Conformationally Restrained Chiral Analogues of Spermine: Chemical Synthesis and Improvements in DNA Triplex Stability†

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The synthesis of novel chiral analogues of spermine, **11** (2*R*,4*S*) and **14** (2*S*,4*R*), is reported starting from *trans*-4-hydroxy-L-proline **3**. These cyclic analogues are generated from linear, achiral spermine by incorporating a pyrrolidine ring on the backbone to effect conformational rigidity, with simultaneous creation of two asymmetric centers. The chiral analogues bind both AT and CG rich DNA duplexes as effectively as spermine and exhibit even better association with DNA triplex than spermine. The newer analogues have features for structural elaboration to novel molecular entities of potential importance in therapeutics and material design.

The linear polyamines—putrescine, spermidine, and spermine (**1**)—are biological cations ubiquitously found in all cells with a diverse role in physiological processes.[1] These range from stabilization/modulation of membrane function and mitochondria[1] and facilitation of DNA transfection by phage[2] to regulation of cell growth and differentiation.[3] They play an important role in proliferative processes, in particular, neoplastic growth and chemical carcinogenesis.[4] The binding of spermine to DNA stabilizes the duplex, causes conformational changes in DNA such as B−Z transition, and induces bending of the helix axis at specific sequences which has importance in chromatic condensation and regulation of transcription.[5] Spermine is known to promote triplex stabilization when externally added,[6] as well as upon covalent conjugation to sugar[7] or nucleobase residues.[8] *In vitro*, polyamines affect many enzymatic systems such as phosphatidylinositol−G and adenylate cyclase−G protein pathways and post-translational modification of proteins by transglutaminases[9] and also inhibit voltage-activated ion channels.[10] The high level of polyamines noticed in transformed cells has led to design of their analogues as inhibitors of polyamine biosynthesis enzymes such as ornithine decarboxylase and *S*-adenosylmethionine decarboxylase.[11] The modulatory activity of spermine on the *N*-methyl-D-glutamate (NMDA) receptor has potential for application of polyamine analogues in therapy of neurological diseases such as epilepsy.[12] Polyamine analogues are also emerging as serious therapeutic options for development of potent new antidiarrheals, in particular, AIDS-related diarrhea.[13] The wide therapeutic potential of polyamines has led to design, synthesis, and biological evaluation of a large number of their analogues toward understanding structure−activity relationships.[14] These studies have pointed out that polyamine analogues at the tetramine level must be charged to be recognized by the cell and analogues with low pK_as that are poorly protonated at pH 7.0 do not compete efficiently with biogenic amines for uptake. The several structural modifications made to the polyamine backbone include variation in the number and distance between nitrogens,[15] terminal N-substitutions of varying sizes,[13] and rigidification via interconnection of secondary amines (cyclopolyamines) based on the cycloputrescine core.[16] Most of these chemical modifications are through N-substitutions and are achiral.

Since many of the polyamine receptor sites (nucleic acids, membranes) are chiral in nature, we reasoned that

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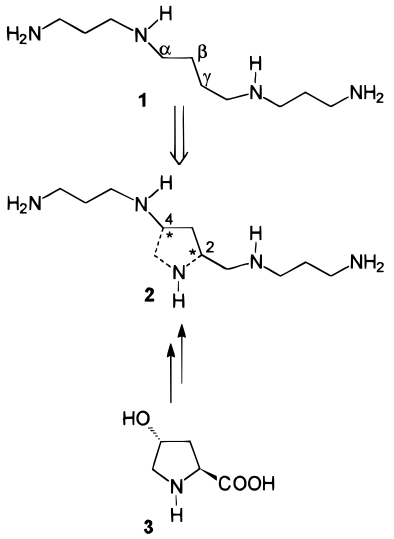
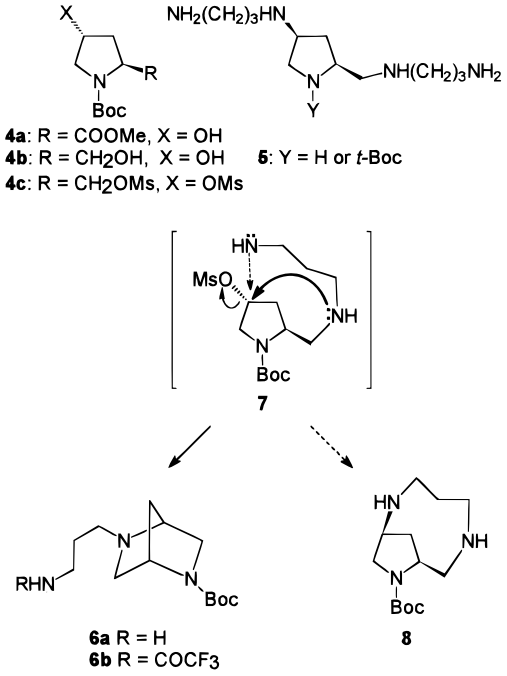
Scheme 1



