6 ppm and in targets **6**, **8**, and **9** exhibit AB patterns with J = 16.9, 16.6, and 16.4 Hz, respectively. Protons α to the more electronegative hydroxyl groups possess signals downfield (3.1–3.8 ppm) to those α to amino groups (2.7–3.1 ppm).

The $\Delta\delta$ of 0.22 observed between isomers **5** and **6** for the $H_4(H_5)$ methine proton resonance signals likely reflects an intramolecular van der Waals' 1,3-diaxial proton–dioxopiperazine interaction, which is possible in the two chair conformers of diastereomer **6**, but not **5**. Such interactions cause a downfield shift in the signal.[18] Also, the $\Delta\delta$ of 0.38 between equatorial ($^eH_3$ and $^eH_6$) and axial ($^aH_3$ and $^aH_6$) methylene protons in diastereomer **5** indicates that the chair-chair interconversion of the cyclohexane ring is slow on the NMR time scale. Conversely, such a conformational interconversion is rapid for diastereomer **6** since both the equatorial and axial methylene protons have the same chemical shift (unresolved multiplets centered at 1.72 ppm). The slower chair–chair interconversion of diastereomer **5** relative to **6** may be attributed to a stabilizing 1,3-diaxial hydrogen bond between the hydroxyl group and the axial C-1 amino nitrogen of the dioxopiperazine ring. Such six-membered ring hydrogen bonding is possible in both conformers of **5**, but in neither conformer of **6**.

The methylene proton resonance signal assignments for diastereomer **7**, established by 2D COSY studies, reveals $^eH_6$ and $^aH_6$ chemical shifts of δ 1.68 and 1.34, respectively, whereas the $^eH_3$ and $^aH_3$ signals are observed as multiplets centered at 1.54 ppm. Protons $^aH_1$ and $^aH_2$ of this isomer are observed as doublets of triplets with J = 10.8 and 3.5 Hz (H_1) and 10.9 and 3.4 Hz (H_2). The $^eH_6$ and $^aH_6$ signals appear as triplets of doublets and doublets of triplets with J = 13.2 and 3.6 Hz ($^eH_6$) and 12.5 and 2.2 Hz ($^aH_6$). Unlike these well-resolved resonance signals, signals attributable to equivalent protons in the other four diastereomers (**5**, **6**, **8**, **9**) appear either as singlets or as broad, complex, and unresolved multiplets. Thus, the NMR data for diastereomer **7** are in agreement with the conformationally constrained structure assigned, a conclusion to be expected based upon a predictable A value for the dioxopiperazine heterocycle in excess of the value for cyclohexyl (2.15 kcal/mol).[19] As expected, ^{1}H NMR spectroscopy also reveals diastereomer **8** to be conformationally constrained. For this bis(dioxopiperazine) the protons ($^aH_4$ and $^aH_5$) α to the hydroxyl groups are held axial and have signals upfield (3.14 ppm) to the equatorial $^eH_5$ signal (3.73 ppm) provided by diastereomer **7**. Owing to anisotropic effects, the equatorial $^eH_5$ proton in isomer **7** also resonates at lower field than the axial $^aH_4$ proton (3.37 ppm). Additionally, with diastereomer **8** the signals for the axial ($^aH_3$ and $^aH_6$) and equatorial ($^eH_3$ and $^eH_6$) protons are separated by approximately 0.61 ppm, a $\Delta\delta$ typical of such signals in rigid six-membered rings.[20]

^{1}H NMR analysis of diastereomer **9** is particularly interesting. The signal attributable to protons $^{e,a}H_{4(5)}$ α to the hydroxyl groups in this compound have a chemical

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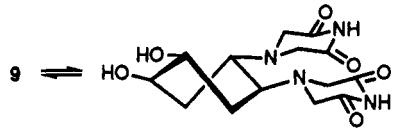
Table II. NOE (270 MHz) Studies for the Bis(dioxopiperazine) Diastereomers 5, 6, 8, and 9

compd	irradn of NCH_2 NOE (% of H_4 or H_5)	irradn of H_1 or H_2 NOE (% of H_4 or H_5)	irradn of H_4 or H_5 NOE (% of NCH_2)	irradn of H_4 or H_5 NOE (% of H_1 or H_2)
5	none	4.11	none	9.86
6	8.6	none	3.52	none
8	none	7.7	none	27.7
9	none	4.51	none	none

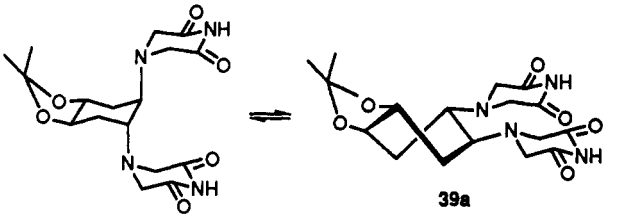
shift (3.62 ppm) between the signals assigned to the equivalent protons in isomers 7 ($^eH_5$, 3.73 ppm) and 8 ($^eH_{4(5)}$, 3.14 ppm). This is consistent with a possible rapid interconversion of conformer 9 and its twist-boat form 9a wherein the $H_{4(5)}$ protons have a quasi-diaxial conformation and the hydroxyl groups provide additional stability to the conformer owing to intramolecular hydrogen bonding. The very small $\Delta\delta$ of < 0.1 ppm between "equatorial" ($^eH_{3(6)}$) and "axial" ($^aH_{3(6)}$ methylene protons in this diastereomer may reflect the 1,3-diaxial ($^aH_{3(6)}$–^{a}OH) intramolecular van der Waal's effect possible in conformer 9, and/or the quasi-diequatorial arrangement possible in conformer 9a.



9a

In contrast to resonance signals assigned to the H_4 and H_5 protons α to hydroxyl groups, signals for protons (H_1 and H_2) α to the amino nitrogens on all five (5–9) diastereomers have relatively similar chemical shifts. Nonetheless, differences do exist and these support the solution conformational analyses previously discussed. Only for conformationally constrained isomer 7 are the centers of these multiplets resolved. Also, the signals for $H_{1(2)}$ of the rapidly interconverting isomer 6 are at lower field (2.91 ppm) than those (2.76 ppm) attributable to the equivalent protons in isomer 5. Furthermore, the $\Delta\delta$ of about 0.14 ppm observed for these protons in diastereomers 9 (3.00 ppm) and 8 (2.86 ppm) may be attributed to an intramolecular van der Waal's effect[18] resulting from 1,3-diaxial interaction between $^aH_1$ (and $^aH_2$) with respective axial hydroxyl groups in 9, not possible in conformationally rigid 8. The similarity in chemical shifts assigned to $H_{1(2)}$ in conformationally interconverting 6 and $^aH_1$ in rigid 7 also may be a function of such 1,3-diaxial interactions.

