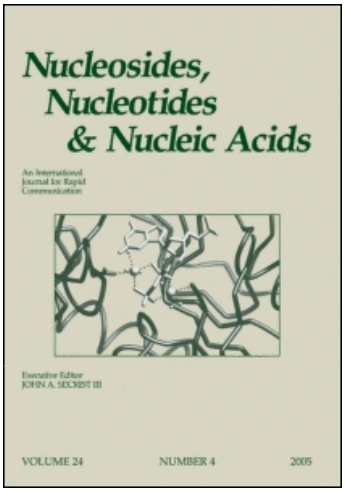

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Sund, Christian , Puri, Nitin and Chattopadhyaya, Jyoti(1997) 'The Chemistry of *C*-Branched Spermine Tethered Oligo-DNAs and Their Properties in Forming Duplexes and Triplexes', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 755 — 760
To link to this Article: DOI: 10.1080/07328319708002946
URL: http://dx.doi.org/10.1080/07328319708002946

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 755-760 (1997)

THE CHEMISTRY OF *C*-BRANCHED SPERMINE TETHERED OLIGO-DNAs AND THEIR PROPERTIES IN FORMING DUPLEXES AND TRIPLEXES

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Abstract: The first synthesis of 2'-, 5'- and centrally tethered *C*-branched spermine oligo-DNAs directly on the solid-support as well as their ability to stabilise DNA duplexes and triplexes are reported.

INTRODUCTION

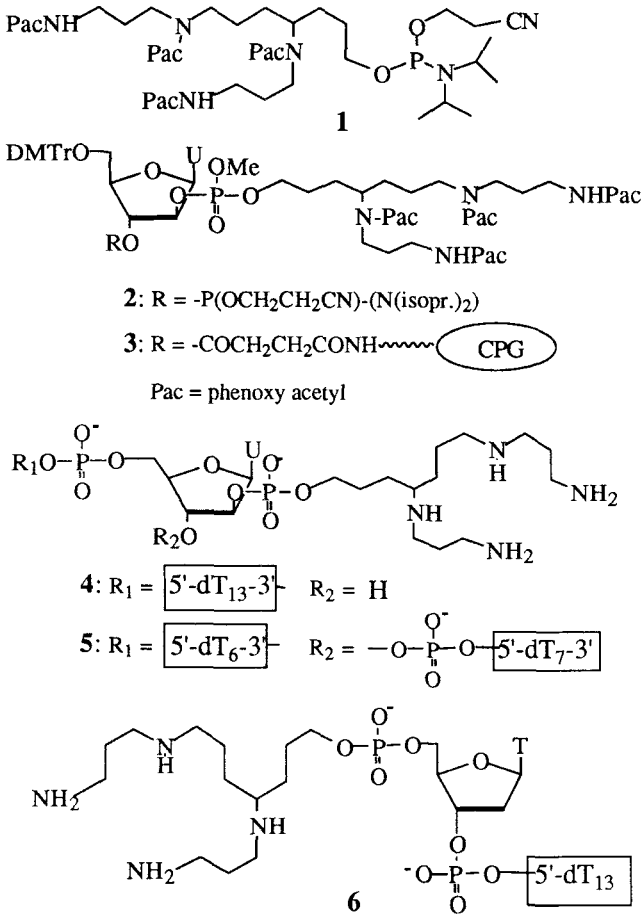
External tetracationic spermine has been shown to stabilise DNA duplexes and triplexes[1,2] and this has raised considerable interest in spermine tethered oligo-DNAs as non-cytotoxic candidates for the antisense and antigene strategies[3,4]. A few reports on spermine tethered oligo-DNAs have emerged over recent years where different methods have been employed for tethering of the spermine to the oligonucleotide, mainly through post-solid phase synthetic tethering to the 5'-end hydroxyl and phosphate[5,6] and through tethering to nucleobases[7,8,9].

In this work[10], we present a hitherto unexplored synthesis of *C*-branched spermine tethered oligo-DNAs with the spermine tethered at the 5'-end or in the center or at the 2'-end of the oligo-DNA, and show their use in the stabilisation of the duplexes and triplexes. This new type of hydroxyalkyl linked *C*-branched spermine tether is unique in that all the amino functions are underivatised and their pK_as unaltered compared to natural spermine (10.97, 10.27, 9.04, 8.03)[11]. The novel nature of *C*-branching of the spermine residue also allowed its coupling to the pentofuranose ring, that enabled us to stereochemically orientate it away from both the minor and major groove of DNA duplex, and thereby placing it in the steric proximity of the phosphate backbone.

