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Pyrrolidyl Polyamines: Branched, Chiral Polyamine Analogues That Stabilize DNA Duplexes and Triplexes

Dendukuri Nagamani and Krishna N. Ganesh*

Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune 411008, India

kng@ems.ncl.res.in

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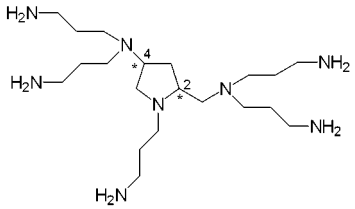
ABSTRACT



III-VI (2R/*S*,4R/*S*)

Pyrrolidyl polyamines (III–VI) are conformationally restricted, chiral analogues of linear spermine elaborated by the addition of aminopropyl chains to yield branched diastereomers. It is demonstrated that in concentrations as low as 0.01 mM, these compounds remarkably stabilize DNA duplexes and triplexes through strong electrostatic interactions. The synthesized compounds are potential dendrons with a chiral pyrrolidine core, and such molecules may have potential as DNA delivery and transfection agents.

The naturally occurring linear polyamines—putrescine. spermidine, and spermine—are biological polycations which are essential for cell growth and division.[1] Their functions range from stabilization/modulation of membrane and mitochondrial activities to facilitating DNA transfection by phage[2] and regulation of cell differentiation.[3] The high level of polyamines noticed in transformed cells has led to the design and synthesis of a number of their analogues as inhibitors of polyamine biosynthetic enzymes.[4] Polyamines have a wide-ranging therapeutic potential ranging from applications in therapy of neurological diseases[5] in development of new antidiarrheals in AIDS related cases[6] and as anticancer agents.[7] This has led to the synthesis and biological evaluation of a large number of their analogues toward an understanding of structure−activity relationships.[8] Most structural modifications[9,10] have involved varying the number and distance between nitrogens, terminal N-substitutions, and rigidification through secondary amines based on the cycloputrescein core.

Recently,[11] we presented a novel strategy for polyamine analogues based on conformational restriction by introducing a CH_2NH bridge between α and γ carbon atoms of the central tetramethylene fragment. This resulted in a five-membered pyrrolidine ring that is chiral at C2 and C4, and the derived analogues were better than linear spermine in selective

* To whom correspondence should be addressed. Telefax: 91 20 589 3153.

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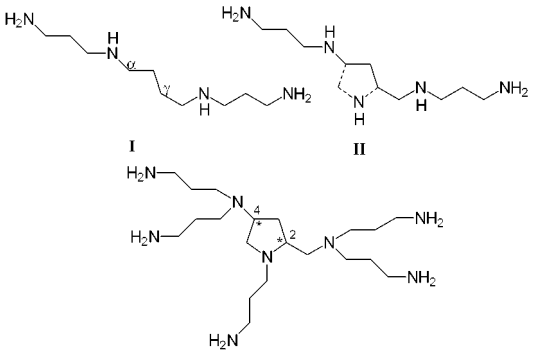
stabilization of DNA triplexes.[11] This principle was subsequently applied to construct rigid carbocyclic analogues by incorporating cyclopropyl, cyclobutyl, and cyclohexyl moieties into *N*,*N*-bisdiethyl spermines, which inhibited growth in several human tumor cell lines.[12] The emerging success of conformationally restricted polyamine analogues has prompted us to further elaborate pyrrolidyl polyamines into novel chiral branched structures, and this paper reports on their synthesis and selectivity in stabilization of DNA duplexes and triplexes.

