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Synthesis and Stereoselective DNA Binding Abilities of New Optically Active Open-Chain Polyamines

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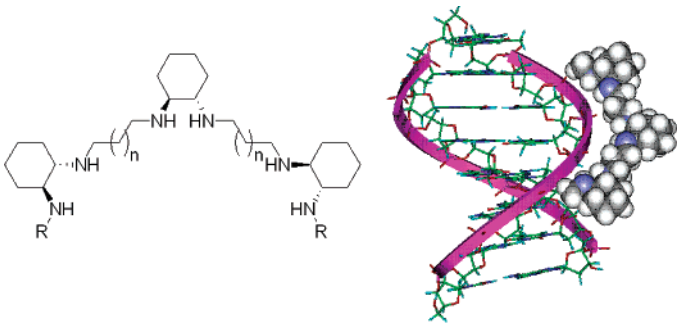

The efficient synthesis of new open-chain enantiopure polyamines bearing (*R*,*R*)- and/or (*S*,*S*)-*trans*-cyclohexane-1,2-diamine moieties is described. The key step for the synthetic procedure is the selective monoalkylation of the cyclohexanebis(sulfonamide) core, which allows the subsequent functionalization of this moiety. Compounds bearing different combinations of absolute configurations, length of the aliphatic spacers and terminal groups have been prepared. As a demonstration of the potential utility of the obtained compounds, the preliminary DNA binding abilities of some of them have been studied by UV-measurements of melting temperatures (T_m). The effects of the absolute configuration of the corresponding chiral centers and the length of the spacer separating the cyclohexanediamine moieties on the strength of the interaction with DNA are also discussed.

Introduction

In general, interest in polyamine compounds is often based on their polycationic nature at physiological pH. In that environment, they play an essential role for many biological processes,[1] ranging from stabilization of membrane and mitochondria functions to facilitation of DNA transfection by phage.[2] Thus, these compounds have a significant therapeutic potential for the treatment of neurological diseases,[3] in the development of new antidiarrheals in AIDS-related cases,[4] and as anti-cancer agents.[5] Most of the biological functions of polyamines relate to their abilities to interact with polyanions such as nucleic acids (DNA and RNA).[6] Spermine, for example, is known to stabilize DNA duplex and triplex[7] and to promote conformational changes of duplex DNA.[8] There is also a proposed structure−function relationship because different molecular architectures display different spatial dispositions of the cationic ammonium

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groups able to interact with the phosphate chain of the oligonucleotides. For instance, both the separation between nitrogen atoms and their grade of substitution dramatically affect the basicity of the compounds, determining their positive charge density under physiological conditions. Accordingly, most of the structural changes studied to date[9] rely on spatial separation of nitrogen atoms,[10] on alkylation of terminal amino groups, or on the construction of dendritic structures.[11] Conformational constrained analogues have been also prepared and tested for biological activities.[12] Especially, derivatives bearing optically active pyrrolidyl moieties stabilize DNA duplexes and triplexes, to an extent that critically depends on the absolute configuration of the chiral centers.[13] In spite of all these studies, a definitive picture of the requirements for the polyamine−DNA supramolecular structures has not yet emerged. In particular, the question of how chirality on the polycationic polyamine can affect the stability of its corresponding nucleic acid complexes has not been investigated thoroughly, probably due to the difficulties experienced in the synthesis of optically active polyamines.[14]

On the other hand, we had previously reported preliminary studies toward the synthesis and DNA-binding abilities of an optically active polyamine, having three cyclohexane rings in its structure.[15] Following on from this work, here we report an extended application of the synthetic studies toward the selective and efficient preparation of a larger family of open chain chiral polyamines. Besides, we have selected some of the newly prepared compounds for preliminary assays in polyamine-oligonucleotide binding experiments.

Results and Discussion

Molecular Design. For the efficient synthesis of the intended polyamine compounds shown in Figure 1, bearing nonsymmetrically substituted cyclohexanediamine moieties, a selective monofunctionalization of this synthon was necessary. We have recently reported the efficient monoalkylation of chiral cyclohexanebis(sulfonamide) in very high yields and selectivities.[15] A thorough study showed that the polarity of the solvent for the reaction is critical for the formation of the monoalkylated derivative, due to the presence of folded conformations in polar solvents, such as acetonitrile.[16] Here, we have used this monoalkylation pathway for the preparation of a new family of chiral polyamines.

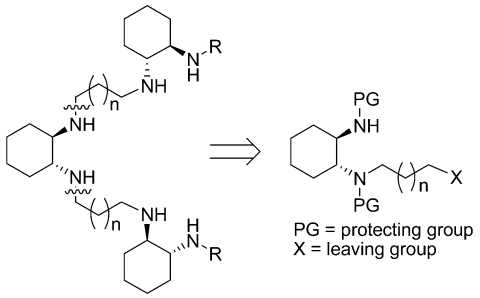

FIGURE 1. Retrosynthetic analysis of the target chiral polyamines.