RESULTS AND DISCUSSION

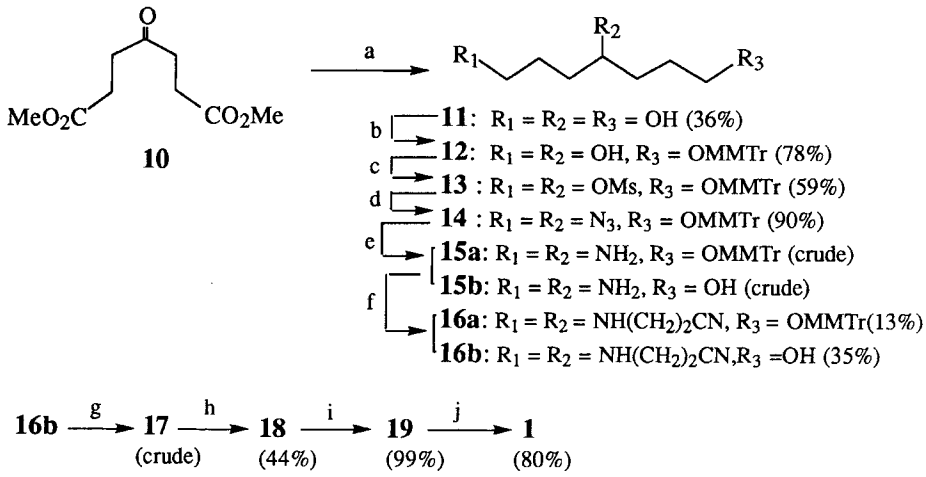
In the present approach, the spermine moiety is introduced directly during solid-phase synthesis in three ways: (i) The synthesis of the *C*-branched tetraphenoxyacetyl spermine phosphoramidite block **1** and subsequently its incorporation at the 5'-end of the oligo-

DNA **6** . A large scale synthesis of **1** was accomplished through a 10 step protocol[10]



*starting from diethyl 3-oxopimelate (Scheme 1). The intermediate N^4, N^7-bis(2-cyanoethyl)diaminoheptan-1-ol (**16b**) was reduced with $LiAlH_4$ / $AlCl_3$ in tetrahydrofuran and diethylether mixture to generate the racemic 6-(3-hydroxypropyl)spermine (**17**) (95% as crude oil), which was then reacted directly with phenoxyacetic (Pac) anhydride in pyridine to generate the penta-Pac intermediate **18** (44%). Compound **18** was then selectively hydrolysed with dry NH_3 in dry MeOH to give the 6-(3-hydroxypropyl) N^1,N^5,N^{10},N^{14}-tetra-Pac spermine **19** in a quantitative yield. An aliquot of **19** was converted into the *O*-(2-cyanoethyl)-(*N*,*N*-diisopropyl)phosphoramidite block **1** (80%) in a standard way[12].

(ii) The synthesis of 5'-*O*-DMTr-2'-phospho (*O*-methyl)-(*O*-(tetraphenoxyacetyl spermine)-6-(3-propyl)) *ara*-uridine 3'-phosphoramidite **2** and its use for incorporation of the *C*-branched spermine into the oligo-DNA **5**, and (iii) the synthesis of the analogous

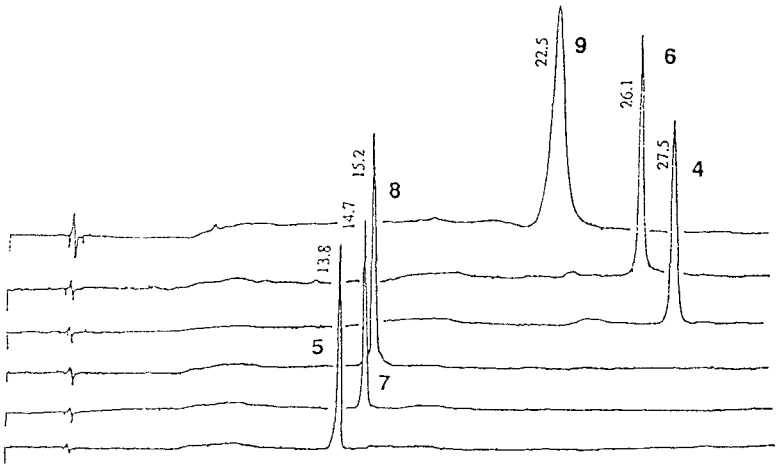


SCHEME 1. (a) $LiAlH_4$ / diethylether, reflux; (b) MMTrCl / pyridine / CH_2Cl_2, 20°C; (c) MsCl / pyridine / CH_2Cl_2, 0-20°C; (d) NaN_3 / DMF, 20°C; (e) H_2 / Pd / C, MeOH, 20°C; (f) CH_2=CHCN / DMF, 20°C; (g) $LiAlH_4$ / $AlCl_3$ (1:1) / THF / diethylether, 20°C; (h) $(Pac)_2O$ / pyridine, 20°C; (i) NH_3 / MeOH, 20°C; (j) (2-CeO)-$((ipr)_2N)$-PCl / $(ipr)_2EtN$ / THF, 20°C.

ara-uridine-3'-succinyl aminoalkyl CPG **3** and its use for incorporation of the *C*-branched spermine residue to the 2'-end of the oligo-DNA **4**. The synthesis[10] of **2** and **3** was straightforeward and high yielding (86-92%) starting from 3', 5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disilyl,1,3-yl) *ara*-uridine (5 steps). All through this work, a racemic mixture of 6-(3-hydroxypropyl)spermine has been used for the oligo-DNA derivatisations.