Scheme 2



introduction of asymmetry into the polyamine backbone may be beneficial to their activity. This and our own studies on stabilization of DNA triplexes at physiological conditions through covalent linking of spermine[8b−d] and use of bisguanidine analogues of spermine[8e] prompted us to synthesize chiral spermine analogues and study their interaction with nucleic acids.

Results and Discussion

Chemical Synthesis of Pyrrolidyl Polyamines. These analogues are derived from spermine (**1**) by introducing a bridge of CH_2-NH between the carbon atoms α and γ of the central tetramethylene fragment as shown in Scheme 1 to generate a five-membered pyrrolidine ring (**2**). This makes the molecule chiral with creation of two asymmetric centers (C2 and C4) and hence two pairs of diastereomers (*cis* 2*S*,4*S*, 2*R*,4*R* and *trans* 2*S*,4*R*, 2*R*,4*S*) with a simultaneous introduction of one additional nitrogen atom. We report here the chemical synthesis of two of the four possible stereoisomers (*trans* 2*S*,4*R* and 2*R*,4*S*) of the designed newer analogues of spermine and demonstrate that these analogues are better than spermine in selectively stabilizing the DNA triple helix.

The spermine analogues **2** were synthesized from the commercially available and versatile chiral starting block *trans*-4-hydroxy-L-proline[17] (**3**) which was treated with thionyl chloride in methanol to yield the 2-carboxymethyl ester that was subsequently reacted with *tert*-butoxy carbazide for protection of the ring N to give *trans*-4-hydroxy-*N*-(*tert*-butoxycarbonyl)-L-proline methyl ester[18] (**4a**). This was reduced with lithium borohydride[19] to *trans*-4-hydroxy-L-prolinol (**4b**) and converted to the corresponding dimesylate **4c** by reaction with mesyl chloride in pyridine. The 2,4-*trans*-dimesylate **4c** was reacted with neat 1,3-diaminopropane with the expectation of obtaining precursor **5** (2*S*,4*S*; Y = Boc) for the *cis* isomer, through a simple displacement of both mesylates by the diamine with a simultaneous inversion at C4. Although the reaction led to a single product, it was not the expected one, and on the basis of spectral data (NMR, mass), it was identified as the bicyclic compound (1*S*,4*S*)-5-(3-aminopropyl)-2-(*tert*-butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptane (**6a**). This product possibly arises from the intermediate **7** resulting from an initial (faster) displacement of primary mesylate at C2 in **4c** by the diamine.[20] A *trans* relationship of the 4-mesylate with the C2 substituent in **7** subsequently leads to a stereochemically favored intramolecular S_N2 attack by the secondary amino group of the C2 side chain on C4. The identity of the resulting bicyclic product **6a** was supported by the presence of a methylene triplet due to the CH_2NH_2 in its ^1H NMR spectrum and by converting **6a** into its monotrifluoroacetamide derivative **6b**. This also ruled out the formation of the isomeric product **8** that lacks a CH_2NH_2 group and forms a ditrifluoroacetamide upon derivatization. The synthesis of 2,4-*trans*-polyamines **11** (2*R*,4*S*) and **14** (2*S*,4*R*) could be achieved alternatively from **3** by selective inversion of configuration at C2 and C4. The epimerization of *trans*-4-hydroxy-L-proline (**3**) at C2 by acetic anhydride/acetic acid treatment[17] followed by reflux in 2 N HCl gave the *cis*-4-hydroxy-D-proline (**9**) whose optical integrity was confirmed by ^{13}C NMR spectrum and optical rotation. The *N*-Boc-*C*2-methyl ester **10a**, obtained from **9**, upon reduction with lithium borohydride gave the *cis*-4-hydroxy-D-prolinol (**10b**) which was derivatized to the *cis*-dimesylate **10c**. This on treatment with 1,3-diaminopropane gave the expected product **11a** unlike in the case of the *trans*-dimesylate **4c**. The *cis* configuration of the C4 mesylate with the C2 side chain amine in the intermediate **10d** does not favor an intramolecular substitution, and as a result, the attack of a second molecule of diamine at C4 eventually occurs, leading to the chiral polyamine **11a**. The successful isolation of the highly water soluble amine **11a** in 95−97% yield was achieved under nonaqueous workup using ethyl acetate (see the Experimental Section for details). The *N*-*t*-Boc function of **11a** was deprotected