The structural variables for the generation of our small library of compounds have been the absolute configurations of the chiral centers of the cyclohexane moiety, the length of the aliphatic spacer (*n* in Figure 1), and to a lesser extent, the terminal alkylating group (R). Regarding the spacer (*n*), preliminary molecular modeling suggested that a propylene spacer ($n = 1$) would separate the consecutive cyclohexanediamine moieties by about 5.0 Å, which is close to the distance between phosphate linkages of the same strand in a DNA molecule (Figure 2). On the other hand, a hexamethylene spacer ($n = 4$) would increase the flexibility of the compound, separating the cylohexanediamine cores up to ca. 9.0−10.0 Å, allowing the binding of the protonated nitrogen atoms from the same polyamine to phosphate anions of complementary strands of the DNA-double helix, within the minor groove. Consequently, these two aliphatic spacers were selected. Also supported by molecular models and previous experimental data,[17] we anticipated that the absolute configuration of the diamine would induce a preferred conformation of the polyamine in a helix of different handness. By preparing both enantiomers of the polyamines, we could also test if the compounds are able to exhibit any steroselectivity in DNA binding, as it is a chiral entity. Apart from the chiral centers of the sugar backbone; the nucleic acid chirality is also expressed through the helicity of the DNA hybridized molecule.[18] Thus, it is well-known that most of the naturally occurring forms of DNA are right-handed (B-DNA) while other secondary structures are left-handed (Z-DNA).[19] The biological role of this change of helicity is still unknown and the

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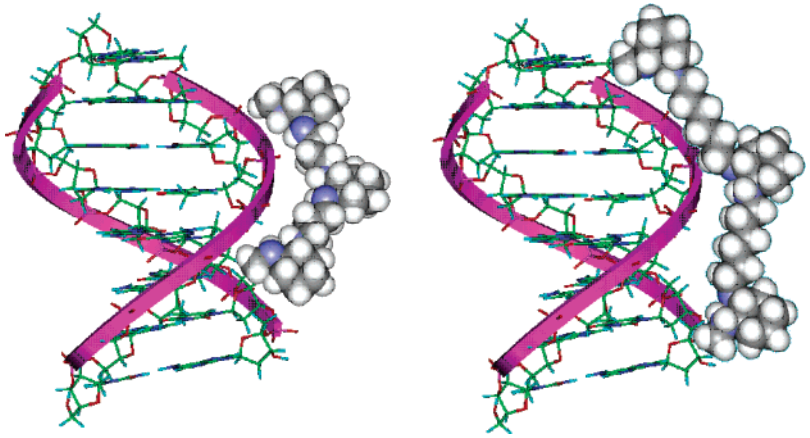
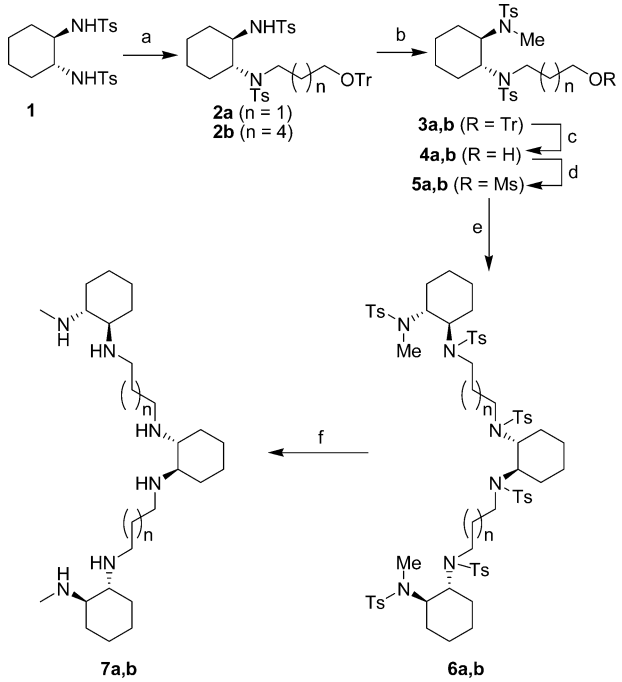


FIGURE 2. Schematic representation for the proposed polyamine−DNA binding modes, where triprotonated polyamines in the minor groove bind to phosphate groups from one strand (left) or both strands (right).

SCHEME 1. Synthesis of Polyamines 7a,b[a]



[a] Reactants and conditions: (a) see refs 15 and 16; (b) MeI, Cs_2CO_3, $PhCH_3$ (quantitative); (c) TFA, MeOH, rt (88−92%); (d) MsCl, NEt_3, DCM (quantitative); (e) 0.5 equiv. of **1**, Cs_2CO_3, $PhCH_3$ (65−70%); (f) aq HBr, PhOH (60−66%).

development of drugs able to stabilize any of the forms or to selectively bind to only one of them is an exciting field of research.[20]