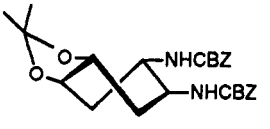
Further evidence supporting these conformational assignments is derived from NOE difference spectra, recorded at 270 MHz and at 60 °C for diastereomers 5, 6, 8, and 9 (Table II). Thus, for conformationally flexible diastereomer 5 and rigid isomer 8 irradiation of the $H_{4(5)}$ protons leads to an expected enhancement of the $H_{1(2)}$ resonance signals and vice versa. In neither conformation of rapidly interconverting isomer 6 are there 1,3-diaxial methine proton interactions, and a NOE is not observed. However, in isomer 6 the axial $H_{5(4)}$ protons are, in their respective flip conformations, in close proximity to the respective methylene protons on the dioxopiperazine rings. Therefore, irradiation of the $H_{5(4)}$ protons produces a positive NOE for the dioxopiperazine methylene signal and vice versa. For conformationally constrained diastereomer 7, the 1,3-diaxial proton–proton ($^eH_2$–$^aH_4$) interaction cannot be analyzed since the H_4 resonance signal overlaps with the methylene signals of the dioxopiperazine ring. However, the NOE difference spectra for isomer 9 confirms its interconversion with twist-boast 9a. Irradiation of $H_{1(2)}$ results in a 4.5% enhancement of the resonance signal attributable to the quasi-diaxial $H_{4(5)}$ protons in conformer 9a. Such a NOE effect is only expected in the twist-boat conformer wherein these quasi-axial protons are in close proximity to the $H_{1(2)}$ quasi-axial protons. A similar positive NOE (6.5%) is observed for bis(dioxopiperazine) 39, the precursor to isomer 9 ⇌ 9a, and this can only occur in twist-boat conformer 39a. Additionally, molecular modeling (Alchemy II) reveals energy differences between conformers 9 and 9a of 1.9 kcal/mol with 9 = −13.3 kcal/mol and 9a = −11.4 kcal/mol, wherein added stability owing to intramolecular hydrogen bonding between hydroxyl groups in the twist-boat form is not even considered.



39 39a

Conclusions

Five (5–9) of the six (5–10) possible diastereomeric 4,4′-(4,5-dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione)s were synthesized from the respective (4,5-dihydroxy-1,2-cyclohexanediyl)bis(carbamate)s (11–16) via their isopropylidine ketals (17–22), which are converted to the precursor diamines 23–28. Diastereomeric diamines 23–27 were successfully transformed into their respective tetraesters 29–33 and acetonide-protected bis-(2,6-dioxopiperazine)s 35–39, and these afford targets 5–9 by methods and in overall yields satisfactory for generation of sufficient quantities for detailed biological study. Only minor modification of reaction conditions are required in some steps. Thus, formation of acetonide 21 requires two NHCBZ groups to assume a high-energy diaxial conformation or the twist-boat form 21a; whereas all other acetonides are synthesized at 0 °C, quantitative yields of intermediate 21 (21a) are only obtained at room temperature. As expected[21–23] cis (17–19) acetonide production requires shorter reaction times than for related trans (20, 22) isomers.

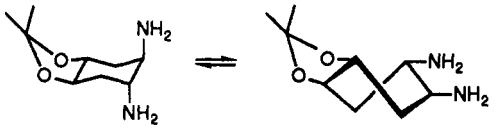


21a

The exclusive formation of *trans-anti-cis*-45 from diamine 28 via tris- rather than tetrakis(N-alkylation) likely is a function of conformationally constrained 28 wherein one amino group is held axial. Equatorial bis(N-alkylation) is favored over axial bis(N-alkylation), and tris(N-alkylated) intermediate 43 undergoes cyclization to produce tricyclic 45 having the axial lactam functionality. In

(21) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; Interscience Publishers: New York, 1981; pp 77 and 104.
(22) Angyal, S. J.; Hoskinson, R. M. *J. Chem. Soc.* **1962**, 2989.
(23) Angyal, S. J.; Macdonald, C. G. *J. Chem. Soc.* **1952**, 686.

contrast, both amino groups in conformationally flexible diastereomer **25** and constrained **26** are diequatorial in their lowest energy conformers and tetrakis(N-alkylation) predominates. Although the amino functions in diastereomers **23** and **24** possess the cis relationship found in diamine **28**, these isomers do not have a conformationally constraining trans-fused 1,3-dioxolane ring. Complete tetrakis(N-alkylation) is facilitated in those interconvertible conformers wherein an amino group or its mono(N-alkylated) derivative assumes the equatorial position. Interestingly, diastereomer **27** also undergoes tetrakis(N-alkylation). This suggests that during alkylation diaxial diamine **27** (or a semialkylated species) exists in equilibrium with its twist-boat conformer **27a** wherein the two sterically hindered quasi diequatorial amino groups are tetrakis(N-alkylated). Possibly, intramolecular hydrogen bonding between amino functions provides stability to the twist-boat form.



27 **27a**

Solution conformations in DMSO-d_6 have been established for the five bis(2,6-dioxopiperazine)s (**5–9**) using ^{1}H NMR and NOE methods. The chair–chair interconversion of *cis-syn-cis*-**5**, but not *cis-anti-cis*-**6**, is slow on the NMR time scale, isomers *cis-anti-trans*-**7** and *trans-syn-trans*-**8** behave as conformationally constrained species at 25 °C, whereas *trans-anti-trans*-**9** likely is in equilibrium with its twist-boat conformer **9a**. Among these five bis(2,6-dioxopiperazine)s only cis-anti-cis diastereomer **6** exhibits enhanced water solubility, 29.3 mg/mL at 25 °C. The remaining four diastereomers have poor water solubility [2.1 (**5**), 1.1 (**7**), 1.3 (**8**), and 1.1 (**9**) mg/mL at 25 °C], and this order is in reverse to their respective melting points [>300 °C (**7**, **8**, **9**) > 279–280 °C (**5**) > 221–223.5 °C (**6**)]. In addition to the relative competition between intra- and intermolecular hydrogen bonding other forces including differences in crystal packing[24] likely affect water solubility. The flip conformers of both isomers **5** and **6** are their respective enantiomorphs and thus these species effectively are meso at room temperature. However, it is the unique 1,4-trans relationship of dioxopiperazine and hydroxyl groups found in *cis-anti-cis*-**6** which provides for enhanced water solubility and lowest melting point likely owing to loose crystal packing of this conformationally flexible species.[25]

Experimental Section

Melting points were determined in open capillaries with a Thomas-Hoover Uni-Melt Apparatus and are uncorrected. Infrared spectra were recorded by either a Beckman Model 4230 or a Laser Precision Analytical RFX-40 spectrometer. Nuclear magnetic resonance spectra were obtained with either an IBM AF-250, an IBM AF-270, or a Bruker AM-500 spectrometer. TMS ($CDCl_3$, DMSO, acetone, or pyridine-d_5) or TSP (D_2O) were used as internal standards. 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. THF was distilled from sodium benzophenone ketyl. Dioxane was distilled first from CaH_2 and then from Na. Acetone was dried over $CaSO_4$, distilled, and stored in a brown bottle. Mass spectra were acquired with either a VG 70-250S or a Nicolet FTMS-2000 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Bis(carbamate)s **11** and **12** were prepared as previously[7] except that the crude products were purified over a silica gel column [$CHCl_3$–MeOH (15:1)] to give **11** (39%), mp 157–158 °C (lit.[7] mp 157–158 °C), and **12** (53%), mp 142–144 °C (lit.[7] mp 142–144 °C). For the large-scale preparation (>5 g) of bis(carbamate) **13** the reaction mixture was dissolved in sufficient $CHCl_3$–MeOH (10:1) solution and washed with a small amount of H_2O (about 1/10 of the volume of the organic solution) until the black color disappeared. If a precipitate appeared, additional CH_3OH was added. The water layers were combined and back extracted several times with $CHCl_3$. The organic layers were combined and dried ($MgSO_4$), and the solvent was removed in vacuo. The white residue was recrystallized from AcOEt to afford compound **13** as a white powder in 95–98% yield, mp 172–173 °C (lit.[7] mp 172–173 °C). Bis(carbamate) **14** was prepared as previously[7] in 72% yield; mp 204–205.5 °C (lit.[7] mp 197–198 °C) and bis(carbamate) **15** was prepared in 54–60‰ yield, except that the white crude product[7] was separated over a silica gel column [CH_3OH–$CHCl_3$ (1:15)]. Fractions containing **15** and bicyclocarbamate side product were combined and further purified by preparative TLC with the same solvent system to give pure **15**, mp 169.5–171.0 °C (lit.[7] mp 171–172 °C). For the synthesis of intermediate **21** impure **15** obtained following reaction solvent removal was used. Bis(carbamate) **16** was prepared as previously[7] in 65–68% yield, except that the oily residue obtain from workup was crystallized from AcOEt and the mother liquor was concentrated and further purified over a silica gel column [$CHCl_3$–MeOH (15:1)], mp 140–141.5 °C (lit.[7] mp 141–142 °C).