By using the above building blocks **1**, **2** and **3**, we synthesised simple T-rich *C*-branched spermine tethered 14-mers **4**, **5** and **6**, together with the reference sequence dT_{14} (**7**) and the target sequences dA_{26} (**9**) and dT_{26} (**8**) and their Hplc profiles are shown below. Oligo-DNAs **4-7** were hybridised with either the dA_{26} alone or with a 1 : 1 complex of dA_{26} : dT_{26} at different pH and salt concentrations, and subsequently studied the melting behavior (T_m) of the resulting double- and triple helical complexes.

Oligomers **4** and **6** have longer Hplc retention times on a hydrophobic C18 column than oligomer **5**, which has a R_t similar to dT_{14} (**7**). This may reflect different degrees of intramolecular electrostatic interactions between the spermine ammonium cations and the phosphodiester linkages in **4-6** and therefore influencing the hydrophopbic character of the oligomers.



The Hplc profiles of purified oligonucleotides **4-9** using the gradient 0-100% B in 30 min at 1 ml/min. Buffer A: 0.1M TEAA, 5% MeCN; Buffer B: 0.1M TEAA, 50% MeCN. The analytical column Nucleosil NC100-5C18. Retention times (Rt) given in minutes.

This observation also appears to correlate to the melting behaviours of **4-6** in their duplexes and triplexes with the target oligomers **8** and **9**. Oligomers **4** and **6** give considerably higher T_ms than **5** in duplex formation (Tables 1 and 2). This may reflect enhanced *inter*molecular electrostatic interactions through the spermine ammonium cations in **4** and **6** with *the opposite target strand.* Only oligomers **4** and **6** give triplexes with the target duplex [dA_{26} + dT_{26}] (Tables 2 and 3).

The results obtained under the experimental conditions used in this work show that: (i) the end-tethered *C*-branched spermine oligo-DNAs **4** & **6** give no or only a small duplex stabilisation (Tables 1&2); (ii) the centrally-tethered *C*-branched spermine oligo-DNA **5** give destabilised duplexes (Tables 1&2); (iii) **4** & **6** promote triplex formation, while **5** gives no triplex formation (Tables 1&3); (iv) comparison of the heating T_ms (^hT_m) and the cooling T_ms (^cT_m) at low salt and with Mg^{2+}, shows that the *C*-branched spermine moiety enhances reassociation of the **4** & **6** to the target duplex **[8+9]** during triplex formation (Table 1); (v) at high salt and no Mg^{2+}, an increased *C*-branched spermine induced triplex stabilisation is observed, possibly due to increase of the hydrophobic interactions ("salting out effect"), as well as due to decrease of water activity in the triplex interior and at the spermine ammonium cations (Table 3); (vi) examination of the ^hT_ms and the ^cT_ms shows that reassociation of the third strands **4** & **6** is slowed down towards high salt (Table 3); (vii) duplex and triplex meltings are practically independent

TABLE 1. Melting temperatures (°C) (heating T_m (^hT_m) & cooling T_m (^cT_m)) of duplexes and triplexes at different pH

Hybrids (μM)	pH 5.5		pH 7.0		pH 7.6	
	^hT_m	^cT_m	^hT_m	^cT_m	^hT_m	^cT_m
Duplex						
9+6 (1:1)	48.0	*a*	48.5	*a*	48.0	*a*
9+5 (1:1)	19.0	*a*	17.5	*a*	17.5	*a*
9+4 (1:1)	46.5	*a*	46.5	*a*	47.0	*a*
9+7 (1:1)	46.0	*a*	46.0	*a*	46.0	*a*
Triplex[c]						
8+9+6 (1:1:2)	19.0	20.0	19.0	19.5	19.0	21.0
8+9+5 (1:1:2)	*b*	*b*	*b*	*b*	*b*	*b*
8+9+4 (1:1:2)	22.5	21.0	23.0	22.0	21.0	21.5
8+9+7 (1:1:2)	16.0	13.5	16.5	14.0	16.0	12.5

Buffer at pH 5.5 : 50 mM AcONa/AcOH, 100 mM NaCl, 20 mM $MgCl_2$; Buffer at pH 7.0 & 7.6: 25 mM TrisHCl, 100 mM NaCl, 20 mM $MgCl_2$; *a* not measured; *b* not detected; *c* ^hT_m of duplex **8+9** was ~58 °C for all pH.