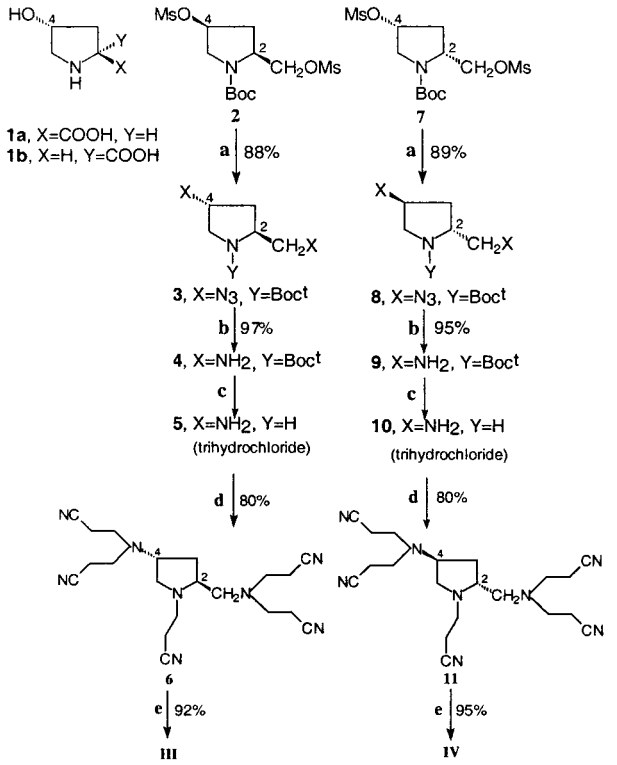
Synthesis of Branched Pyrrolidyl Polyamines. The target compounds (2*S*,4*R*)-*trans* **III**, (2*R*,4*S*)-*trans* **IV**, (2*S*,4*S*)-*cis* **V**, and (2*R*,4*R*)-*cis* **VI** are four diastereomers in which the



III, (2*S*,4*R*); **IV**, (2*R*,4*S*); **V**, (2*S*,4*S*); **VI**, (2*R*,4*R*)

five aminopropyl chains are distributed on the three nitrogens of 2-aminomethyl-4-aminopyrrolidene. The polyamine **III** was synthesized according to Scheme 1 starting from the commercially available *trans*-4-hydroxy-L-proline **1a**, which was converted to the (2*S*,4*S*)-*cis*-dimesylate **2** by following the earlier procedure.[11] After reaction with sodium azide in DMF, the dimesylate furnished (2*S*,4*R*)-*trans*-diazide **3**, accompanied by an inversion at C4. The diazide on catalytic hydrogenation using Pd−C in methanol gave the (2*S*,4*R*) *trans*-diamine **4**. The deprotection of the *N*-*tert*-BOC function in **4** was effected by treatment with 5 M aqueous HCl, to isolate the trihydrochloride salt **5** which was neutralized with NEt_3 and reacted with excess of acrylonitrile to give the *trans* isomer (2*S*,4*R*)-2-[*N*,*N*-bis(2-cyanoethyl)aminomethyl]-4-[*N*,*N*-bis(2-cyanoethyl)amino]-*N*1-(2-cyanoethyl)pyrrolidine **6**. This was transformed into the desired (2*S*,4*R*)-L-*trans* octamine (**III**) by catalytic hydrogenation over Raney Ni and NaOH.[13] The synthesis of the (2*R*,4*S*)- *trans* octamine **IV** was similarly accomplished starting from *cis*-4-hydroxy-D-proline[14] **1b** and following a route identical to that in Scheme 1. The (2*S*,4*R*)-*trans* dimesylate obtained from *trans*-4-hydroxy-L-proline by standard procedures[11] was used for the synthesis of (2*S*,4*S*)-L-*cis* cctamine **V**, while starting from the (2*R*,4*S*)-*trans* dimesylate, (2*R*,4*R*)-D-*cis*-polyamine **VI** was synthesized. The complete reduction of the pentacyano derivatives to the corresponding polyamines was followed

Scheme 1



Reagents: **a**. NaN_3 - DMF; **b**. H_2/Pd-C/MeOH; **c**, 5 M HCl; **d**, CH_2=CH-CN; **e**, H_2/Ra-Ni, NaOH.

12 *d*TTCTTTTTTCTTTTTTCT
13 *d*GCCAAGAAAAAAGAAAAAAGACGC
14 CGGTTCTTTTTTCTTTTTTCTGCG*d*

and confirmed by the complete disappearance of the C≡N band in the IR at 2247 cm^{-1} and finally by mass spectral data. All compounds were characterized by spectral analysis.[15] The oligonucleotides **12**−**14** needed for duplex (**13**:**14**) and triplex (**12***:**13**:**14**) binding studies with chiral, branched polyamines were synthesized on an automated DNA synthesizer by standard protocols[11] and purified by HPLC.