(3aα,5β,6β,7aα)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (17). Diol **11** (2.07 g, 5 mmol) was dissolved in dry acetone (65 mL) under N_2, and 2-methoxypropene (0.72 g, 10 mmol) was added. The mixture was cooled to −70 °C (dry ice–acetone), and TsOH (10 mg) was added. The mixture was stirred at −70 °C for 10 min, slowly warmed to 0 °C, and further stirred at that temperature for 0.5 h. The reaction was monitored by TLC (CH_3OH–$CHCl_3$, 1:15) and quenched by pyridine (20 drops). Removal of solvent in vacuo yielded a colorless oil, which was dissolved into AcOEt (100 mL). The solution was washed with H_2O (50 mL × 3) and brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The oily residue was dissolved into Et_2O and crystallized slowly by evaporating the Et_2O at room temperature. Compound **17** was produced as white shiny crystals (2.02 g, 89%): mp 86–87.5 °C; IR (KBr) 3412, 3248, 1727, 1700, 1552, 1524, and 1034 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 7.34 (s, 10 H, aromatic), 5.88 (d, 2 H, NH, J = 6.1 Hz), 5.13 (d, 2 H, H_A of AB, methines, J = 12.1 Hz), 5.06 (d, 2 H, H_B of AB, methines, J = 12.1 Hz), 4.12 (deceptively simple s, 2 H, methines), 3.84 (m, 2 H, methines), 2.01 (m, 4 H, methylenes), 1.53 (s, 3 H, methyls), 1.32 (s, 3 H, methyls). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 65.95; H, 6.53; N, 6.06.

(3aα,5α,6α,7aα)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (18). Diol **12** (3.4 g, 8.2 mmol) was treated with 2-methoxypropene (1.18 g, 16.4 mmol) and TsOH (20 mg) in dry acetone (100 mL) as described for the synthesis of **17**. The crude white solid was dissolved in $CHCl_3$ (20 mL) and concentrated to about 2 mL. After the addition of Et_2O (100 mL), the solution was kept in a refrigerator overnight to provide compound **16** (3.6 g, 88%) as white shiny crystals. These were dried at room temperature for 2 days in air to remove trace amounts of pyridine: mp 136–137 °C; IR (KBr) 3334, 3287, 2936, 1726, 1695, 1684, 1539, 1064, and 1039 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 7.31 (s, 10 H, aromatic), 5.0–5.4 (m, 6 H, 2 NH, exch. with D_2O, and 4 benzylic), 4.17 (deceptively simple t, 2 H, methines, J = 3.7 Hz), 4.10 (m, 2 H, methines), 1.97 (m, 4 H, methylenes), 1.47 (s, 3 H, methyls), 1.30 (s, 3 H, methyls). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 66.27; H, 6.62; N, 6.11.

(3aα,5α,6β,7aα)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (19) was prepared from diol **13** according to the procedure used to synthesize **17**. The oily residue was purified over a silica gel [$CHCl_3$–acetone (10:1)] to afford a colorless oil that crystallized from Et_2O–hexane providing compound **19** in 83% yield: mp

(24) Hempel, A.; Camerman, N.; Camerman, A. *J. Am. Chem. Soc.* **1982**, *104*, 3453.

(25) Grant, D. J. W.; Higuchi, T. *Techniques of Chemistry*; Weissberger, A., Founding Ed.; Saunders, W. H., Jr., Series Ed.; John Wiley and Sons: Inc., New York, 1990; Vol. 21.

107.5–109 °C; IR (KBr) 3350, 1684, 1542, 1530, and 1270 cm^{-1}; NMR (500 MHz, $CDCl_3$) δ 7.31 (s, 10 H, aromatic), 5.23 (d, 1 H, NH, J = 6.7 Hz), 5.05 (m, 5 H, 4 benzylic and 1 NH), 4.25 (m, 1 H, methine), 4.19 (m, 1 H, methine), 3.82 (m, 1 H, methine), 3.48 (m, 1 H, methine), 2.39 (deceptively simple d, 1 H, methylene, J = 14.0 Hz), 2.12 (m, 1 H, methylene), 1.63 (m, 2 H, methylenes), 1.49 (s, 3 H, methyls), 1.31 (s, 3 H, methyls). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 65.95; H, 6.60; N, 6.20.

(3aα,5β,6α,7aβ)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (20). Diol **14** (3.8 g, 9.2 mmol) and 2-methoxypropene (2.65 g, 36.8 mmol) were dissolved in dry acetone (300 mL) and stirred under N_2 at room temperature. After the mixture was cooled to −70 °C, TsOH (20 mg) was added. The solution turned brown after being allowed to warm to 0 °C. The reaction was quenched after 3 h by the addition of pyridine (20 drops). Workup was the same as described for compound **17**. The yellow solid obtained was washed with Et_2O to provide compound **20** as a white solid (3.4 g, 81%), which did not require further purification: mp 182–183 °C; IR (KBr) 3337, 2950, 1685, 1529, and 1268 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 7.31 (s, 10 H, aromatic), 5.15 (m, 2 H, NH, exch with D_2O), 5.10 (d, 2 H, H_A of AB, benzylic, J = 12.3 Hz), 5.04 (d, 2 H, H_B of AB, benzylic, J = 12.3 Hz), 3.65 (m, 2 H, methines), 3.40 (m, 2 H, methines), 2.45 (deceptively simple d, 2 H, methylenes, J = 11.7 Hz), 1.49 (m, 6 H, methyls and 2 H, methylenes). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 65.73; H, 6.71; N, 6.23.

(3aα,5α,6β,7aβ)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (21). Diol **15** (828 mg, 2 mmol) was dissolved in dry acetone (30 mL) and stirred under N_2 at room temperature. 2-Methoxypropene (580 mg, 8 mmol) and TsOH (8 mg) were added. The solution turned brown, and the reaction was quenched after 5 min by the addition of pyridine (10 drops). Workup was the same as described for compound **17**. The resulting pale yellow solid was purified by silica gel column chromatography [Et_2O–hexane (4:1)], providing a white solid, which was crystallized from AcOEt (very small amount)–Et_2O to furnish 862 mg of compound **21** as a white powder (95%): mp 165.5–167 °C; IR (KBr) 3366, 3267, 1706, 1695, 1685, 1522, 1346, 1388, 1230, and 1075 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 7.36 (s, 10 H, aromatic), 5.10 (s, 4 H, benzylic), 4.89 (m, 2 H, NH, exch, with D_2O), 4.13 (m, 2 H, methines), 3.41 (deceptively simple d, 2 H, methines, J = 10.5 Hz), 2.25 (deceptively simple d, 2 H, methylenes, J = 12.3 Hz), 1.81 (m, 2 H, methylenes), 1.41 (s, 6 H, methyls). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 66.26; H, 6.85; N, 6.23.

