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Solvent dependent selective alkylation of a bis(sulfonamide) for the synthesis of a DNA-binding chiral polyamine

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Abstract—A new optically active linear polyamine has been efficiently prepared and its DNA binding abilities studied by UV melting experiments. The key step for this synthesis was the selective monoalkylation of the corresponding bis(sulfonamide). Thus, it is possible to obtain mono or dialkylated compounds by simply changing the reaction conditions. These results are explained in terms of conformational preferences and solvophobic effects.

Polyamines are polycations at physiological pH and 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, this family of compounds has a great therapeutic potential for neurological diseases,[3] in the development of new antidiarrheals in AIDS related cases[4] and as anticancer agents.[5] Many of the biological applications of polyamines are related to their ability to interact with polyanions such as nucleic acids. For instance, spermine is known to stabilize DNA duplex and triplex,[6] and to promote conformational changes of duplex DNA, like B–Z transitions.[7] There is also a structure–function relationship, as different molecular architectures display different spatial dispositions of the cationic ammonium groups, which are able to interact with the phosphate chain of the oligonucleotides. Most of the structural changes studied to date[8] rely on spatial separation of nitrogen atoms,[9] alkylation of terminal amino groups, or the construction of dendritic structures.[10] Conformational restricted analogues have also been prepared and tested for biological activities.[11] Especially, derivatives bearing optically active pyrrolidyl moiety stabilize DNA duplexes and triplexes, in a different extent depending on the absolute configuration of the chiral centers.[12] In spite of all these studies, there is not yet a clear picture of the requirements for the polyamine–DNA supramolecular structures. In particular, the question how chirality on the polycationic polyamine can affect the stability of its corresponding nucleic acid complexes has not been investigated thoroughly.[13]

On the other hand, chiral cyclohexane-1,2-diamine has been used for the preparation of receptors based on macrocyclic polyamines for the molecular recognition of optically active polyanions.[14] Compounds bearing this diamine are also known to induce helices of different topicity depending on the cyclohexane-1,2-diamine absolute configuration.[15] Continuing our earlier studies, we envisioned that optically active linear polyamines could be interesting synthetic targets for the complexation of chiral anions.

Inspired by preliminary molecular modeling, we decided to prepare open chain polyamines with three cyclohexane-1,2-diamine fragments joined by linkers of different length. For this purpose, one of the key synthetic intermediates should be a monoalkylated protected diamine (Fig. 1). To achieve secondary amino groups in the final product, protection of the diamine is desirable. Among

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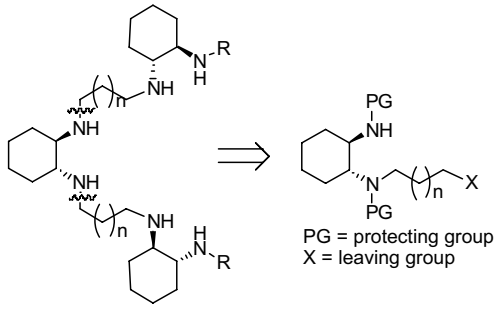


Figure 1. Retrosynthetic analysis of the target chiral polyamines.

different options, we found tosyl (Ts) as the most suitable protecting group for our purposes. Despite that monoalkylation or monoprotection of cyclohexane-1,2-diamine is not an easy task,[16] here we report an easily tunable methodology for obtaining mono- or dialkylated bis(sulfonamide) in high yields and selectivity depending on the reaction conditions. Finally, we have used this strategy for the synthesis of a DNA-binding chiral polyamine.

In a previous paper,[17] we had carried out the alkylation of (*R*,*R*)-**1** with 3-bromopropanol (Table 1, entry 1). Over all the reaction conditions tested, we always obtained a mixture of mono and dialkylated bis(sulfonamide), readily separable after flash chromatography.