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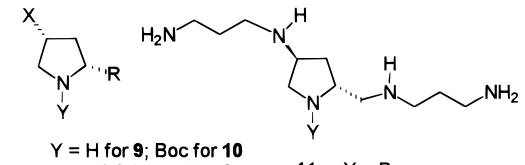
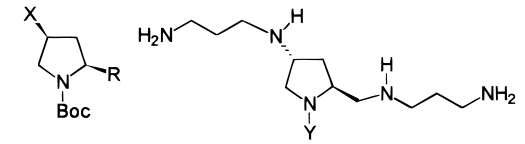
using 5 N HCl to obtain the 2,4-*trans* (2*R*,4*S*) analogue as its pentahydrochloride salt **11b**.



Y = H for **9**; Boc for **10**

9: R = COOH, X = OH
10a: R = COOMe, X = OH
10b: R = CH_2OH, X = OH
10c: R = CH_2OMs, X = OMs
10d: R = $CH_2NH(CH_2)_3NH_2$, X = OMs,

11a: Y = Boc
11b: Y = H



12: R = COOMe, X = OBz
13a: R = CH_2OH, X = OH
13b: R = CH_2OMs, X = OMs
13c: R = $CH_2NH(CH_2)_3NH_2$, X = OMs

14a: Y = Boc
14b: Y = H

The inversion of configuration of hydroxyl at C4 in **4** was achieved via Mitsunobu reaction[21] with benzoic acid affording the *cis*-4-*O*-benzoyl-*N*-(*tert*-butoxycarbonyl)-L-proline methyl ester (**12**). This ester on reduction with lithium borohydride in dry THF gave the *cis*-diol **13a** that was mesylated to the *cis*-dimesylate **13b** followed by reaction with neat 1,3-diaminopropane to give the polyamine **14a**. The removal of *t*-Boc protection in **14a** was achieved by treatment with 5 N HCl to furnish 2,4-*trans* (2*S*,4*R*) polyamine **14b**, isolated as its pentahydrochloride salt.

DNA Duplex and Triplex Stabilization with Spermine Analogues 11b and 14b. Spermine is well known to stabilize DNA duplexes and triplexes,[5e−j,22] although the exact molecular details are not yet clear. The terminal ammonium cations may bind to anionic phosphates of DNA, while the internal amino moieties may be involved in specific hydrogen bonding with nucleobases in the major groove, with a cross groove binding for A:T base pairs and a down groove mode among G:C base pairs.[22] In the case of triplexes, stabilization by cationic polyamines is ascribed to proceed via electrostatic neutralization of large negative charge density on triplexes.[6c] Spermine, a linear aliphatic chain, enjoys enormous conformational freedom, and theoretical calculations have suggested a preferential occurrence of *trans*/*gauche* conformations at C−N and N−C bonds.[23] In the presently designed analogues, conformational constrain imposed by the five-membered chiral pyrrolidine ring (2*R*,4*S* and 2*S*,4*R*) on the spermine backbone may influence its interaction with DNA, and to examine such structural effects, we carried out UV−t_m DNA melting studies of DNA duplexes and triplexes in the presence of these analogues at 1 mM concentration; the results are presented in Table 1. Since Mg^{2+} is well known to stabilize duplexes and triplexes, all melting experiments were carried out under identical conditions, in both the presence and absence of Mg^{2+}.

Table 1. UV−t_m (°C) of DNA Duplex/Triplex in the Presence of Spermine and Its Analogues[a]

no.	duplex/ triplex	control	**1**	**11a**	**11b**	**14a**	**14b**
1	**15**	50	56	53.0	58.0	55.0	57.0
2	**16:17**	43	50	49.5	51.5	48.5	51.0
3	**17*18:19**	nd[b]	30	nd[b]	42.0	nd[b]	45.0

[a] For conditions, see Experimental Section; [amine] = 1 mM, no Mg^{2+}. [b] Not detectable.