(3aα,5α,6α,7aβ)-Bis(phenylmethyl) (Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(carbamate) (22). Diol **16** (4.14 g, 10 mmol) was treated with 2-methoxypropene (2.9 g, 40 mmol) and TsOH (10 mg) in dry acetone (120 mL) as described for the preparation of **20**. Workup was the same as described for compound **17**. The resulting yellow semisolid residue was purified over silica gel [Et_2O–hexane (4:1)] to yield **22** (3.8 g, 85%) as a white solid: mp 61.5–63.5 °C; IR (KBr) 3338, 3333, 2984, 1733, 1701, 1534, 1261, and 1091 cm^{-1}; NMR (500 MHz, $CDCl_3$) δ 7.34 (s, 10 H, aromatic), 4.9–5.5 (m, 6 H, 4 benzylic and 2 NH), 4.30 (deceptively simple s, 1 H, methine), 3.86 (deceptively simple s, 1 H, methine), 3.35 (deceptively simple s, 2 H, methines), 2.25 (m, 2 H, methylenes), 1.70 (deceptively simple s, 1 H, methylene), 1.40 (s, 6 H, methyls), 1.36 (m, 1 H, methylene). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.17. Found: C, 65.79; H, 6.76; N, 5.97.

(3aα,5β,6β,7aα)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (23). Bis(carbamate) **17** (4.13 g, 9.1 mmol) was dissolved in HPLC-grade MeOH (200 mL containing <0.001% acetone). After addition of 10% Pd/C (724 mg) under N_2, the bottle was alternately evacuated (H_2O aspirator) and refilled to 20 psi with H_2 gas. The reaction mixture was shaken under 20 psi of H_2 for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo at room temperature to yield **23** (1.7 g; ~95%) as a colorless oil, which was used immediately and without further purification (reaction workup needs to be rapid in order to minimize formation of the acetone-derived aminal): IR (neat) 3395 (br), 3320 (br), 2985, 2936, 1381, 1224, and 1038 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.13 (m, 2 H, methines), 2.8 (m, 2 H, methines), 1.97 (m, 2 H, methylenes), 1.82 (m, 2 H, methylenes), 1.75 (s, 4 H, 4 NH, 2 OH, exch. with D_2O), 1.52 (s, 3 H, methyls), 1.32 (s, 3 H, methyls); FAB/MS, exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1492.

(3aα,5α,6α,7aα)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (24) was prepared from compound **18** (940 mg, 2 mmol) by a method identical with the one employed for the synthesis of isomer **23**. Diamine **24** was obtained in ~95% yield (372 mg) as a colorless oil, which was used immediately and without further purification: IR (neat) 3360 (br), 3288, 2982, 2933, 1456, 1245, and 1045 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.28 (m, 2 H, methines), 3.16 (m, 2 H, methines), 1.87 (m, 4 H, methylenes and 4 H, NH, exch. with D_2O), 1.48 (s, 3 H, methyls), 1.33 (s, 3 H, methyls); FAB/MS, exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1458.

(3aα,5α,6β,7aα)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (25). Bis(carbamate) **19** (5.0 g, 0.11 mmol) was treated with 10% Pd/C (878 mg) in MeOH (220 mL) as described for the synthesis of **23**. After 4 h, the reaction mixture was filtered and the solid was washed repeatedly in manually stirred MeOH (300 mL) to remove all product from the catalyst. The solution was again filtered through Celite. The filtrate was concentrated in vacuo at room temperature to afford **25** (2.1 g, ~100%) as a white solid, which was used immediately and without further purification: mp 56–60 °C; IR (KBr) 3854, 3744, 3341, 2984, 2930, 1653, 1600, 1387, 1241, and 1048 cm^{-1}; NMR [250 MHz, pyridine-d_5 + D_2O (1 drop) δ 4.18 (m, 2 H, methine), 2.88 (m, 1 H, methine), 2.47 (m, 2 H, 1 H methine and 1 H methylene), 2.17 (m, 1 H, methylene), 1.65 (m, 2 H, methylenes), 1.52 (s, 3 H, methyls), 1.34 (s, 3 H, methyls); FAB/MS, exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1470.

(3aα,5β,6α,7aβ)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (26) was obtained as a colorless oil (1.1 g, ~100%) from **20** (2.63 g, 5.6 mmol) and 10% Pd/C (462 mg) in MeOH (175 mL) according to the methodology used for synthesis of compound **23**: IR (neat) 3380, 3294, 2986, 2936, 1663, 1646, 1592, 1370, 1120, and 1057 cm^{-1}; NMR [250 MHz, $CDCl_3$ + D_2O (1 drop)] δ 3.46 (m, 2 H, methines), 2.66 (m, 2 H, methines), 2.44 (m, 2 H, methylenes), 1.47 (s, 6 H, methyls), 1.55 (m, 2 H, methylenes); FAB/MS exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1461.

(3aα,5α,6β,7aβ)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (27). Compound **21** (2.6 g, 5.8 mmol) was treated with 10% Pd/C (459 mg) in MeOH (170 mL) as described for the synthesis of **23**. Diamine **27** (1.08 g, ~100%) was obtained as a colorless oil, which was used immediately and without further purification: IR (neat) 3351, 3280, 2985, 1603, 1456, 1235, 1034 cm^{-1}; NMR [250 MHz, $CDCl_3$ + D_2O (1 drop)] δ 3.75 (m, 2 H, methines), 3.19 (m, 2 H, methines), 1.98 (m, 4 H, methylenes), 1.44 (s, 6 H, methyls); FAB/MS, exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1480.

(3aα,5α,6α,7aβ)-Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diamine (28). Compound **22** (2.87 g, 6.1 mmol) and 10% Pd/C (504 mg) in HPLC-grade MeOH (45 mL, containing <0.001% acetone) was treated as described for the synthesis of **23**. Diamine **28** was obtained (1.13 g, ~95%) as a colorless oil, which was used immediately and without further purification: IR (KBr) 3385, 3355, 2985, 2935, 1597, 1455, 1234, and 1072 cm^{-1}; NMR [500 MHz, $CDCl_3$ + D_2O (1 drop)] δ 3.67 (m, 1 H, methine), 3.30 (m, 1 H, methine), 3.19 (m, 1 H, methine), 2.90 (m, 1 H, methine), 2.10 (m, 1 H, methylene), 2.00 (m, 1 H, methylene), 1.63 (m, 2 H, methylenes), 1.39 (s, 3 H, methyls), 1.38 (s, 3 H, methyls); FAB/MS, exact mass calcd for $C_9H_{19}N_2O_2$ (M + H)$^+$ 187.1447, found 187.1442.

(3aα,5β,6β,7aα)-Diethyl *N,N'*-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (29). To a mixture of diamine **23** (1.69 g, 9.1 mmol) and PMP (5.6 g, 36.5 mmol) in DMF (35 mL) was added ethyl bromoacetate (22.59 g, 0.14 mol). The reaction mixture was stirred at room temperature for 24 h, and AcOEt (35 mL) was added. The precipitate was removed by filtration and washed with AcOEt. The combined filtrates were concentrated in vacuo (0.5 Torr, 35 °C) to produce a yellow residue, which was dissolved in AcOEt (200 mL), washed (brine, 50 mL × 4), and dried ($MgSO_4$). Removal of the solvent in vacuo yielded a yellow oil, which was purified over silica gel [AcOEt–hexane (1:2.5)] to provide 3.48

g (72.%) of **29** as a pale yellow oil: IR (neat) 2983, 2936, 1740, 1170, and 1030 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.14 (q, 8 H, methylenes, J = 7.1 Hz), 4.08 (m, 2 H, methines), 3.96 (d, 4 H, H_A of AB, methylenes, J = 17.9 Hz), 3.70 (d, 4 H, H_B of AB, methylenes, J = 17.9 Hz), 2.91 (m, 2 H, methines), 2.18 (m, 2 H, methylenes) 1.90 (m, 2 H, methylenes), 1.43 (s, 3 H, methyls), 1.30 (s, 3 H, methyls), 1.26 (t, 12 H, methyls, J = 7.1 Hz). Anal. Calcd for $C_{25}H_{42}N_2O_{10}$: C, 56.59; H, 7.98; N, 5.28. Found: C, 56.68; H, 7.78; N, 5.41.