Synthesis of Optically Active Polyamines. The proposed compounds were prepared following the synthetic pathway outlined in Scheme 1. The unsymmetrical derivatives **2a**,**b** were obtained by monoalkylation of **1** as previously described.[15,16] Methylation of **2a**,**b** was quantitatively performed with MeI in the presence of an excess of cesium carbonate as a base. Conventional deprotection of trityl group in **3a**,**b** followed by mesylation of the obtained alcohol yielded the corresponding electrophiles **5a**,**b** in excellent overall yields (88−90%). Coupling of 2 equiv of these cyclohexane-derived electrophiles with 1 equiv of bis(sulfonamide) **1** led to the pertosylated polyamines (**6a**,**b**) in very good (65−70%) isolated yields after chromatographic purification. Finally, acidic hydrolysis of all of the sulfonamide groups gave the desired polyamines (**7a**,**b**), which were transformed into the corresponding hydrochloric salt for an easier manipulation and storage. All of these compounds were characterized by their full spectroscopic and analytical data, showing the expected C_2 symmetries in their corresponding ^1H and ^{13}C NMR spectra and confirming that no epimerization of any chiral center occurred during our synthetic procedure. Besides, no significant differences in yields or reaction times were observed for $n = 1$ and 4. The obtained overall yields for the final polyamines **7a**,**b** starting from bis(sulfonamide) **1** was ca. 28%, in a six-step synthetic procedure.

To explore the stereochemical space (only all-*R* isomer represented in Scheme 1), we have used either (*R*,*R*)-**1** or (*S*,*S*)-**1** as starting materials, finally leading to both enantiomers of homochiral **7a**,**b** compounds for every case [namely (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, (*S*,*S*,*S*,*S*,*S*,*S*)-**7a**, (*R*,*R*,*R*,*R*,*R*,*R*)-**7b**, and (*S*,*S*,*S*,*S*,*S*,*S*)-**7b**] in comparable yields. Conversely, coupling of 2 equiv of (*S*,*S*)-**5a** with one of (*R*,*R*)-**1** led, after final Ts deprotection, to (*S*,*S*-,*R*,*R*,*S*,*S*)-**7a**, in very similar yield to its diastereomeric counterpart. Thus, it is noteworthy that our modular methodology allows the introduction of different stereochemistries (and even combinations of them) within the polyamino backbone.

In order to expand the synthetic application of our methodology, we have finally tried to introduce functionality in the terminal alkylating groups (R in Figure 1). With this aim in mind, we prepared the polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-**13** featuring terminal primary amino groups (Scheme 2). Alkylation of **2b** with *N*-(3-bromopropyl)phthalimide using cesium carbonate as a base and in dry toluene led to compound **8**, with an unsymmetrically substituted cyclohexane-1,2-diamine moiety. Conventional transformation of **8** into the electrophile **10** was carried out in very good overall isolated yield (90%). Although the subsequent coupling between **10** and **1** (2:1 molar ratio) occurred in moderate yield, the starting materials could be easily recovered after flash chromatography. An explanation for this low reactivity could be the intermediate polarity of phthalimide

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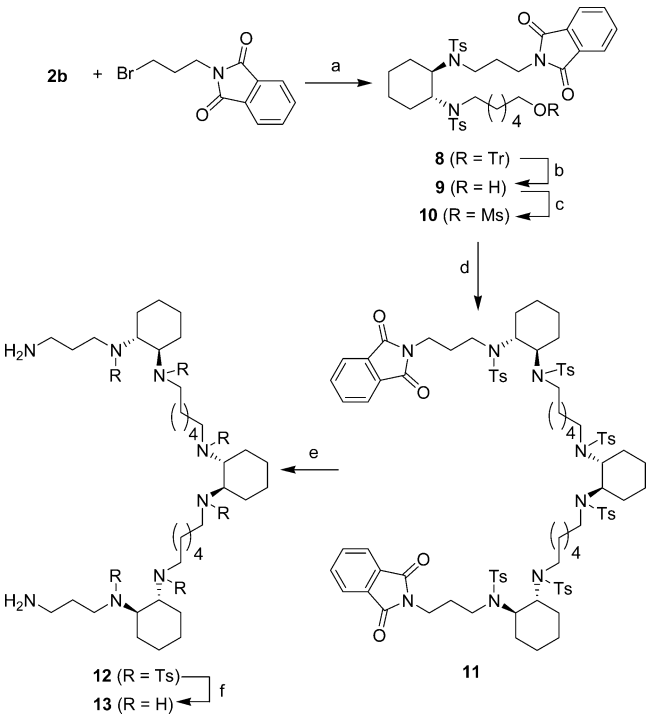
SCHEME 2. Syntheses of Polyamine 13[a]



[a] Reactants and conditions: (a) *N*-(3-bromopropyl)phthalimide, Cs_2CO_3, cat. TBABr, $PhCH_3$ (75%); (b) TFA, MeOH, rt (90%); (c) MsCl, NEt_3, DCM (quantitative); (d) 0.5 equiv of **1**, Cs_2CO_3, $PhCH_3$ (30%); (e) hydrazine, MeOH (75%); (f) aq HBr, PhOH (77%).

group which could facilitate folded conformers both in polar and nonpolar environments, as previously observed for the same residue.[16] The protected polyamine **11** is a very valuable compound as it contains orthogonally protected secondary and primary nitrogen atoms. Thus, hydrazinolysis of **11** selectively deprotected the terminal amino groups, leading to the diaminopolysulfonamide **12**. This intermediate could be easily used for further transformations, such as elongation, polymerization of dendrimer synthesis. Instead, we finally deprotected the secondary amino groups to yield the desired polyamino compound **13**. Once again, the spectroscopic data confirms the lack of epimerization during the synthetic route.