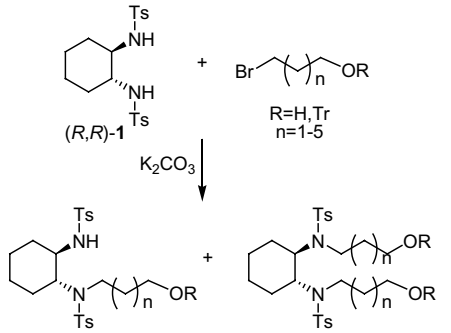
However, for selectively obtaining mono- or dialkylated compounds in good yields, a more elaborated synthetic strategy had to be found. We firstly checked if protection of the terminal hydroxy group could affect the reactivity on the electrophilic center. Thus, when the reaction was carried out with *O*-trityl derivative (Table 1, entry 2), the monoalkylated sulfonamide (*R*,*R*)-**2b** was obtained as the major product.[18] This result could be initially explained by steric hindrance between Ts and Tr protecting groups. We extended the process using *O*-Tr-α,ω-bromoalcohols with different number of methylenes (*n* = 1–5). Surprisingly, in all the tested examples, the reaction was highly selective towards the formation of the monoalkylated products, showing minor variations in yield with increasing length of the alkyl chain.

Considering the flexibility of aliphatic linear chains, we ruled out steric repulsion as the only source for this selectivity. We then suspected that the conformation of the monoalkylated sulfonamide **2** could play an important role in the reactivity of the second NH group. With the alkyl chain in an extended conformation (I in Fig. 2), Tr group would be far away from the second sulfonamide. However, in a folded conformation (II), aromatic rings of Ts and Tr groups would be close to each other. This arrangement would protect the free NH group in **2** from reaction with a second electrophile.

An experimental evidence for the existence of folded conformations of the monoalkylated derivatives was obtained from 1D NOESY experiments on (*R*,*R*)-**2e** (*n* = 4). For instance, selective pulse field gradient irradiation on the *ortho* protons of the Tr group yielded NOE effects on ortho protons of the Ts group of the non-alkylated sulfonamide and on both Tosyl-CH_3 (Fig. 3). This would set the two protecting groups at a distance shorter than 5 Å, [19] supporting the folded conformation of (*R*,*R*)-**2e**.

The above-mentioned conformational equilibrium is expected to be highly dependent on the polarity of the medium. Polar solvents (such as acetonitrile) would

Table 1. Alkylation of bis(sulfonamide) (*R*,*R*)-**1**



(*R*,*R*)-**2a** (R=H, n=1)
(*R*,*R*)-**2b-f** (R=Tr, n=1-5)

(*R*,*R*)-**3a** (R=H, n=1)
(*R*,*R*)-**3b-f** (R=Tr, n=1-5)

Entry	*n*	R	Solvent	Yield **2**[a] (%)	Yield **3**[a] (%)
1	1	H	CH_3CN	21[b]	50[b]
2	1	$C(Ph)_3$	CH_3CN	70	11
3	2	$C(Ph)_3$	CH_3CN	80	—
4	3	$C(Ph)_3$	CH_3CN	75	—
5	4	$C(Ph)_3$	CH_3CN	67	12
6	5	$C(Ph)_3$	CH_3CN	75	5
7	1	$C(Ph)_3$	$PhCH_3$[c]	—	72
8	5	$C(Ph)_3$	$PhCH_3$[c]	—	82

[a] Isolated yields after flash chromatography.
[b] Taken from Ref. 17.
[c] A catalytic amount of *n*-Bu_4NBr was added.

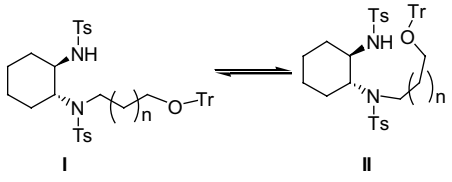


Figure 2. Proposed equilibrium between extended (I) and folded (II) conformations of **2**.