(3aα,5α,6α,7aα)-Diethyl *N,N'*-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (30). Method A. Diamine **24** (372 mg, 2 mmol) was treated with PMP (1.29 g, 8 mmol) and ethyl bromoacetate (5.4 g, 32 mmol) in DMF (8 mL) as described for the synthesis of **29**. Silica gel chromatography [AcOEt–hexane (1:2)] afforded 562 mg (53%) of diamine **30** as a colorless oil: IR (neat) 2983, 2906, 1740, 1243, and 1193 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.29 (m, 2 H, methines), 4.14 (q, 8 H, methylenes, J = 7.2 Hz), 3.81 (d, 4 H, H_A of AB, methylenes, J = 17.8 Hz), 3.70 (d, 4 H, H_B of AB, methylenes, J = 17.8 Hz), 3.48 (m, 2 H, methines), 2.10 (m, 2 H, methylenes), 1.80 (m, 2 H, methylenes), 1.45 (s, 3 H, methyls), 1.30 (s, 3 H, methyls), 1.26 (t, 12 H, methyls, J = 7.2 Hz). Anal. Calcd for $C_{25}H_{42}N_2O_{10}$: C, 56.59; H, 7.98; N, 5.28. Found: C, 56.34; H, 7.76; N, 5.33. **Method B.** Diol tetraester **42** (2.9 g, 5.86 mmol) was dissolved in dry acetone (58 mL) under N_2 with stirring. After addition of 2-methoxypropene (1.13 g, 15.7 mmol), the reaction mixture was cooled to −70 °C and TsOH (15 mg) was added. The solution was allowed to slowly warm to room temperature. After 1 h, the reaction was quenched by addition of pyridine (10 drops) and worked up as in the procedure employed for the synthesis of compound **18**. The resulting oily residue was purified over a silica gel [hexane–AcOEt (2:1)] to yield compound **30** (3.1 g, ~100%) as a colorless oil identical with the compound produced by method A.

(3aα,5α,6β,7aα)-Diethyl *N,N'*-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (31) was prepared from diamine **25** (2.05 g, 11.0 mmol), PMP (6.86 g, 44.2 mmol), and ethyl bromoacetate (28.6 g, 0.17 mol) in DMF (42 mL) according to the procedure used for the synthesis of its isomer **29**. The product was purified on silica gel [hexane–AcOEt–NEt_3 (2 mL:1 mL:1 drop); due to considerable tailing, the column should have a relatively large diameter (5 cm) and short length] to produce **31** (4.5 g, 77.1%) as a colorless oil: IR (neat) 2983, 2936, 1747, 1379, 1179, and 1030 cm^{-1}; NMR (500 MHz, $CDCl_3$) δ 4.24 (m, 1 H, methine), 4.14 (q, 9 H, methylenes, J = 7.1 Hz, includes 1 H, methine, hidden), 3.67 (d, 2 H, $H_{A'}$ of AB methylenes, J = 17.5 Hz), 3.66 (d, 2 H, $H_{B'}$ of AB methylenes, J = 17.5 Hz), 3.66 (d, 2 H, H_A, of A'B' methylenes, J = 17.4 Hz), 3.62 (d, 2 H, H_B, of A'B' methylenes, J = 17.4 Hz), 3.14 (dt, 1 H, H_4, methine), 2.84 (m, 1 H, methine), 2.26 (m, 1 H, methylene), 2.12 (m, 1 H, methyhlene), 1.64 (m, 1 H, methylene), 1.53 (m, 1 H, methylene), 1.46 (s, 3 H, methyls), 1.30 (s, 3 H, methyls), 1.26 (t, 12 H, methyls, J = 7.2 Hz). Anal. Calcd for $C_{25}H_{42}N_2O_{10}$: C, 56.59; H, 7.98; N, 5.28. Found: C, 56.49; H, 8.00; N, 5.43.

(3aα,5β,6α,7aβ)-Diethyl *N,N'*-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (32). Diamine **26** (1.04 g, 5.6 mmol), PMP (3.5 g, 22.5 mmol), and ethyl bromoacetate (14.8 g, 89.5 mmol) in DMF (20 mL) were treated as described for the synthesis of **29**. The crude product was purified over silica gel [hexane–AcOEt (2:1)] to yield **32** (2.11 g, 69%) as a yellow oil: IR (neat) 2983, 1747, 1179, 1163, and 1030 cm^{-1}; IR (neat) 2983, 1747, 1179, 1163, and 1030 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.14 (q, 8 H, methylenes, J = 7.2 Hz), 3.74 (d, 4 H, H_A of AB, methylenes, J = 17.4 Hz), 3.58 (d, 4 H, H_B of AB, methylenes, J = 17.4 Hz), 3.27 (m, 2 H, methines), 2.96 (m, 2 H, methines), 2.45 (m, 2 H, methylenes), 1.43 (m, 2 H, methylenes), 1.41 (s, 6 H, methyls), 1.26 (t, 12 H, methyls, J = 7.2 Hz). Anal. Calcd for $C_{25}H_{42}N_2O_{10}$: C, 56.59; H, 7.98; N, 5.28. Found: C, 56.43; H, 8.12; N, 5.07.

(3aα,5α,6β,7aβ)-Diethyl *N,N'*-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (33). Diamine **27** (1.08 g, 5.8 mmol) was treated with PMP (3.45 g, 22.2 mmol) and ethyl bromoacetate (14.7 g, 88 mmol) in DMF (22 mL) as described for the synthesis of **29**. The crude yellow oil was purified over silica gel [hexane–AcOEt (2:1)] to yield)33 (2.2 g, 75%) as a colorless oil: IR (neat) 2982, 2936, 2905, 1735, 1466, 1369, and 1161 cm^{-1}; NMR (500 MHz, $CDCl_3$) δ 4.14 (q, 8 H, methylenes, J = 7.1 Hz), 3.60 (m, 2 H, methines), 3.58 (s, 8 H, methylenes), 3.51 (broad s, 2 H, methines), 1.99 (m, 4 H, methylenes), 1.43 (s, 6 H, methyls), 1.26 (t, 12 H, methyls, J = 7.1 Hz). Anal. Calcd for $C_{25}H_{42}N_2O_{10}$: C, 56.59; H, 7.98; N, 5.28. Found: C, 56.65; H, 8.15; N, 4.95.

***cis*-4-Cyclohexene-1,2-diamine dihydrochloride (40)** was prepared according to a modified method of Witiak et al.[7] except that the vigorously stirred solution was slowly heated to 85–95 °C in a water bath. Ice was placed into the water bath to lower the reaction temperature when N_2 gas evolved too rapidly. The hydrochloride precipitate was filtered and washed with acetone, giving a white powder (52%): mp 267–268 °C (lit.[7] mp 255–265 °C); IR (KBr) 2900 and 1490 cm^{-1}; NMR (250 MHz, D_2O) δ 5.73 (s, 2 H, olefinic), 3.97 (t, 2 H, methines), 2.70 (m, 2 H, methylenes), 2.40 (m, 2 H, methylenes).