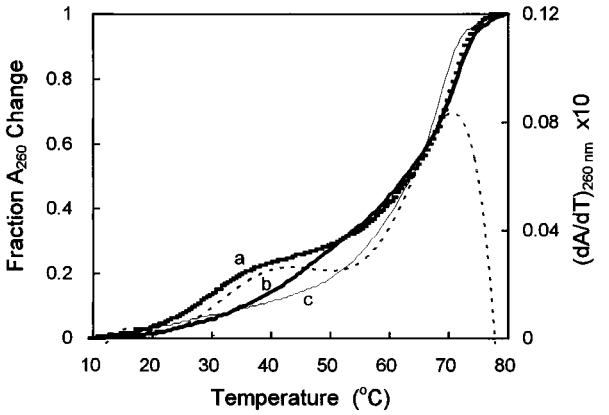


Figure 1. UV−melting profiles of triplex **17*18:19** in TRIS buffer containing 0.1 M NaCl at pH 7.3, in the presence of 1 mM (a) spermine (**1**), (b) **11b**, and (c) **11a**. Dotted curve is the first derivative of profile b.

It is seen from the experimental data that the self-complementary CG rich Dickerson 12-mer duplex **15** is stabilized by spermine **1** ($\Delta t_m \approx 6$ °C), as well as the free amine analogues **11b** and **14b** ($\Delta t_m \approx 7-8$ °C) compared to the control (entry 1). Although no significant differences were noticed among the free amines, the *N*-Boc derivatives **11a** and **14a** exhibited a relatively lower stabilizing effect ($\Delta t_m \approx 3-5$ °C) arising from either steric hindrance by the Boc substituent upon complex formation with DNA double/triple helices or lack of protonation on the ring N. The addition of 20 mM $MgCl_2$ raised the t_m by 3−5 °C, in the case of control and duplex containing Boc derivatives **11a** and **14a** (data not shown) as expected, but with spermine **1** or its analogues **11b** and **14b**, the increase was not appreciable. The non-self-complementary AT rich duplex (**16:17**) showed a similar trend of stabilization with spermine and its analogues (Table 1, entry 2). Thus the constrained spermine analogues are as good as spermine in effecting duplex stability of both AT and CG rich sequences, and structural specificity effects are minimal on duplex stabilization which is largely governed by the number of positive charges.

15 *d* C G C G A A T T C G C G
16 A A G A A A A A A G A A A A A A G A *d*
17 *d* T T C T T T T T T C T T T T T T C T
18 *d* G C C A A G A A A A A A G A A A A A A G A C G C
19 C G G T T C T T T T T T C T T T T T T C T G C G *d*

In comparison, considerable specificity differences were noticed among spermine and its constrained analogues in their interaction with the triplex (**17*18:19**). In the absence of Mg^{2+}, while no triplex was detected with the control (Table 1, entry 3), spermine and its analogues **11b** and **14b** exhibited well-recognizable triplex formation (Figure 1) with the lower transition corresponding to triplex ⇔ duplex and the higher one to duplex melting.

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Further, the analogues showed remarkably improved stabilization of triplexes ($\Delta t_m \approx 12-15$ °C) over that by spermine, with (2*S*,4*R*)-**14b** isomer being slightly better than (2*R*,4*S*)-**11b** isomer. Interestingly, the *N*-Boc analogues **11a** and **14a** failed to promote triplexation in the absence of Mg^{2+}. As expected, all including control and Boc derivatives, showed the triplex in the presence of Mg^{2+} (not shown). These results indicate that the constrained spermine analogues **11b** and **14b**, while stabilizing duplexes as effectively as spermine, are significantly better than spermine in selectively stabilizing DNA triplexes. The topology of polyamine−DNA interactions in duplexes differs considerably from that in triplexes where polyamine structural effects (number and distribution of positive charges) have profound consequences on stability.[6b] The magnitude of observed triplex stability with **11b** and **14b** cannot be solely attributed to the effect of an additional positive charge in them as compared to spermine. It is more likely that the rigid conformations of the constrained analogues **11** and **14** are more suitable for triplex binding than the flexible conformation of spermine.[23]

In summary, novel chiral analogues of spermine, incorporating a pyrrolidine ring on its backbone with consequential conformational restraint, have been synthesized from *trans*-4-hydroxy-L-proline, and these are shown to substantially improve DNA triplex stability over that by spermine. Further studies are aimed at rational fine tuning of stereochemistry in the polyamine analogues through molecular modeling and derivatization (e.g., guanidylation[8e]) to develop more efficient and selective DNA-binding agents. The newer spermine analogues reported here have features for extension to branched structures through the ring N, and such new entities add to an expanding library of polyamines required for drug and novel material designs.