Studies on the Effect of Polyamine Analogues on DNA Duplex Stabilities. The comparative thermal stabilities of DNA duplex (**13**:**14**) with and without spermine **I** and the

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(15) **III** (2*S*,4*R*): $[\alpha]_D$ 25 °C, −54.5° (*c* = 0.389, H_2O); ^{1}H NMR δ (D_2O) 3.30−3.10 (m, 2H), 3.00−2.70 (m, 11H), 2.70−2.40 (m, 10H), 2.40−2.35 (m, 1H), 2.35−2.20 (m, 2H), 2.15−2.05 (m, 1H), 1.95−1.55 (m, 11H). **IV** (2*R*,4*S*): $[\alpha]_D$ 25 °C, +54.8° (*c* = 0.22, H_2O); ^{1}H NMR δ (D_2O) 3.60−3.50 (m, 1H), 3.30−3.20 (m, 4H), 3.15−2.85 (m, 12H), 2.75−2.45 (m, 8H), 2.45−2.20 (m, 2H), 2.15−1.95 (m, 3H), 1.95−1.60 (m, 8H). **V** (2*S*,4*S*): $[\alpha]_D$ 25 °C, −23.6° (*c* = 0.75, H_2O); ^{1}H NMR δ (D_2O) 3.70−3.45 (m, 1H), 3.10−3.00 (m, 2H), 3.00−2.90 (t, 10H), 2.88−2.70 (m, 3H), 2.70−2.45 (m, 10H), 2.35−2.25 (m, 1H), 2.10−1.95 (m, 1H), 1.90−1.70 (t, 10H).^{13}C NMR δ (D_2O): 62.45, 57.98, 55.90, 54.67, 52.07, 50.59, 47.59, 38.00, 37.94, 37.76, 33.94, 24.75, 23.67, 23.26. See Supporting Information for other details.
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branched pyrrolidyl polyamines **III**−**VI** were determined under identical conditions of buffer, pH, and salt by UV absorbance−temperature plots (Figure 1) and the melting

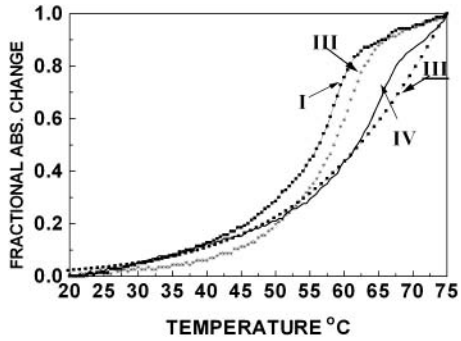

Figure 1. UV−melting profiles of DNA duplex **13**:**14** in the presence of 0.01 mM polyamines **I**, **III**, **IV**, and **III** (1 mM of **III**).

data summarized in Table 1. In the absence of any salt, spermine increased the duplex stability by 3.9° and the branched polyamines enhanced the duplex stability by different magnitudes: **III** 10.9°, **IV** 8.3°, **V** 11.7°, and **VI** 5.2°. As expected, in the absence and presence of spermine, addition of NaCl (10 mM/50 mM) increased the duplex T_m by 7.2° and 3.7°, respectively. Such an effect was less pronounced (<3°) for the DNA containing the polyamines **III**−**VI**, suggesting the cause of higher duplex stabilities observed with the branched polyamines to be mostly electrostatic in origin. In the absence of salt, among the four polyamine diastereomers, the (2*S*,4*S*) *cis*-isomer **V** appeared to be best in stabilizing the duplex, followed by (2*S*,4*R*)-*trans* **III**, (2*R*,4*S*)-*trans* **IV**, and (2*R*,4*R*)-*cis*-isomer **VI**. In the presence of 50 mM NaCl, the relative differences were diminished, with the (2*S*,4*S*)-*cis* isomer **V** still superior to others in duplex stabilization. The results suggest that the chiral polyamines stabilize DNA duplex by electrostatic interactions, with the degree of stabilization dependent on their stereochemistry. The duplex T_ms measured as a function of increasing polyamine concentrations (0.01−1 mM) indicated a ΔT_m of +14.5° for spermine, and for the branched pyrrolidyl polyamines **III**−**VI**, above 0.01 mM, the melting was accompanied by expected hyperchromicity effects, but no upper plateau was reached even beyond 80° in the melting curves. This is a consequence of incomplete duplex melting due to very strong DNA complexation with the pyrrolidyl polyamines **III**−**VI**.