***cis*-Diethyl *N,N'*-(4-Cyclohexene-1,2-diyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (41). Method A.** To a suspension of cyclohexenediamine hydrochloride salt **40** (370 mg, 2 mmol) and anhydrous K_2CO_3 (1.65 g, 12 mmol) in DMSO (4 mL) was added dropwise ethyl bromoacetate (0.98 mL, 8.8 mmol). The mixture was stirred at room temperature for 24 h and diluted with 40 mL of EtOAc. The mixture was washed out with brine (40 mL × 3), dried ($MgSO_4$), and concentrated in vacuo. The resulting yellow oil was purified over silica gel [hexane–AcOEt (5:1)] to afford 953 mg (96%) of pale yellow oil: IR (neat) 2950, 1735, 1370, and 1180 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 5.55 (m, 2 H, olefin), 4.14 (q, 8 H, methylenes, J = 7.2 Hz), 3.66 (s, 8 H, methylenes), 3.03 (deceptively simplet t, 2 H, methines), 2.43 (m, 2 H, methylenes), 2.00 (m, 2 H, methylenes), 1.26 (t, 12 H, methyls, J = 7.2 Hz). Anal. Calcd for $C_{22}H_{36}N_2O_8$·0.5DMSO: C, 55.73; H, 7.93; N, 5.65. Found: C, 56.04; H, 7.54; N, 5.45. **Method B.** To a mixture of diamine hydrochloride salt **40** (7.0 g, 37.8 mmol) and PMP (42.9 g, 0.28 mol) in DMF (110 mL) was added dropwise ethyl bromoacetate (49 mL, 0.44 mol). The mixture was stirred at room temperature for 24 h and diluted with 500 mL of AcOEt. The precipitate was filtered and washed with AcOEt, and the filtrate was concentrated in vacuo (0.5 Torr) at 35 °C. The pale yellow oil was dissolved in AcOEt (300 mL), washed with brine (100 mL × 3), dried ($MgSO_4$), and concentrated to afford a oily residue, which was purified as in method A to furnish 16.5 g (94.5%) of compound **41** as a colorless oil.

(1α,2α,4β,5β)-Diethyl *N,N'*-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis[*N*-(2-ethoxy-2-oxoethyl)glycine ester] (42). Tetraester **41** (6.06 g, 13.3 mmol) was added with stirring and at room temperature to a mixture of acetone (95 mL), distilled H_2O (13 mL) and *t*-BuOH (8.5 mL). *N*-Methymorpholine *N*-oxide monohydrate (3.26 g, 23.0 mmol) and OsO_4 (24 mg in 2.4 mL of CCl_4) were added. The reaction mixture was stirred at room temperature for 24 h, and OsO_4 was decomposed by addition of $NaHSO_3$ (1 g). After 15 min, the solvent was removed in vacuo, and the dark brown oily residue was dissolved in AcOEt (300 mL). The solution was washed with H_2O (60 mL × 3) and brine (60 mL × 3), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified over silica gel [$CHCl_3$–acetone (3:1)] to afford a light yellow oil, which crystallized from AcOEt–hexane to provide **42** (4.62 g, 70%) as white thin needles: mp 79–80.5 °C; IR (KBr) 3444, 3378, 2987, 2956, 1749, 1737, 1371, 1198, and 1033 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 4.06 (q, 8 H, methylenes, J = 7.1 Hz), 3.92 (m, 2 H, methines), 3.76 (d, 4 H, H_A of AB, methylenes, J = 17.9 Hz), 3.61 (d, 4 H, H_B of AB, methylenes, J = 17.9 Hz), 3.41 (deceptively simple d, 2 H, methines), 2.68 (br s, 2 H, OH, exch. with D_2O), 1.88 (m, 2 H, methylenes), 1.74 (m, 2 H, methylenes), 1.19 (t, 12 H, methyls, J = 7.1 Hz). Anal. Calcd for $C_{22}H_{38}N_2O_{10}$: C, 53.86; H, 7.81; N, 5.71. Found: C, 53.60; H, 8.05; N, 5.68.

(3aα,5β,6β,7aα)-4,4'-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(2,6-piperazinedione) (35). To mixture of tetraester **29** (1.36 g, 2.57 mmol) and formamide (913 mg, 20.3 mmol) in dry dioxane (26 mL) was added under N_2 and with vigorous stirring 97% NaH (595 mg, 24 mmol). The reaction mixture was further stirred under N_2 at room temperature for 24 h. Anhydrous TsOH (~5 g) [prepared by dissolving TsOH·H_2O in 2-propanol (150 mL) and evaporating the resulting solution to dryness at 90 °C in vacuo for 1 h] in dry dioxane was added with vigorous stirring to the reaction mixture immersed in a water

bath (room temperature). When the pH (wet pH paper) of the mixture reached 4.1, sufficient MeOH was added to provide a clear solution. The solution, which should have a pH of 4.1 (wet pH paper), was concentrated in vacuo and the precipitate removed by filtration. This procedure was repeated 4–5 times with the filtrate until there was only about 5 mL of solution remaining. The individual solid fractions were individually washed with MeOH to remove undesired NaOTs. The MeOH insoluble solids were combined with any solid obtained from the 5-mL solution. Product from the remaining 5 mL was obtained by diluting with MeOH and storing in a freezer overnight. The precipitate was collected and washed with MeOH. The combined MeOH insoluble solids were recrystallized from DMF/H_2O, providing 444 mg (45%) of **35** as white shiny crystals: mp 273–274 °C; IR (KBr) 3204, 2993, 1746, 1716, 1696, and 1296 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 11.05 (s, 2 H, NH, exch. with D_2O), 4.06 (m, 2 H, methines), 3.54 (s, 8 H, methylenes), 2.70 (m, 2 H, methines), 1.87 (m, 4 H, methylenes), 1.37 (s, 3 H, methyls), 1.24 (s, 3 H, methyls). Anal. Calcd for $C_{17}H_{24}N_4O_6$: C, 53.67; H, 6.36; N, 14.73. Found: C, 53.78, H, 6.50; N, 14.75.

(3aα,5α,6α,7aα)-4,4'-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(2,6-piperazinedione) (36). To a mixture of tetraester **30** (1.55 g, 2.9 mmol) and formamide (1.04 g, 23.1 mmol) in dry dioxane (30 mL) under N_2 and with stirring was added 97% NaH (676 mg, 28 mmol) at room temperature. The mixture was heated to 70–75 °C and vigorously stirred for 24 h. The reaction mixture was cooled to room temperature and worked up by a procedure similar to the one employed for the preparation of bis(dioxopiperazine) **35**. However, in this case the desired product **36** was only found in the final 20 mL of solution following concentration in vacuo. The 20-mL solution was diluted to 40 mL with MeOH and placed in a freezer for 24 h to afford a pale yellow solid (547 mg). Recrystallization from DMF–H_2O provided **36** (400 mg, 36%) as white powder: mp 243–244 °C; IR (KBr) 3246, 2957, 1745, 1686, 1317, and 1056 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 11.12 (s, 2 H, NH, exch. with D_2O), 4.22 (s, 2 H, methines), 3.51 (d, 4 H, H_A of AB, methylenes, J = 17.5 Hz), 3.48 (d, 4 H, H_B of AB, methylenes, J = 17.5 Hz), 2.90 (deceptively simple d, 2 H, methines), 1.95 (m, 2 H, methylenes), 1.87 (m, 2 H, methylenes), 1.37 (s, 3 H, methyls), 1.23 (s, 3 H, methyls). Anal. Calcd for $C_{17}H_{24}N_4O_6$: C, 53.67; H, 6.36; N, 14.73. Found: C, 53.70, H, 6.40; N, 14.75.