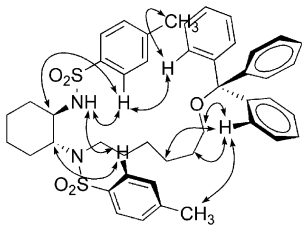


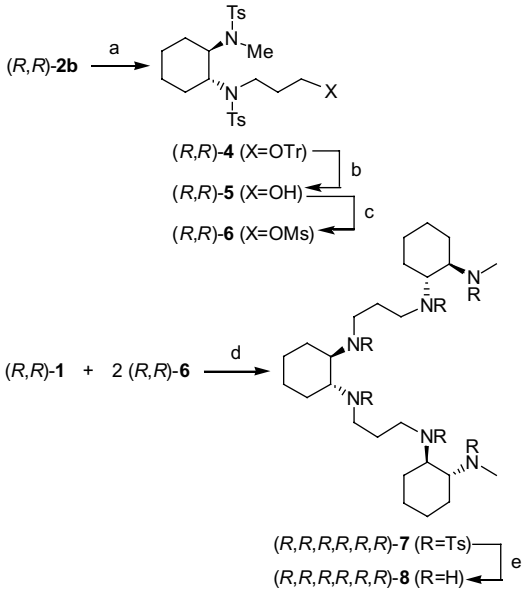
Figure 3. NOE contacts obtained from 1D NOESY experiments on (*R*,*R*)-**2e** ($CDCl_3$, 500 MHz).

favor folded conformers while hydrophobic environments would stabilize extended structures. When running the reaction (for both the shortest and longest electrophiles) in an aromatic hydrophobic solvent, only dialkylated derivatives were isolated in good yields (Table 1, entries 7 and 8). We thus demonstrated that the effect of Tr group depends on the hydrophobicity of the medium. Solvophobic effects governing conformation[20] and reactivity[21] have been previously reported in the literature and it is a critical parameter for protein folding.[22] We present here an interesting example of solvophobic effects where a terminal group prevents the reaction in a remote center of the molecule. From the synthetic point of view, the interest of this effect is the possibility of selectively obtaining mono- or dialkylated bis(sulfonamide) by simply changing the solvent.

As the final goal, we attempted to use one of the monoalkylated compounds for the synthesis of a new optically active polyamine ($n = 1$, R = Me in Fig. 1). The synthetic procedure is outlined in Scheme 1. Conventional nitrogen alkylation of (*R*,*R*)-**2b**, followed by Tr cleavage and mesylation of the resulting alcohol led to (*R*,*R*)-**6** in very high overall yield. Coupling of (*R*,*R*)-**1** with 2 equivalents of (*R*,*R*)-**6** in Cs_2CO_3/CH_3CN afforded the hexatosylated polyamine (*R*,*R*,*R*,*R*,*R*,*R*)-**7** in ca. 50% yield. Applying the same rational than for the alkylation reaction, the isolated yield of this coupling process was increased up to 70% by using toluene as solvent. Acidic deprotection of all the Ts groups led to the desired polyamine which was isolated as the hydrochloride salt.[23] Both ^1H and ^{13}C NMR spectra revealed C_2 symmetry in solution, supporting the lack of epimerization throughout the synthetic sequence.

The DNA binding ability of (*R*,*R*,*R*,*R*,*R*,*R*)-**8** has also been investigated. The presence of the polyamine increased the UV-melting temperatures of three different oligonucleotides up to $\Delta T_m = 7.3$ °C (Table 2 and Fig. 4). Addition of sodium chloride decreased the stabilization produced by the polycation, and the effect was even more pronounced when ionic strength was increased and dicationic metal salts were added. This suggests that the interaction is mainly electrostatic and agrees with reported data in related systems.

At this moment, it remains unknown if there is any conformational preference for the polyamine to bind the



Scheme 1. Synthesis of (*R*,*R*,*R*,*R*,*R*,*R*)-**8**. Reagents and conditions: (a) MeI, Cs_2CO_3, $PhCH_3$, n-Bu_4NBr, 110 °C (90%). (b) TFA, CH_2Cl_2, MeOH (80%). (c) MsCl, NEt_3, CH_2Cl_2 (quantitative). (d) Cs_2CO_3, $PhCH_3$, n-Bu_4NBr (70%). (e) HBr (aq), PhOH (60%).