Table 1. UV−T_m Data of DNA Duplex **13**:**14** in the Presence of Polyamines[a]

	no salt	salt[b]		polyamine[c]	
		10 mM	50 mM	0.1 mM	1 mM
12:**13**	53.8	55.1	61.0	53.8	53.8
I spermine	57.7	57.9	61.4	67.0	72.2
III (2*S*,4*R*)	64.7	63.2	63.2	melting incomplete	
IV (2*R*,4*S*)	62.1	60.0	62.7	melting incomplete	
V (2*S*,4*S*)	65.5	65.9	65.7	melting incomplete	
VI (2*R*,4*R*)	59.0	59.2	62.4	melting incomplete	

[a] All in 25 mM Tris buffer at pH 7.3. [b] Polyamines used as hydrochlorides in 0.01 mM concentration. [c] No added salt. All T_ms were determined from three separate experiments and are accurate to ±0.5 °C.

Studies on the Effect of Polyamine Analogues on DNA Triplex Stabilities. The melting data of DNA triplexes obtained from mixing equimolar amounts of oligonucleotides **12**−**14** in the presence and absence of 0.01 mM polyamines **III**−**VI** are shown in Table 2. At pH 7.3, triplex formation was not found with or without spermine but materialized upon addition of NaCl with a T_m of 40.7° in 50 mM NaCl. In comparison, the pyrrolidyl polyamines **III**−**VI** (0.01 mM) facilitated triplex formation even without the addition of any salts. The T_m of triplexes (Table 2) showed significant dependence on the stereochemistry of the polyamines, with a decrease in the order **III** (2*S*,4*R*) > **V** (2*S*,4*S*) > **VI** (2*R*,4*R*) > **IV** (2*R*,4*S*). In the presence of 50−100 mM NaCl, the T_m of the control significantly increased and the above differences almost vanished. This is due to an effective competition from salt at higher concentration that nulls the effect of polyamines present in much lower concentrations (0.01 mM).

Table 2. pH-Dependent UV−T_m Data for DNA Triplex **12***13:14 in the Presence of Polyamines[a]

	no salt	pH 7.3			pH 5.5	
		10 mM	50 mM	100 mM	10 mM	100 mM
12*13:14		33.8	40.1	44.9	35.7	44.2
I		35.2	40.7	44.5	37.0	44.2
III	42.7	38.7	41.0	44.2	40.7	44.5
IV	36.8	36.6	41.0	44.9	39.2	44.5
V	41.1	41.3	45.1	44.9		44.6
VI	35.1	34.8	42.2	44.9		43.0

[a] Salt refers to NaCl. Polyamines used as hydrochlorides in 0.01 mM concentrations, precipitation of DNA samples. All T_ms were determined from three separate experiments and are accurate to ±0.5 °C.

For DNA sequences with CG bases, the triplex stability is a function of pH with a maximum around pH 5.5 at which N3 of C is protonated.[16] At pH 5.5, the triplexes were formed in all cases, except with *cis* polyamines **V** and **VI**, where aggregation induced by strong binding[17] precipitated DNA out of solution. The polyamine (2*S*,4*R*)-*trans* **III** exhibited a 5° increase in T_m over that of control in comparison to 3.5° by (2*R*,4*S*)-*trans* polyamine **IV** and 1.3° by spermine. At higher NaCl concentration (100 mM), triplexes showed an increase in T_m but the relative differences among the polyamines disappeared, suggesting the weak effect of polyamines in the presence of competing salt.

The weakly fluorescent ethidium bromide upon intercalation into DNA exhibits a strong fluorescence (λ_{ex} 475 nm; λ_{em} 595 nm), and a competent ligand upon DNA binding can displace the intercalated ethidium bromide, leading to a decrease in the fluorescent intensity.[18] Such an experiment

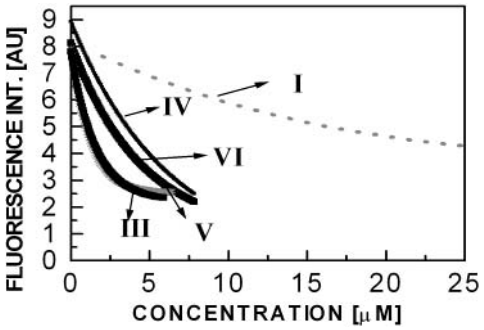

Figure 2. Ethidium bromide displacement assay monitored by fluorescence: ethidium bromide 1 μM, λ_{ex} 475 nm, λ_{em} 595 nm, DNA 20 μM, pH 7.3, 20 °C.