(3aα,5α,6β,7aα)-4,4'-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(2,6-piperazinedione) (37) was prepared from tetraester **31** (2.3 g, 4.3 mmol), formamide (1.5 g, 33.3 mmol), and 97% NaH (981 mg, 40.9 mmol) in dry dioxane (38 mL) according to a procedure similar to the one used for the synthesis of **35**. The white crude product (756 mg) was recrystallized from DMF–H_2O to yield 550 mg (34%) of white solid **37**: mp >280 °C; IR (KBr) 3258, 1712, 1331, and 1287 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 10.99 (s, 2 H, NH, exch. with D_2O), 4.20 (br s, 1 H, methine), 4.10 (m, 1 H, methine), 3.49 (d, 4 H, H_A of AB, methylenes, J = 17.4), 3.38 (d, 4 H, H_B of AB, methylenes, J = 17.4 Hz), 2.98 (m, 1 H, methine), 2.87 (m, 1 H, methine), 1.88 (m, 2 H, methylenes), 1.55 (m, 2 H, methylenes), 1.42 (s, 3 H, methyls), 1.23 (s, 3 H, methyls). Anal. Calcd for $C_{17}H_{24}N_4O_6$: C, 53.67; H, 6.36; N, 14.73. Found: C, 53.98, H, 6.39; N, 14.64.

(3aα,5β,6α,7aβ)-4,4'-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(2,6-piperazinedione) (38) was prepared from tetraester **32** (1.9 g, 35 mmol), formamide (1.2 g, 27 mmol), and 97% NaH (806 mg, 33.6 mmol) in dry dioxane (40 mL) using a method similar to the one used for the synthesis of **35**. In this case, the final 15-mL volume obtained during concentration in vacuo was diluted with 30 mL of MeOH to afford a white precipitate (823 mg, extremely soluble in DMF), which recrystallized from DMF–H_2O, providing 567 mg (43%) of **38** as white powder: mp 288–289 °C; IR (KBr), 3446, 3272, 2991, 1717, 1382, and 1070 cm^{-1}; NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 2 H, NH, exch. with D_2O), 3.47 (d, 4 H, H_A of AB, methylenes), 3.43 (d, 4 H, H_B of AB, methylenes), 3.28 (m, 2 H, methines), 3.05 (m 2 H, methines), 2.00 (deceptively simple d, 2 H, methylenes), 1.43 (m, 2 H, methylenes), 1.32 (s, 6 H, methyls). Anal. Calcd for $C_{17}H_{24}N_4O_6 \cdot 0.09H_2O$: C, 53.44; H, 6.38; N, 14.66. Found: C, 53.10; H, 6.16; N, 14.52.

(3aα,5α,6β,7aβ)-4,4'-(Hexahydro-2,2-dimethyl-1,3-benzodioxole-5,6-diyl)bis(2,6-piperazinedione) (39) was synthesized from tetraester **33** (1.9 g, 3.9 mmol), formamide (1.4 g, 30.5 mmol), and 97% NaH (814 mg, 33.9 mmol) in dry dioxane (42 mL) according to a procedure similar to the one used for the synthesis of **35**. However, in this case, after stirring for 36 h the pH of the reaction mixture was adjusted to 4.4 (at lower pH, i.e., 4.1, a 1:1 mixture of acetonide **39** and hydrolysis product **9** is obtained). The crude solid was recrystallized from DMSO–H_2O to yield 536 mg (39%) of **39** as a white solid: mp 280–282 °C; IR (KBr) 3264, 1716, 1386, 1272, and 1074 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 11.08 (s, 2 H, NH, exch. with D_2O), 3.56 (m, 2 H, methines) 3.36 (s, 8 H, methylenes), 3.26 (s, 2 H, methines), 2.01 (m, 2 H, methylenes), 1.62 (m, 2 H, methylenes), 1.31 (s, 6 H, methyls). Anal. Calcd for $C_{17}H_{24}N_4O_6$: C, 53.67; H, 6.36; N, 14.73. Found: C, 53.67; H, 6.42; N, 14.48.

(1α,2α,4α,5α)-4,4'-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione) (5). Bis(dioxopiperazine) **35** (100 mg, 0.26 mmol) was suspended in 1.5 N HCl (2 mL). The mixture was stirred at room temperature until all solid dissolved (~3 min). The solution was cooled in a ice bath, and the pH was carefully adjusted to 4.1 with NaOH–H_2O (1:1). The solution was kept in an ice bath for 1.5 h and the solid was filtered and washed with cold H_2O and MeOH (10 mL) to produce 60 mg (64%) of **5** as white shiny crystals: mp 279–280 °C; IR (KBr) 3513, 3169, 2940, 1729, 1693, 1377, 1220, and 1076 cm^{-1}; NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 2 H, NH, exch. with D_2O), 4.62 (s, 2 H, OH, exch. with D_2O), 3.57 (s, 8 H, methylenes), 3.49 (s, 2 H, methines), 2.76 (s, 2 H, methines), 1.90 (m, 2 H, methylenes), 1.52 (deceptively simple d, 2 H, methylenes). Anal. Calcd for $C_{14}H_{20}N_4O_6 \cdot 1.0H_2O$: C, 46.92; H, 6.19; N, 15.64. Found: C, 46.76; H, 6.27; N, 15.42.

(1α,2α,4β,5β)-4,4'-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione) (6) was prepared from bis(dioxopiperazine) **36** (110 mg, 0.29 mol) using 1.5 N aqueous HCl solution (1 mL) according to the method used for the synthesis of **5**; white powder (40 mg, 40%) was obtained: mp 221–223.5 °C; IR (KBr) 3451, 3212, 2930, 1743, 1692, 1275, and 1013 cm^{-1}; NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 2 H, NH, exch. with D_2O), 4.45 (br s, 2 H, OH, exch. with D_2O), 3.71 (br s, 2 H, methines), 3.51 (d, 4 H, H_A of AB, methylenes, J = 16.9 Hz), 3.49 (d, 4 H, H_B of AB, methylenes, J = 16.9 Hz), 2.91 (deceptively simple s, 2 H, methines), 1.72 (m, 4 H, methylenes). Anal. Calcd for $C_{14}H_{20}N_4O_6 \cdot 0.1H_2O$: C, 49.14; H, 5.95; N, 16.38. Found: C, 48.90; H, 6.07; N, 16.12.

(1β,2α,4α,5α)-4,4'-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione) (7) was synthesized from bis(dioxopiperazine) **37** (162 mg, 0.43 mmol) in 1.5 N aqueous HCl solution according to the procedure used for the synthesis of **5**; a white powder (122 mg, 82%) was obtained; mp >300 °C; IR (KBr) 3494, 3230, 2925, 1709, 1688, 1319, and 1073; NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 1 H, NH, exch. with D_2O), 10.92 (s, 1 H, NH, exch. with D_2O), 4.45 (br s, 1 H, OH, exch. with D_2O), 4.39 (br d, 1 H, OH, exch. with D_2O), 3.73 (s, 1 H, methine), 3.40 (m, 8 H, methylenes), 3.37 (m, 1 H, methine), 3.01 (deceptively simple dt, 1 H, methine, splitting pattern of 10.8 and 3.5 Hz), 2.82 (deceptively simple dt, 1 H, methine, splitting pattern of 10.9 and 3.4 Hz), 1.68 (deceptively simple td, 1 H, methylene, splitting pattern 13.2 and 3.6 Hz), 1.54 (m, 2 H, methylenes), 1.34 (deceptively simple dt, 1 H, methylene splitting pattern 12.5 and 2.2 Hz). Anal. Calcd for $C_{14}H_{20}N_4O_6$: C, 49.40; H, 5.92; N, 16.48. Found: C, 49.43; H, 6.13; N, 16.21.