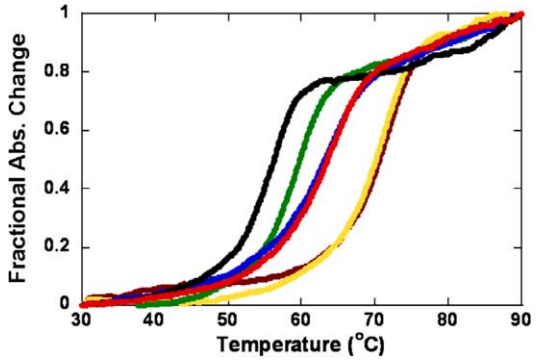


Figure 4. UV-melting profiles of B1/B1C DNA oligonucleotides (2 μM in 20 mM Tris buffer, pH 7.4): (a) alone (black); (b) +0.1 mM (*R*,*R*,*R*,*R*,*R*,*R*)-**8** (red); (c) 20 mM NaCl (green); (d) like in (c) +0.1 mM (*R*,*R*,*R*,*R*,*R*,*R*)-**8** (blue); (e) 100 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ (brown); (f) like in (e) +0.1 mM (*R*,*R*,*R*,*R*,*R*,*R*)-**8** (yellow).

Table 2. UV (260 nm) melting temperatures (T_m in °C) of DNA (2 μM) sequences in the absence (−) and in the presence (+) of 0.1 mM (*R*,*R*,*R*,*R*,*R*,*R*)-**8**

	20 mM Tris (pH 7.4)		20 mM Tris, 20 mM NaCl (pH 7.4)		20 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, $CaCl_2$ (pH 7.4)	
	−	+	−	+	−	+
A16/T16	34.0	37.2	40.0	40.9	39.2	39.3
B1/B1C	56.3	63.6	59.8	63.2	70.6	70.2
Hairpin	42.7	45.2	46.8	47.0	53.3	54.1

B1: dCCTGTCGCCTCGCACATAGCC.
B1C: GGACAGCGGAGCGTGTATCGGd.
Hairpin: dAGTCTATGGGTTAGACT.

polyphosphate backbone. However, our modular synthetic approach allows us to prepare a complete battery of isomeric polyamino derivatives for binding assays, which would give us interesting information about the supramolecular structure. Other structural variables such as the terminal alkylating group or separation between conformationally rigid moieties will be also investigated.

In summary, we report here a selective alkylation of the bis(sulfonamide) (*R*,*R*)-**1**. The corresponding mono- or dialkylated derivatives can be easily prepared by simply changing the reaction conditions. These results have been rationalized in terms of conformational preferences and solvophobic effects. The applications of the obtained synthons have been illustrated with the efficient synthesis of a new optically active open chain polyamine, which is able to interact with DNA molecules. Further synthetic studies are underway for the preparation of different chiral polyamino structures. The potential of these new molecules ranges from chiral anion recognition to separation and transport, as well as in non-viral gene transfection technology.[24]

Acknowledgments

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18. *General procedure for the alkylation reaction.* In a flask under nitrogen atmosphere 2 mmol of (*R*,*R*)-**1** and 20 mmol of anhydrous K_2CO_3 were suspended in 12 mL of dry CH_3CN and the mixture heated to 70 °C for half an hour. Then, 8 mmol of the corresponding electrophile were added drop wise and the obtained mixture stirred at 70 °C for 2 days (TLC AcOEt:Hexan 3:2). After that, the reaction was allowed to room temperature, acidified with 15 mL of 3 N HCl and extracted with CH_2Cl_2. The combined organic layers were dried and evaporated to dryness. The final monoalkylated product was isolated by flash chromatography. For obtaining the dialkylated compound, the reaction was carried out like in the monoalkylation procedure but in dry toluene and a catalytic amount of tetrabutylammonium bromide was also added.
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23. Spectral data for the hydrochloride of (*R*,*R*,*R*,*R*,*R*,*R*)-**8**: decompose without melt; $[\alpha]_{Hg}^{578}$ − 48.4, (*c* = 0.5, 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) 21.22 (CH_2), 21.33 (CH_2), 21.39 (CH_2), 22.86 (CH_2), 24.80 (CH_2), 25.14 (CH_2), 25.33 (CH_2), 30.55 (CH_3), 42.47

(CH_2), 42.58 (CH_2), 57.04 (CH), 57.52 (CH), 57.62 (CH); HRMS/EI calculated for $C_{26}H_{54}N_6$: 450,4410; found 450,4414.

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