Experimental Section

All the melting points are uncorrected. All solvents used were purified according to literature procedures.[24] *trans*-4-Hydroxy-L-proline, *tert*-butyl carbazate, DEAD, and 1,3-diaminopropane were obtained form Aldrich Chemical Co. ^{1}H NMR spectra were recorded at 200 MHz and ^{13}C NMR at 50 MHz, and all the chemical shifts are referred to internal TMS or 1,4-dioxane. Silica gel column chromatographic separations were followed by TLC analysis using precoated silica gel TLC plates (Merck Kieselgel 60 F_{254}) and visualized by ninhydrin spray.

Oligonucleotide Synthesis and UV−Melting Experiments. All oligonucleotides were synthesized on 1.3 μmol scale on a Pharmacia GA Plus DNA synthesizer and purified according to literature procedure.[12c,d] Duplex and triplex melting experiments were carried out in TRIS (25 mM) buffer at pH 7.3, containing NaCl (100 mM) in the presence or absence of $MgCl_2$ (20 mM). Appropriate oligonucleotides, each at a strand concentration of 1 μmol based on UV absorbance at 260 nm calculated using molar extinction coefficients of respective nucleosides, were mixed in the buffer containing polyamines (1 mM), heated at 80 °C for 3 min, and cooled to room temperature followed by overnight storage at 4 °C. A_{260} values at various temperatures were recorded using a Perkin Elmer Lambda 15 UV/vis spectrophotometer, fitted with a water jacketed 5-cell holder and Julabo temperature programmer with a heating rate of 0.5 °C/min over the range 5−75 °C. Dry nitrogen gas was flushed in the spectrophotometer chamber to prevent moisture condensation at temperatures below 15 °C. The triplex dissociation temperature (t_m) was determined from the midpoint of the first transition from the plot of fraction absorbance change versus temperature and was further confirmed by differential [$(dA/dT)_{260\ nm}$ vs T] curves. The t_m values are accurate to ±0.5 °C over the reported values.

***trans*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-L-prolinol (4b).** To an ice cold solution of **4a** (1.2 g, 5.0 mmol) in THF (20 mL) was added lithium borohydride (0.45 g, 21 mmol), and the mixture was stirred at 5−10 °C for 4 h, continued at ambient temperature for 36 h. The reaction was quenched by addition of saturated ammonium chloride solution (10 mL) at 0 °C with stirring for a further 2 h. It was concentrated to a pasty mass under reduced pressure and extracted into $CHCl_3$ followed by evaporation to give a colorless viscous liquid. This was purified by column chromatography on silica gel (eluent: dichloromethane/EtOAc, 1:1) to yield the diol **4b** as a colorless oil (1 g, 94%). IR ($CHCl_3$): 3500−3300, 1670 cm^{-1}. ^{1}H NMR δ ($CDCl_3 + D_2O$): 4.33 (bs, 1H), 4.12−4.05 (m, 1H), 3.62−3.35 (m, 4H), 2.08−1.99 (bm, 1H), 1.75−1.59 (bs, 1H), 1.45 (s, 9H). $[\alpha]^{25}_D = -56.2 \pm 0.5$ ($c = 0.56$, $CHCl_3$) [lit.[25] $[\alpha]^{20}_D = -58.87$ ($c = 1.01$, EtOH)].

***cis*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-D-prolinol (10b).** The reduction of **10a** (1.25 g, 5.1 mmol) using lithium borohydride (0.8 g, 37 mmol) in THF (20 mL) as in the case of **4a** afforded **10b** (0.8 g, 72%) as a white solid, mp = 91−93 °C. IR (Nujol): 3300−3100, 2920−2850, 1670 cm^{-1}. ^{1}H NMR δ ($CDCl_3 + D_2O$): 4.28 (bt, 1H), 4.05−3.99 (bm, 2H), 3.55−3.44 (bm, 3H), 2.37−2.26 (bm, 1H), 1.94−1.80 (bm, 1H), 1.46 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 155.5, 154.7, 79.8, 69.4, 68.7, 63.7, 63.2, 58.3, 56.3, 37.7, 37.0, 28.3. $[\alpha]^{25}_D = +46.3 \pm 0.5$ ($c = 0.16$, $CHCl_3$). Anal. for $C_{10}H_{19}NO_4$ Calcd: C, 55.29; H, 8.75; N, 6.45. Found: C, 55.65; H, 8.39; N, 6.77.