(1β,2α,4α,5β)-4,4'-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione) (8) was synthesized from bis(dioxopiperazine) **38** (51 mg, 0.14 mmol) in 0.1 N aqueous HCl solution (2 mL) according to a procedure similar to the one used for the preparation of **5**. In this case, the pH of the solution was adjusted to 3.5. Compound **8** was obtained as a white powder (36 mg, 74%): mp >300 °C; IR (KBr) 3509, 3039, 2927, 1733, 1711, 1653, and 1283 cm^{-1}; NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 2 H, NH, exch. with D_2O), 4.65 (br s, 2 H, OH, exch. with D_2O), 3.44 (d, 4 H, H_A of AB, methylenes, J = 16.6 Hz), 3.39 (d, 4 H, H_B of AB, methylenes, J = 16.6 Hz), 3.14 (m, 2 H, methines), 2.86 (m, 2 H, methines), 1.75 (m, 2 H, methylenes), 1.14 (m, 2 H, methylenes). Anal. Calcd for $C_{14}H_{20}N_4O_6 \cdot 0.5H_2O$: C, 48.13; H, 6.06; N, 16.04. Found: C, 48.22; H, 6.21; N, 16.35.

(1α,2β,4α,5β)-4,4'-(4,5-Dihydroxy-1,2-cyclohexanediyl)bis(2,6-piperazinedione) (9) was synthesized from bis(dioxopiperazine) **39** (100 mg, 0.26 mmol) in 1.5 N aqueous HCl solution (2 mL) according to the procedure employed for the synthesis

of 5; a white powder (72 mg, 78%) was obtained: mp >300 °C; IR (KBr) 3539, 3397, 3180, 3082, 1714, 1652, 1287, and 1050 cm^{-1}; NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 2 H, NH, exch. with D_2O), 4.72 (s, 2 H, OH, exch. with D_2O), 3.62 (s, 2 H, methines), 3.44 (d, 4 H, H_A of AB, methylenes, J = 16.4 Hz), 3.40 (d, 4 H, H_B of AB, methylenes, J = 16.4 Hz), 3.00 (m, 2 H, methines), 1.63 (m, 2 H, methylenes), 1.55 (m, 2 H, methylenes). Anal. Calcd for $C_{14}H_{20}N_4O_6 \cdot 0.5H_2O$: C, 48.13; H, 6.06; N, 16.04. Found: C, 48.00; H, 6.12; N, 16.01.

(3aα,4aβ,8aβ,9aβ)-Diethyl Octahydro-2,2-dimethyl-6-oxo-1,3-dioxolo[4,5-g]quinoxaline-5,8-diacetic Acid Ester (45). Diamine 28 (1.1 g, 6.1 mmol) was treated with PMP (3.8 g, 25 mmol) and ethyl bromoacetate (16.2 g, 97 mmol) in DMF (25 mL) as described for the synthesis of 29. The yellow oily residue was purified by preparative TLC (silica gel, hexane–AcOEt (2:1)] to furnish a colorless oil (1.8 g), which was identified as **(3aα,5β,6β,7aβ)-ethyl N-(2-ethoxy-2-oxoethyl)-N-[6-[(2-ethoxy-2-oxoethyl)amino]hexahydro-2,2-dimethyl-1,3-benzodioxol-5-yl]glycine ester (43)** and spontaneously converted to 45 at room temperature. For 43: FAB/MS calcd for $C_{21}H_{37}N_2O_8$ $(M + H)^+$ 445.53, found 445.51; NMR (500 MHz, $CDCl_3$) δ 4.20 (m, 6 H, methylenes, overlap with equivalent methylene protons of 45), 3.89 (m, 1 H, methine), 3.69 (d, 3 H, H_A of AB, methylenes, J = 18.6 Hz), 3.60 (d, 3 H, H_B of AB, methylenes, J = 18.6 Hz), 3.34 (m, 1 H, methine), 3.20 (m, 1 H, methine), 3.16 (m, 1 H, methine), 2.75 (br. s, 1 H, NH, exch. with D_2O), 2.21 (m, 1 H, methylene), 2.08 (m, 1 H, methylene), 1.85 (m, 1 H, methylene), 1.32 (m, 1 H, methylene), 1.38 (s, 6 H, methyls, overlap with equivalent methyl protons of 45), 1.28 (m, 9 H, methyls, overlap with equivalent methyl protons of 45). For 45: IR (neat) 2990, 2935, 1775, 1650, and 1200 cm^{-1}; ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 26.7, and 26.8 (methyls), 23.2, 28.6, 43.3, 51.7, 55.2, 61.0, and 61.3 (methylenes), 54.4, 57.3, 74.7, 78.4 (methines), and 110.8 (quaternary C), 168.6, 168.7, 169.7 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 4.70 (d, 1 H, methylene, J = 17.6 Hz), 4.26 (m, 1 H, methine), 4.20 (m, 4 H, methylenes), 3.88 (d, 1 H, methylene, J = 17.6 Hz), 3.59 (d, 1 H, H_A of AB, methylene, J = 18.2 Hz), 3.46 (d, 1 H, H_B of AB, methylene, J = 18.2 Hz), 3.45 (d, 1 H, $H_{A'}$ of A'B', methylene, J = 16.7 Hz), 3.43 (m, 1 H, methine), 3.37 (m, 1 H, methine), 3.33 (d, 1 H, $H_{B'}$ of A'B', methylene, J = 16.7 Hz), 3.28 (td, 1 H methine, J = 11.9 and 4.0 Hz), 2.35 (td, 1 H, methylene, J = 14.2 and 3.0 Hz), 2.20 (td, 1 H, methylene, J = 11.6 and 4.0 Hz), 1.91 (q, 1 H, methylene, J = 11.7 Hz), 1.63 (ddd, 1 H, methylene, J = 14.2, 12.4, and 3.5 Hz), 1.38 (s, 6 H, methyls), 1.28 (m, 6 H, methyls); FAB/MS, exact mass calcd for $C_{19}H_{31}N_2O_7$ $(M + H)^+$ 399.2131, found 399.2122.

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Aza- and Diazaannulenones. Influence of Nitrogen Position on Their Reactivity and Stability

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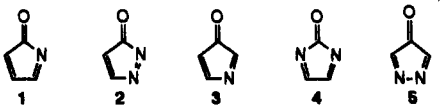
Three new elusive intermediates, 3-aza-2,4-cyclopentadienone, 2,5-diaza-2,4-cyclopentadienone, and 3,4-diaza-2,4-cyclopentadienone, are reported. The three species can act either as dienes or as dienophiles in Diels–Alder reactions. The influence of the number and position of nitrogen atoms on their stability is studied.

We have previously demonstrated the existence in solution of 2-aza-2,4-cyclopentadienone, 1, and 2,3-diaza-2,4-cyclopentadienone, 2, and studied their reactivity in Diels–Alder reactions.[1,2] In order to study the influence of nitrogen position on their stability and reactivity, we presently report the study of three new intermediates, 3-aza-2,4-cyclopentadienone, 3, 2,5-diaza-2,4-cyclopentadienone, 4, and 3,4-diaza-2,4-cyclopentadienone, 5 (Chart I).

The literature provides some information on 2,5-disubstituted 3,4-diaza-2,4-cyclopentadienones in Diels–Alder reactions, but the parent compound has not been studied to date. On the other hand, no experimental evidence for the existence of compounds related to 3 and 4 exists. To study this aspect, and the reactivity and stability of these intermediates, we have employed the three-phase test. Accordingly, the intermediate is generated from an insoluble polymer-bound precursor and trapped by a second solid phase by using Diels–Alder reactions.[3] Isolation of an adduct gives positive evidence for existence of the postulated intermediate. We used this test successfully to demonstrate the transient existence of several reactive intermediates.[4]

Chart I



We were interested in providing evidence for the existence of 3–5 and showing that they may behave either as dienes or as dienophiles in Diels–Alder reactions. Thus, we prepared a suitable polymer-bound precursor for each intermediate, as shown below. Polymer-bound monoester of acetylenedicarboxylic acid,[5] 17, and polymer-bound ester of 2-furoic acid,[5] 21, were used as dienophilic and dienic trapping agents in the three-phase test.

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