DNA Binding Studies. In order to study potential biological and therapeutic applications of this family of compounds, we have investigated their DNA binding abilities. As the polyamine−oligonucleotide interaction increases the thermal stability of the double helix, we have performed preliminary UV melting experiments of DNA strands in the absence and in the presence of some of the synthesized polyamines. A melting temperature shift in the presence of the different polyamines is indicative of the strength of their interaction with DNA. For a clearer interpretation, we have selected the polyamines having different length of the aliphatic spacers ($n = 1, 4$) and different absolute configurations of the chiral centers, but all of them having a terminal methyl group and being homochiral. Thus, we could directly compare the effects of two variables (spacer length and chirality) in the DNA binding abilities. Besides, two different oligonucleotides were checked: one AT-rich and another CG-rich sequences, which are expected to favor B and Z-DNA forms, respectively.[18,19] As, in some cases, smooth differences in the heating and the annealing melting processes were observed, both temperatures are given for all of the examples in Table 1.

Almost all of the tested polyamines stabilized the DNA double helix, as shown by the increasing melting temperature effects ($\Delta T_m > 0$, Table 1). This stabilization most likely arises from the electrostatic interaction between phosphate backbone of the DNA and partially protonated polyamine. While all of the studied polyamines are expected to be triprotonated at neutral pH, the observed differences in the DNA binding must come from other structural factors, different to the charge density.[21] In general, polyamines **7a**,**b** bound more strongly to AT-rich than to CG-rich sequences, as the increase in the melting temperatures is higher ($\Delta T_m \approx 10-11$ °C for AT-rich sequence but $\Delta T_m \approx 0-6$ °C for CG-rich sequence). Regarding the chirality of the polyamine, interesting differences were found, which are highlighted in Figure 3. For the AT-rich sequence, when comparing entries 2 and 3, a subtle difference was obtained for the binding with both enantiomers of **7a**. Although the difference in melting of diastereomeric pairs is small ($\Delta\Delta T_m \approx 1.5$ °C), it is above the experimental error and suggests that the *all-R* enantiomer of **7a** binds slightly more strongly to the oligonucleotide than the *all-S* isomer. Besides, when the aliphatic spacer is longer (entries 4 and 5) this stereoselectivity is suppressed, probably due to larger flexibility of **7b** or to a different mode of binding, as proposed in Figure 2. Interestingly, for the CG-rich sequence, the observed stereoselectivity is much higher and reversed. Thus, in this case, when comparing results with both enantiomers of the short polyamine **7a**, we found that the *all-R* isomer has little effect on the CG-rich DNA melting, while the *all-S* isomer produced a $\Delta\Delta T_m \approx 5$ °C, which is remarkable for an energetic difference of diastereomeric polyamine−DNA noncovalent complexes.[22] These results support that, in this case, (*S*,*S*,*S*,*S*,*S*,*S*)-**7a** binds much more strongly to a CG-rich sequence than (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**. Once again, this difference is reduced (almost abolished) for the longer derivatives **7b**. Thus, the larger aliphatic spacer does not seem to improve the binding but rather reduced the stereoselectivity observed for the shorter derivatives.

In order to check the effect of the electrostatic interactions between the phosphate backbone and the polyamonium moieties, measurements at high salt concentration (5 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, pH = 7.11) were performed in the case of the system with the best enantiodiscrimination, the CG-rich sequence and both enantiomers of polyamine **7a**. In the absence of polyamine, but in high salt concentration, a stabilization of the double helix was observed [$T_m = 72.0$ °C (heating) and $T_m = 71.8$ °C (annealing)] as expected. In the presence of 40 μM (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, there is no appreciable effect [T_m=

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TABLE 1. UV (260 nm) Melting Temperatures ($T_m \pm 0.5$ °C) of DNA (2 μM) Sequences in the Absence and in the Presence of 40 μM of Different Polyamines

			5′-GCC AAG AAA GAA AAA AGA CGC 3′-CGG TTC TTT CTT TTT TCT GCG			5′-CCT TCG CCT CGC ACA TAG CC 3′-GGA AGC GGA GCG TGT ATC GG		
entry	polyamine		T_m (°C)	ΔT_m[a] (°C)	$\Delta\Delta T_m$[b] (°C)	T_m (°C)	ΔT_m[a] (°C)	$\Delta\Delta T_m$[b] (°C)
1		heating	44.8	0		54.2	0	
		annealing	44.5	0		53.0	0	
2	(*R*,*R*,*R*,*R*,*R*,*R*)-**7a**	heating	56.6	11.8	1.5	54.3	0.1	
		annealing	56.0	11.5	1.5	54.2	1.2	
3	(*S*,*S*,*S*,*S*,*S*,*S*)-**7a**	heating	55.1	10.3		59.3	5.1	5.0
		annealing	54.5	10.0		58.8	5.8	4.6
4	(*R*,*R*,*R*,*R*,*R*,*R*)-**7b**	heating	56.5	11.7	0.5	59.3	5.1	
		annealing	55.9	11.4	0.4	58.0	5.0	
5	(*S*,*S*,*S*,*S*,*S*,*S*)-**7b**	heating	56.0	11.2		60.4	6.2	1.1
		annealing	55.5	11.0		58.2	5.2	0.2