***cis*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-L-prolinol (13a).** The reduction of **12** (1.8 g, 5.2 mmol) with lithium borohydride (0.75 g, 34 mmol) in THF (25 mL) followed by silica gel column chromatography as in the case of **4b** yielded **13a** (0.65 g, 58%) as a white solid, mp = 88−90 °C. IR (Nujol): 3300−3200, 2920−2860, 1685 cm^{-1}. ^{1}H NMR δ ($CDCl_3 + D_2O$): 4.29 (bs, 1H), 4.08−4.01 (bm, 2H), 3.61−3.45 (bm, 3H), 2.37−2.26 (bm, 1H), 1.95−1.75 (bm, 1H), 1.46 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 155.7, 154.9, 80.0, 69.6, 68.9, 64.0, 63.4, 58.5, 56.6, 38.0, 37.3, 28.5. $[\alpha]^{25}_D = -44.6 \pm 0.5$ ($c = 0.4$, $CHCl_3$). Anal. for $C_{10}H_{19}NO_4$ Calcd: C, 55.29; H, 8.75; N, 6.45. Found: C, 55.53; H, 8.51; N, 6.47. FAB MS: 218 ($M^+ + 1$, 32), 188 (100).

***trans*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-L-prolinol Dimethanesulfonate (4c).** To a precooled, stirred solution of diol **4b** (0.95 g, 4.5 mmol) in pyridine (10 mL) was added methanesulfonyl chloride (1 mL, 12.9 mmol) dropwise under nitrogen. The stirring was continued at 0 °C for 4 h. The solvent was removed under vacuum at 30 °C; the residue was extracted into ethyl acetate (20 mL) and upon workup yielded **4c** as a yellow solid (1.5 g, 91%). This solid was used directly for the next reaction without further purification. ^{1}H NMR δ ($CDCl_3$): 5.29−5.26 (m, 1H), 4.70−4.55 (bm, 1H), 4.40−4.28 (m, 2H), 3.88−3.76 (m, 1H), 3.62−3.48 (bm, 1H), 3.08 (s, 3H), 3.05 (s, 3H), 2.47−2.28 (m, 2H), 1.48 (s, 9H).

***cis*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-D-prolinol Dimethanesulfonate (10c).** The reaction of compound **10b** (0.5 g, 2.5 mmol) with mesyl chloride (0.6 mL, 7.8 mmol) in pyridine (5 mL) gave **10c** (0.85 g, 99%) as a pale yellow solid. This solid was used for the next reaction without further purification. ^{1}H NMR δ ($CDCl_3$): 5.32−5.28 (m, 1H), 4.46−4.44 (m, 1H), 4.42−4.16 (bm, 2H), 3.71−3.69 (m, 2H), 3.10 (s, 3H), 3.06 (s, 3H), 2.47−2.37 (bm, 2H), 1.48 (s, 9H).

***cis*-4-Hydroxy-*N*-(*tert*-butoxycarbonyl)-L-prolinol Dimethanesulfonate (13b).** The compound **13a** (0.55 g, 2.5 mmol) was mesylated using mesyl chloride (0.6 mL, 8 mmol) in dry pyridine (5 mL) to yield **13b** (0.94 g, 99%). ^{1}H NMR δ ($CDCl_3$): 5.31−5.26 (m, 1H), 4.47−4.43 (bm, 1H), 4.36−4.14 (bm, 2H), 3.70 (bs, 2H), 3.08 (s, 3H), 3.04 (s, 3H), 2.41−2.31 (bm, 2H), 1.48 (s, 9H).

(1*S*,4*S*)-5-(3-Aminopropyl)-2-(*tert*-butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptane (6a): Procedure 1. The dimesylate **4c** (0.8 g, 2 mmol) was stirred in neat 1,3-diaminopro-

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pane (5 mL) at ambient temperature for 4 h followed by stirring at 60 °C for 24 h. The excess diamine was removed under reduced pressure at 50 °C. The residue was dissolved in dichloromethane (30 mL) followed by washing with water (20 mL). The dichloromethane extract was dried over anhydrous sodium sulfate and concentrated to yield a pale yellow oil of **6a** (0.45 g, 40%).