(Figure 2) performed with spermine **I** and the pyrrolidyl polyamines **III**−**VI** indicated that the polyamine concentrations required for a 50% displacement of ethidium bromide (C_{50}) to be in the order (2*S*,4*S*)-*cis* **V** (0.96 μM), (2*S*,4*R*)-*trans* **III** (1.1 μM), (2*R*,4*S*)-*trans* **IV** (2.55 μM), (2*R*,4*R*)-*cis* **VI** (2.9 μM), and spermine (16.4 μM). Within the experimental limitations, (2*S*,4*S*)-*cis* polyamine **V** was the strongest DNA binder followed by the polyamines **III**, **IV**, and **VI**. The order is in agreement with that found from the UV melting temperatures, and all polyamines bound duplex about 8−15 times stronger than spermine. Adding saturating concentrations of intercalating agent could not reverse the binding of polyamines.

Spermine, a linear polyamine, is well-known to stabilize DNA duplexes and triplexes, with the terminal ammonium cations interacting electrostatically with the anionic phosphate on DNA backbone.[19] The internal amino moieties may be involved in specific hydrogen bonding with the nucleobases in the major groove, with a cross groove binding for AT base pairs and down groove binding for GC base pairs.[18c] In triplexes, where the negative charge density is higher, stabilization by cationic polyamines assumes a major role. Substitution of terminal primary amino groups of spermine by guanidino functions led to selective enhancement in DNA triplex stability.[20] Restricting the enormous conformational freedom in linear polyamines by introduction of a pyrrolidine ring improved the stability of DNA triplexes, due to a preorganization effect.[11] The present approach constitutes a combination of the above in which additional aminopropyl side chains are introduced as branches on the pyrrolidyl nucleus to act as sources of spatially distributed positive charges. The UV melting data indicated that the branched pyrrolidyl polyamines **III**−**VI**, even at a low concentration of 0.01 mM, imparted considerable duplex and triplex thermal stabilities. These effects arise predominantly via electrostatic interactions of their polycationic charges with anionic phosphates of DNA. Such are the stabilizing effects of the branched polyamines that above 0.1 mM concentration, the duplexes do not completely melt even beyond 80 °C. DNA triplex formation occurs at low polyamine concentrations (0.01 mM) and at physiological pH, and there are no salt conditions under which triplex formation occurred with or without spermine.

Preliminary molecular modeling studies indicated that the stereochemistry of the inner core of pyrrolidine nucleus determines the topological dispositions of terminal amino functions and hence the positive charges.[21] This implies a dependence of the stability of complexes on the relative stereochemistry of the side chains, which determine the spatial arrangement of charges for DNA interaction. The topology of polyamine−DNA interactions seems to differ in duplexes and triplexes since the *cis* analogue **V** is most efficient for duplex stabilization while, the (2*S*,4*R*)-*trans* isomer **III** is better in triplex stabilization.

In summary, we have the synthesized conformationally restricted chiral analogues of spermine on the basis of the pyrrolidine nucleus as DNA stabilizing agents. The chiral, branched analogues exhibit DNA duplex and triplex stabilizing properties, superior to the previous analogues.[11] Extension of the synthesis to the next generation by repeating acrylonitrile condensation and reduction of the nitrile to introduce more alkylamino branches and build chiral dendrimeric polyamines is under progress. Such novel dendrimers in which the chiral core determines the arrangement of the growing branches may have potential tailored applications, for example, in material chemistry and chiral separations.[22] Polycations that bind DNA efficiently may also have utility as DNA transfection agents.[23]

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Supporting Information Available: ^{1}H and ^{13}C NMR and FABMS of **III**, **IV**, **V**, and pentacyano derivatives, molecular modeling of **III**−**VI**, UV−T_m curves for **V**and **VI**, and CD of polyamine−DNA complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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