[a] Observed increase of T_m in the presence of the corresponding polyamine. [b] Difference of ΔT_m produced by enantiomers of the each corresponding polyamine (given at the isomer inducing the highest T_m shift).

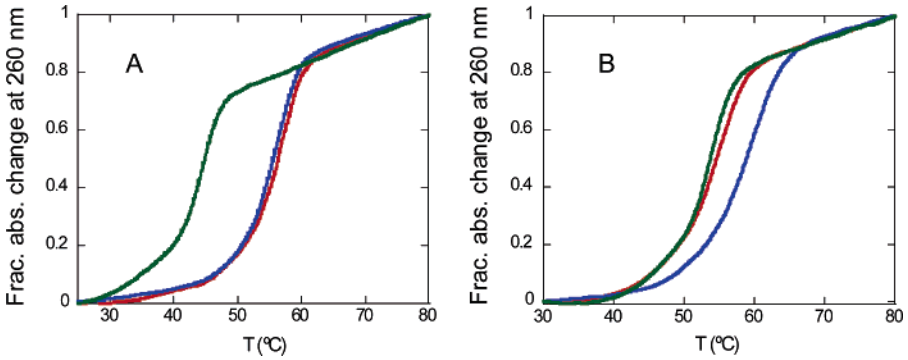

FIGURE 3. Selected UV-melting traces for (A) AT-rich and (B) CG-rich model sequences in the absence (green) and in the presence of polyamines (*R*,*R*,*R*,*R*,*R*,*R*)-**7a** (red) and (*S*,*S*,*S*,*S*,*S*,*S*)-**7a** (blue).

72.1 °C (heating) and $T_m = 71.8$ °C (annealing)]. In addition, the melting experiment with its enantiomer gave very similar results [$T_m = 73.1$ °C (heating) and $T_m = 71.6$ °C (annealing)]. These results support the importance of the electrostatic interaction in the polyamine binding to the DNA strands. On the other hand, the stereoselectivity previously observed was almost abolished after elimination of this charge−charge interaction, by competition with an excess of both sodium and magnesium cations.

Although we are aware that the data here presented does not allow extracting definitive conclusions, the current work reports a remarkable stereoselective DNA molecular recognition event produced by a chiral polyamine compound. Our preliminary data seems to indicate that the DNA with an AT-rich sequence binds preferentially to polyamines containing (*R*,*R*)-cyclohexane-1,2-diamine moieties while the CG-rich sequence prefers to bind to the (*S*,*S*) enantiomer. However, more synthetic and analytical work has to be done to optimize the strength and selectivity of binding to DNA and to verify the proposed model for the interaction.

Conclusions

We describe in this paper the efficient synthesis of a new family of open-chain optically active polyamines, bearing three fragments of *trans*-cyclohexane-1,2-diamine in their stuctures. Different combinations of the absolute configurations of the chiral centers, aliphatic spacers between cyclohexanediamine moieties, and terminal groups are implemented within the polyamino backbone. Preliminary DNA binding studies showed an interesting stereoselective interaction between some of the new polyamino derivatives and model olygonucleotides. The easy synthesis and high modularity of our synthetic approach open up possibilities for expanding the methodology to longer and more elaborated systems which could improve the selectivity and stability of the interaction with DNA strands and, thus, present interesting biological functions. The potential of these new molecules ranges from chiral anion recognition[21] to separation and transport.[23] Biotechnological applications in nonviral gene transfection technology[24] as well as in medicinal chemistry for new anti-cancer drugs[25] are also foreshadowed for this family of compounds. Further studies regarding these topics are underway in our laboratories.

Experimental Part

Synthesis of Different Stereoisomers of Polyamines 7a,b. Tosylated Polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-6a. To a solution of 0.5 mmol of (*R*,*R*)-**1** in 10 mL of dry toluene was added 10 mmol (3.1 g) of Cs_2CO_3, and the suspension thus obtained was stirred at 75 °C for