Procedure 2. The dimesylate **4c** (0.95 g, 2.5 mmol) was stirred in neat diaminopropane (6 mL) under argon atmosphere at ambient temperature for 4 h and then at 60 °C for 8 h. The excess diamine was removed under low pressure at 40 °C, the residue was dried with anhydrous sodium sulfate (10 g), and the product was extracted into ethyl acetate (3 × 10 mL) and filtered. Evaporation of the filtrate under vacuum gave **6a** as a pale yellow oil (0.9 g, 100%). ^{1}H NMR δ ($CDCl_3$ + D_2O): 4.34 (bs, 0.5H), 4.21 (bs, 0.5H), 3.55−3.43 (bt, 2H), 3.16−3.11 (bd, 1H), 2.95−2.86 (t, 1H), 2.79−2.73 (t, 2H), 2.64−2.43 (m, 3H), 1.79−1.52 (bm, 4H), 1.45 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 153.7, 78.6, 60.9, 60.3, 59.6, 57.3, 56.3, 51.8, 51.4, 49.2, 48.4, 40.0, 35.7, 35.0, 32.1, 28.1. $[\alpha]^{25}_D = -47.5 \pm 0.5$ (c = 0.64, MeOH). FAB MS: 256 ($M^+ + 1$, 100).

(2*R*,4*S*)-2-[[*N*-(3-Aminopropyl)amino]methyl]-4-[*N*-(3-aminopropyl)amino]-1-(*tert*-butoxycarbonyl)pyrrolidine (11a). Compound **10c** (0.8 g, 2.1 mmol) was treated with neat 1,3-diaminopropane (6 mL) at 60 °C for 24 h and worked up according to procedure 2 for the preparation of **6a** to get **11a** (0.6 g, 85%) as a pale yellow oil. ^{1}H NMR δ ($CDCl_3$ + D_2O): 3.97 (bs, 1H), 3.53−3.48 (m, 1H), 3.47−3.06 (bm, 2H), 2.79−2.58 (m, 10H), 2.09−1.99 (bm, 1H), 1.80−1.47 (m, 5H), 1.46 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 154.3, 78.6, 77.5, 55.8, 55.5, 52.7, 51.8, 47.2, 45.6, 39.7, 39.1, 36.4, 36.3, 33.1, 32.1, 27.9. $[\alpha]^{25}_D = +45.3 \pm 0.5$ (c = 0.64, MeOH).

(2*S*,4*R*)-2-[[*N*-(3-Aminopropyl)amino]methyl]-4-[*N*-(3-aminopropyl)amino]-1-(*tert*-butoxycarbonyl)pyrrolidine (14a). Compound **13b** (0.9 g, 2.4 mmol) was treated with neat 1,3-diaminopropane (6 mL) at 60 °C for 24 h and worked up according to procedure 2 for the preparation of **6a** as above to get **14a** (0.66 g, 83%) as a pale yellow oil. ^{1}H NMR δ ($CDCl_3$ + D_2O): 3.95 (bs, 1H), 3.55−3.48 (m, 1H), 3.38−3.10 (bm, 2H), 2.81−2.50 (m, 10H), 2.1−1.99 (bm, 1H), 1.83−1.57 (m, 5H), 1.46 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 154.4, 78.7, 77.6, 55.9, 55.5, 52.9, 52.0, 47.3, 45.7, 39.8, 39.2, 36.1, 33.1, 33.0, 28.0. $[\alpha]^{25}_D = -45.9 \pm 0.5$ (c = 0.64, MeOH). FAB MS: 330 ($M^+ + 1$, 100).

(1*S*,4*S*)-5-[3-(Trifluoroacetamido)propyl]-2-(*tert*-butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptane (6b). The compound **6a** (0.75 g) was treated with ethyl trifluoroacetate (0.5 mL) in ethanol (10 mL) followed by standard work up and column chromatography on silica gel (elution: dichloromethane/ethyl acetate, 4:1) to yield the trifluoroacetamide derivative **6b** as a yellow oil (1.1 g, 67.6%). ^{1}H NMR δ ($CDCl_3$): 9.67−9.58 (bd, 1H), 4.39 (bs, 0.6H), 4.25 (bs, 0.4H), 3.58−3.35 (bm, 4H), 3.21 (d, 0.6H, J = 2.0 Hz), 3.16 (d, 0.4H, J = 2.0 Hz), 2.95−2.79 (m, 3.5H), 2.52−2.47 (bd, 0.5H), 1.76−1.65 (m, 4H), 1.46 (s, 9H). ^{13}C NMR δ ($CDCl_3$): 157.4, 156.9, 156.5, 154.0, 121.7, 117.9, 114.1, 79.4, 61.7, 61.2, 59.6, 57.3, 56.3, 54.5, 53.6, 53.3, 49.9, 48.4, 40.8, 36.4, 35.9, 34.7, 28.2. $[\alpha]^{25}_D = -28.8 \pm 0.5$ (c = 0.84, MeOH). FAB MS: 352 (M^+, 100).