TABLE 2. Melting temperatures (°C) (heating T_m (^hT_m)) of duplexes at various NaCl concentrations at pH 7.0 in 10 mM NaH_2PO_4 / Na_2HPO_4, 0.1 mM EDTA $2Na^+$.

Hybrids (μM)	0.05M	0.1M	0.2M	0.4M	0.8M	1.2M
8+9 (1:1)	44.0	48.0	53.0	58.0	62.0	64.5
9+6 (1:1)	31.0	36.0	40.5	46.0	50.0	*a*
9+5 (1:1)	*a*	8.0	13.5	18.0	21.5	*a*
9+4 (1:1)	29.0	33.5	38.5	43.5	48.0	*a*
9+7 (1:1)	29.5	34.0	39.0	44.0	48.0	*a*

a not measured; *b* not detected.

TABLE 3. Melting temperatures (°C) (heating T_m (^hT_m) & cooling T_m (^cT_m)) of triplexes at various NaCl concentrations at pH 7.0 in 10 mM NaH_2PO_4 / Na_2HPO_4, 0.1 mM EDTA $2Na^+$.

Hybrids (μM)	^hT_m/ ^cT_m	0.2M	0.4M	0.8M	1.0M	1.2M	1.4M
8+9+6		*b*	<7.0	20.0	25.0	32.5	38.0
(1:1:2)		*b*	*a*	18.5	24.5	31.0	36.0
8+9+5		*b*	*b*	*b*	*b*	*b*	*b*
(1:1:2)		*b*	*b*	*b*	*b*	*b*	*b*
8+9+4		*b*	<7.0	20.0	25.0	32.5	38.0
(1:1:2)		*b*	*a*	19.0	23.5	29.5	35.0
8+9+7		*b*	<7.0	15.0	21.5	25.5	28.5
(1:1:2)		*b*	*a*	12.5	19.5	24.0	29.0

a not measured; *b* not detected.

of pH in the range 5.5 - 7.6, showing that the *C*-branching of spermine at the C6 position gives no alterations in the pK_as of the ammonium groups compared to natural spermine.

CONCLUSION

Our study has shown that 2'- and 5'-end tethered *C*-branched spermine oligo-DNA, exemplified by **4** and **6**, promote uniform triple helix stabilisation over the pH range of 5.5 to 7.6 with 0.1M NaCl and Mg^{2+}, with $^{h}T_m$s 2.5 to 3 °C and $^{c}T_m$s 5.5 to 9 °C. Under the same conditions, oligomers **4** and **6** form duplexes with target dA_{26} (**9**) with only 0.5 to 2.5 °C stabilisation compared to the reference dT_{14} (**7**). This study has shown that *C*-branched spermine tethered oligo-DNAs such as **4** and **6** also promote triplex stabilisation[1,5,9], facilitated by an enhanced rate of reassociation of the third strand. They are less potent for duplex stablisations[7], but this order may be changed depening on the choice of base sequence of the oligo-DNAs in the area of the spermine interaction[8].

ACKNOWLEDGEMENTS

Authors thank Swedish Natural Science Research Council (NFR) and Engineering Research Council (TFR) for generous funding.

REFERENCES

1. Hanvey, J.; Williams, E.; Besterman, J. *Antisense Res. Development* **1991**, *1*, 307-317.
2. Thomas, T.; Thomas, T. *Biochemistry* **1993**, *32*, 14068-14074.
3. Hélène, C. *Eur. J. Cancer* **1991**, *27*, 1466-1471.
4. Matteucci, M.; Bischofberger, N. *Annual Reports in Medicinal Chemistry* **1991**, *26*, 287-296.
5. Tung, C.; Breslauer, K.; Stein, S. *Nucleic Acids Res.* **1993**, *21*, 5489-5494.
6. Bigey, P.; Pratviel, G.; Meunier, B. *J. Chem. Soc., Chem. Comm.* **1995**, 181-182.
7. Prakash,T.; Barawkar, D.; Kumar, V.; Ganesh, K. *Bioorg.Med.Chem. Lett.* **1994**, *4*, 1733-1738.
8. Schmid, N.; Behr, J. *Tetrahedron Lett.* **1995**, *36*, 1447-1450.
9. Barawkar, D.; Rajeev, K.; Kumar, V.; Ganesh, K. *Nucleic Acids Res.* **1996**, *24*, 1229-1237.
10. Sund, C.; Puri, N; Chattopadhyaya, J. *Tetrahedron* **1996**, *52*, 12275-12290.
11. Takeda, Y.; Samejima, K.; Nagano, K.; Watanabe, M.; Sugeta, H.; Kyogoku, Y. *Eur. J. Biochem.* **1983**, *130*, 383-389.
12. Sinha, N.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846.