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0.5 h. After that time, 1 mmol of (*R*,*R*)-**5a** was added, and the mixture was stirred at the same temperature for 5 days. Then, the reaction was extracted with CH_2Cl_2 and 1 N HCl, and the organic layers were dried and evaporated. The title compound was purified by flash chromatography with 5.8% AcOEt in CH_2Cl_2 as eluent: yield = 70%; white foamy solid; mp 138−142 °C; $[\alpha]^{20}_D = +7.1$ ($c = 0.59$, $CHCl_3$); R_f (5.8% AcOEt/CH_2Cl_2) 0.1; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 0.71−1.81 (bm, 23H), 1.96−2.23 (m, 5H), 2.43 (m, 18H), 2.54 (s, 6H), 2.87−3.40 (bm, 8H), 3.60−4.24 (m, 6H), 6.97−7.55 (m, 12H), 7.55−8.19 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 20.9 (CH_3), 24.0 (CH_2), 24.6 (CH_2), 24.9 (CH_2), 27.9 (CH_2), 27.9 (CH_3), 28.2 (CH_2), 30.2 (CH_2), 31.0 (CH_2), 41.3-(CH_2), 56.0 (CH_2), 57.5 (CH), 58.0 (CH), 126.7 (CH), 126.8 (CH), 126.9 (CH), 129.0 (CH), 129.0 (C), 136.2 (C), 137.9 (C), 138.1 (C), 142.3 (C), 142.5 (C); ESI-MS (*m*/*z*) 1397.2 [(M + Na)$^+$, 100]. Anal. Calcd for $C_{68}H_{90}N_6O_{12}S_6$ C, 59.36; H, 6.59; N, 6.11. Found: C, 59.03; H, 6.75; N, 6.01

Tosylated Polyamine (*S*,*S*,*S*,*S*,*S*,*S*)-6a. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*)-**6a**, but coupling (*S*,*S*)-**1** with (*S*,*S*)-**5a**, showing the expected spectroscopic and analytical data: yield 70%; $[\alpha]^{20}_D = -7.2$ ($c = 0.48$, $CHCl_3$).

Tosylated Polyamine (*R*,*R*,*R*,*R*,*R*)-6b. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*)-**6a**, but coupling (*R*,*R*)-**1** with (*R*,*R*)-**5b**: yield 68%; white solid; mp = 111−115 °C; $[\alpha]^{20}_D = -9.4$ ($c = 0.65$, $CHCl_3$); R_f (5.8% AcOEt/CH_2Cl_2) 0.1; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 0.98−1.90 (m, 44H), 2.39 (s, 18H), 2.59 (s, 6H), 2.85−3.30 (m, 8H), 3.65−4.00 (m, 6H), 7.25 (m, 12H,), 7.59−7.84 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 21.2 (CH_3), 24.6 (CH_2), 25.2 (CH_2), 26.6 (CH_2), 26.8 (CH_2), 28.6 (CH_3), 29.6 (CH_2), 30.2 (CH_2), 32.0 (CH_2), 32.3 (CH_2), 44.0 (CH), 44.2 (CH), 56.8 (CH_2), 57.4 (CH), 58.3 (CH_2), 127.0 (CH), 127.1 (CH), 129.3 (CH), 129.4 (CH), 136.3 (C),138.6 (C), 138.8 (C), 142.6 (C),143.0 (C); ESI-MS (*m*/*z*) 1481.2 [(M + Na)$^+$, 80]. Anal. Calcd for $C_{74}H_{102}N_6O_{12}S_6$: C, 60.88; H, 7.04; N, 5.76. Found: C, 60.56; H, 7.34; N, 5.65.

Tosylated Polyamine (*S*,*S*,*S*,*S*,*S*)-6b. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*)-**6a**, but coupling (*S*,*S*)-**1** with (*S*,*S*)-**5b**, showing the expected spectroscopic and analytical data: yield 65%; $[\alpha]^{20}_D = +9.8$ ($c = 0.52$, $CHCl_3$).

Tosylated Polyamine (*S*,*S*,*R*,*R*,*S*,*S*)-6a. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*)-**6a**, but coupling (*R*,*R*)-**1** with (*S*,*S*)-**5a**, showing the expected spectroscopic and analytical data: yield 68%; $[\alpha]^{20}_D = +1.4$ ($c = 0.4$, $CHCl_3$); R_f (5.8% AcOEt/CH_2Cl_2) 0.1; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 1.00−1.35 (m, 10H), 1.35−1.81 (m, 12H), 1.96−2.26 (m, 6H), 2.42 (s, 18H), 2.56 (s, 6H), 3.22−3.64 (m, 7H), 3.69−4.00 (m, 7H), 7.31 (dd, $J = 8.1$ Hz, $J = 2.2$ Hz,12H), 7.68 (d, $J = 8.3$ Hz ,6H), 7.75 (d, $J = 8.3$ Hz ,6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 21.4 (CH_3), 24.7 (CH_2), 25.2 (CH_2), 28.4 (CH_2), 28.6 (CH_3), 31.1 (CH_2), 33.7 (CH_2), 40.8 (CH_2), 57.4 (CH), 58.3 (CH_2), 60.2 (CH_2), 65.8 (CH_2), 77.2 (CH), 127.3 (CH), 127.3 (CH), 129.6 (CH), 129.7 (CH), 136.4 (C),138.0 (C), 143.2 (C), 143.3 (C); ESI-MS (*m*/*z*) 1397.2 [(M + Na)$^+$, 20]. Anal. Calcd for $C_{74}H_{102}N_6O_{12}S_6$: C, 60.88; H, 7.04; N, 5.76. Found: C, 60.75; H, 7.40; N, 5.53.

Polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-7a. Compound (*R*,*R*,*R*,*R*,*R*,*R*)-**6a** (0.1 mmol) was dissolved in HBr (48% in water) and phenol (1.6 mmol). The mixture was heated to reflux for 5 days, and then the cold mixture was extracted with CH_2Cl_2. The aqueous layer was basified with NaOH and thoroughly washed with CH_2Cl_2. The combined organic fractions were evaporated to dryness giving rise to the title compound. For longer storage and better characterization, it was more convenient to transform the polyamine into the corresponding HCl salt, which was prepared by the treatment of (*R*,*R*,*R*,*R*,*R*,*R*)-**7a** in methanolic HCl solution: yield = 60%; white hygroscopic solid; decompose without melt; $[\alpha]^{25}_{Hg} = -48.4$ ($c = 0.50$, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ (ppm) 1.18−1.81 (bm, 18H), 1.98−2.67 (m, 10H), 2.69 (s, 6H), 2.99−3.22 (m, 4H), 3.22−3.64 (bm, 10H); ^{13}C NMR (D_2O, 75 MHz) δ (ppm) 21.2 (CH_2), 21.3 (CH_2), 21.4 (CH_2), 22.9 (CH_2), 24.8 (CH_2), 25.1 (CH_2), 25.3 (CH_2), 30.6 (CH_3), 42.5 (CH_2), 42.6 (CH_2), 57.0 (CH), 57.5 (CH), 57.6 (CH); HRMS/EI calcd for $C_{26}H_{54}N_6$ 450.4410, found 450.4414.

Polyamine (*S*,*S*,*S*,*S*,*S*,*S*)-7a. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, but starting from (*S*,*S*,*S*,*S*,*S*,*S*)-**6a**, showing the expected spectroscopic and analytical data: yield 61%; $[\alpha]^{25}_{Hg} = +48.5$ ($c = 0.32$, MeOH).

Polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-7b. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, but starting from (*R*,*R*,*R*,*R*,*R*,*R*)-**6b**: yield 66%; $[\alpha]^{20}_{Hg} = -36.7$ ($c = 0.49$, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ (ppm) 1.10−1.76 (m, 34H), 1.95−2.23 (m, 6H), 2.66 (s, 6H), 2.80−3.00 (m, 4H), 3.01−3.24 (m, 4H), 3.24−3.49 (m, 4H); ^{13}C NMR (D_2O, 75 MHz) δ (ppm) 20.8 (CH_2), 20.9 (CH_2), 21.1 (CH_2), 24.6 (CH_2), 24.6 (CH_2), 24.8 (CH_2), 24.8 (CH_2), 25.0 (CH_2), 30.0 (CH_3), 43.0 (CH_2), 45.0 (CH_2), 57.1 (CH), 56.7 (CH), 57.2 (CH); ESI-MS (*m*/*z*) 767.5 [(M + Na)$^+$, 50]. Anal. Calcd for $C_{32}H_{82}N_6Cl_6$: C, 50.32; H, 10.82; N, 11.00. Found: C, 50.15; H, 10.98; N, 10.80.

Polyamine (*S*,*S*,*S*,*S*,*S*,*S*)-7b. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, but starting from (*S*,*S*,*S*,*S*,*S*,*S*)-**6b**, showing the expected spectroscopic and analytical data: yield 60%; $[\alpha]^{20}_{Hg} = +36.5$ ($c = 0.89$, MeOH).

Polyamine (*S*,*S*,*R*,*R*,*S*,*S*)-7a. The synthesis of the title compound was performed as for (*R*,*R*,*R*,*R*,*R*,*R*)-**7a**, but starting from (*S*,*S*-,*R*,*R*,*S*,*S*)-**6a**: yield 68%; decompose without melt; $[\alpha]^{20}_{Hg} = +8.8$ ($c = 0.25$, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ (ppm) 0.99−2.29 (m, 28H), 2.70 (s, 6H), 2.90−3.49 (m, 14H); ^{13}C NMR (D_2O, 75 MHz) δ (ppm) 21.3 (CH_2), 21.4 (CH_2), 21.6 (CH_2), 23.3 (CH_2), 24.8 (CH_2), 25.4 (CH_2), 25.6 (CH_2), 30.4 (CH_3), 42.5 (CH_2), 54.1 (CH_2), 57.0 (CH), 57.7 (CH), 57.8 (CH); HRMS-EI (*m*/*z*) calcd for $C_{26}H_{54}N_6$ 450.4410, found 450.4414.