***cis*-4-*O*-Benzoyl-1-(*tert*-butoxycarbonyl)-L-proline Methyl Ester (12).** To a solution of **4** (2.0 g, 8 mmol), triphenylphosphine (2.8 g, 10.5 mmol), and benzoic acid (1.3 g, 10.5 mmol) in acetonitrile (30 mL) at 0 °C was added diisopropyl azodicarboxylate (2.15 g, 10.6 mmol) as a neat liquid. The reaction mixture was stirred at 0 °C for 2 h and then at ambient temperature for 24 h. After addition of methanol (1 mL) the reaction mixture was concentrated to a pasty mass which was dissolved in diethyl ether (30 mL), triturated with petroleum ether (40−60 °C), and kept at 5 °C overnight. The triphenylphosphine oxide formed was filtered off, the filtrate was concentrated to dryness, and the residue was chromatographed over silica gel using petroleum ether/dichloromethane (2:3; R_f = 0.4) as eluent to obtain **12** (2.2 g, 77%) as a white solid, crystallized from EtOAc/petroleum ether (40−60 °C), mp 85−88 °C. IR (Nujol): 2950−2850, 1745, 1710, 1690 cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 7.98 (d, 2H), 7.61−7.40 (m, 3H), 5.54 (bm, 1H), 4.64−4.58 (m, 0.5H), 4.51−4.46 (m, 0.5H), 3.87−3.68 (m, 5H), 2.59−2.42 (m, 2H), 1.47 (d, 9H). ^{13}C NMR δ ($CDCl_3$): 172.4, 172.1, 165.7, 154.1, 153.7, 133.3, 129.7, 128.4, 80.3, 73.5, 72.4, 57.9, 57.6, 52.5, 52.1, 36.6, 35.7, 28.3. $[\alpha]^{25}_D = -28.0 \pm 0.5$ (c = 0.5, $CHCl_3$). Anal. for $C_{18}H_{23}NO_6$ Calcd: C, 61.86; H, 6.64; N, 4.01. Found: C, 61.78; H, 6.76; N, 4.01. FAB MS: 350 ($M^+ + 1$, 18), 250 (100).

(2*R*,4*S*)-2-[[*N*-(3-Aminopropyl)amino]methyl]-4-[*N*-(3-aminopropyl)amino]pyrrolidine Pentahydrochloride (11b). Compound **11a** (0.2 g, 0.6 mmol) was treated with 5 N HCl (10 mL) at ambient temperature for 8 h. Water and HCl were removed under vacuum, and the residue was dried over anhydrous P_2O_5 under vacuum to obtain the pentahydrochloride salt **11b** (0.2 g, 80%) as a white hygroscopic solid. ^{1}H NMR δ (D_2O): 4.40−4.25 (m, 2H), 4.14−4.03 (m, 1H), 3.73−3.55 (m, 3H), 3.31−3.08 (m, 8H), 2.73−2.68 (m, 1H), 2.59−2.48 (m, 1H), 2.21−2.06 (m, 4H). ^{13}C NMR δ (D_2O): 56.4, 56.2, 48.6, 48.2, 46.7, 45.2, 37.6, 32.5, 24.8, 24.6.

(2*S*,4*R*)-2-[[*N*-(3-Aminopropyl)amino]methyl]-4-[*N*-(3-aminopropyl)amino]pyrrolidine Pentahydrochloride (14b). Compound **14a** (0.2 g, 0.6 mmol) was treated with 5 N HCl at ambient temperature for 8 h. Workup as above yielded the pentahydrochloride salt **14b** (0.18 g, 73%) as a white hygroscopic solid. ^{1}H NMR δ (D_2O): 4.44−4.18 (m, 2H), 4.13−4.02 (m, 1H), 3.70−3.53 (m, 3H), 3.30−2.96 (m, 8H), 2.74-2.64 (m, 1H), 2.58−2.45 (m, 1H), 2.20−2.05 (m, 4H). ^{13}C NMR δ (D_2O): 56.1, 55.9, 48.3, 47.9, 46.4, 44.9, 37.4, 32.3, 24.6, 24.4.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **6a**, **12**, and **14b**, mass spectra for compounds **6a**, **12**, and **14a**, and UV−melting profile for duplex **16:17** in the presence of **1** and **14a,b** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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