Synthesis of Polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-13. Pertosylated Octaamine (*R*,*R*,*R*,*R*,*R*,*R*)-11. In a flask under nitrogen atmosphere, 0.3 mmol of (*R*,*R*)-**1**, a large excess of Cs_2CO_3 (1.1 g, 3.3 mmol), and catalytic TBABr (0.18 mg, 0.06 mmol) were suspended in 10 mL of dry toluene. The reaction mixture was stirred and heated to reflux for 0.5 h. After that, 0.6 mmol of (*R*,*R*)-**10** was added. After 5 days at reflux, the solvent was evaporated, and the crude product extracted with 1 N HCl and CH_2Cl_2. The combined organic layers were dried and concentrated in vacuum. The title compound was finally isolated after flash chromatographic purification: yield 30%; white foamy solid; mp 117−119 °C; $[\alpha]^{20}_D = -32.7$ ($c = 0.40$, $CHCl_3$); R_f (CH_2Cl_2/AcOEt 10:1) 0.28; IR (cm^{-1}) (KBr) 1710; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 1.06−2.20 (m, 44H), 2.35 (m, 18H), 2.90−3.85 (m, 22H), 7.20 (m, 12H), 7.66−7.80 (m, 16H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 21.3 (CH_3), 25.2 (CH_2), 26.8 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 31.7 (CH_2), 32.0 (CH_2), 42.0 (CH_2), 44.1 (CH_2), 44.3 (CH_2), 58.4 (CH), 58.6 (CH), 60.2 (CH_2), 123.0 (CH), 127.0 (CH), 127.2 (CH), 127.3 (CH) ,129.4 (CH), 132.0 (C), 133.7 (C), 137.9 (C), 138.7 (C), 142.8 (C), 143.0 (C), 167.1 (C); ESI-MS (*m*/*z*) 1825.5 [(M + Na)$^+$, 80]. Anal. Calcd for $C_{94}H_{116}N_8O_{16}S_6$: C, 62.50; H, 6.47; N, 6.20. Found: C, 62.30; H, 6.80; N, 6.11.

Hexatosylated Octaamine (*R*,*R*,*R*,*R*,*R*,*R*)-12. In a flask under nitrogen atmosphere, 0.051 mmol of (*R*,*R*,*R*,*R*,*R*,*R*)-**11** was dissolved in 0.3 mL of toluene, and an excess of hydrazine (0.04 mL, 0.51 mmol) was added. The mixture was heated at 120 °C for 1 day. Then, the crude reaction was filtered, and the final compound was obtained after evaporation of the solvent to dryness. Also, for suitable storage, the HCl salt was prepared by addition of concentrated HCl to a methanolic solution: yield 75%, pale red hygroscopic solid, decompose without melt; $[\alpha]^{20}_D = -15.2$ ($c = 0.37$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 1.14−2.10 (m, 44H), 2.39−2.69 (m, 22H), 2.69−4.00 (m, 18H), 7.10−7.42 (m, 12H), 7.61−7.80 (m, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) (all of the aliphatic signals seemed to be broad) 21.3 (CH_3), 25.2 (CH_2), 26.8 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 31.7 (CH_2), 32.0 (CH_2), 42.0 (CH_2), 44.1 (CH_2), 44.3 (CH_2), 58.4 (CH), 58.6 (CH), 60.2 (CH_2), 123.0 (CH), 127.0 (CH), 127.2 (CH), 127.3 (CH), 129.4 (CH), 132.0 (C), 133.7 (CH), 137.9 (C), 138.7 (C), 142.8

(C), 143.0 (C), 167.1 (C); ESI-MS (*m*/*z*) 774.2 [(M + 2H)$^{+2}$, 20]. Anal. Calcd for $C_{78}H_{114}Cl_2N_8O_{14}S_6$: C, 56.74; H, 6.96; N, 6.79. Found: C, 56.35; H, 7.23; N, 6.58.

Polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-13. Tosyl group deprotection was carried out as in (*R*,*R*,*R*,*R*,*R*,*R*)-**6a**. Also in this case, the title compound was isolated and stored as the HCl salt: Yield: 77%; white hygroscopic solid; $[\alpha]^{20}_D = -39.9$ (*c* = 0.51, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ (ppm) 1.20−1.84 (m, 34H), 1.93−2.30 (m, 10H), 2.88−3.50 (m, 22H); ^{13}C NMR (D_2O, 75 MHz) δ (ppm) 21.0 (CH_2), 23.6 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 36.3 (CH_2), 42.5 (CH_2), 45.4 (CH_2), 57.0 (CH), 57.4 (CH); ESI-MS (*m*/*z*) 311 [(M + 2H)$^{+2}$, 65], 208.0 [(M + 3H)$^{+3}$, 100]. Anal. Calcd for $C_{36}H_{84}Cl_8N_8$: C, 47.37; H, 9.28; N, 12.28. Found: C, 47.12; H, 9.55; N, 12.03.

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Supporting Information Available: General experimental methods, detailed procedures, and characterization for all of the synthetic intermediates [(*R*,*R*)-**3a**,**b**, (*S*,*S*)-**3a**,**b**, (*R*,*R*)-**4a**,**b**, (*S*,*S*)-**4a**,**b**, (*R*,*R*)-**5a**,**b**, (*S*,*S*)-**5a**,**b**, (*R*,*R*)-**8**, (*R*,*R*)-**9**, and (*R*,*R*)-**10**], as well as copies of ^{1}H, ^{13}C, and DEPT NMR spectra for all of the described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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