Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 691–693

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Solid-Phase Synthesis of Oligomeric Deoxynucleic-Thiourea (DNT) and Deoxynucleic *S*-Methylthiourea (DNmt): a Neutral/Polycationic Analogue of DNA

Dev P. Arya† and Thomas C. Bruice*

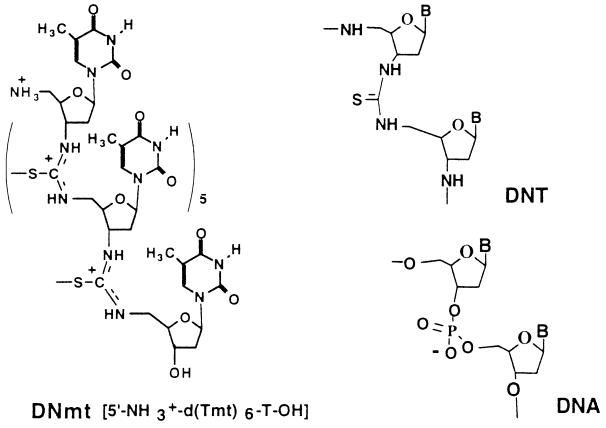
Department of Chemistry, University of California, Santa Barbara, CA 93106, USA

Received 9 December 1999; accepted 14 January 2000

Abstract—A solid-phase synthesis for oligomeric DNmt is reported. Synthesis proceeds in 3′–5′ direction and involves coupling of a protected 3′-isothiocyanate with the corresponding 5′-amine of the growing oligo chain. The difference in oligomeric thiourea/*S*-methylthiourea binding to DNA is investigated.

Introduction

In recent years, much interest has been expressed in antisense agents for the regulation of gene expression in living cells.[1,2] Among other requirements, successful development of antisense therapeutics presupposes the oligos to (a) be stable in vivo, (b) have improved permeability and cellular uptake and (c) have greater binding affinity with high specificity.[3–5] We have previously reported the replacement of the phosphate linkages in DNA and RNA by achiral guanido and *S*-methylthiourea groups which we identify as DNG[6–8] and DNmt respectively.[9–11] A 5 unit oligomer of thymidyl DNmt (NH_3^+-(Tmt)$_4$-T-OH) binds to complementary poly(rA) with a melting temperature (Tm) for dissociation of the double helix estimated to be close to 85 °C and yet no base specific H-bonding (Watson–Crick) is observed with poly(rC), (rG), (U), (dT) or (I).[9] Due to this strong binding, specificity and resistance to nucleases, DNmt represents an extremely interesting putative genetic regulatory agent. The stepwise synthesis of oligomeric DNmt strands in solution has been reported by us recently.[9] In order to facilitate the development of longer sequences of DNmt, a synthesis that involved stepwise construction on a solid support was needed. In this paper, we report the first solid-phase synthesis of a DNmt oligomer.



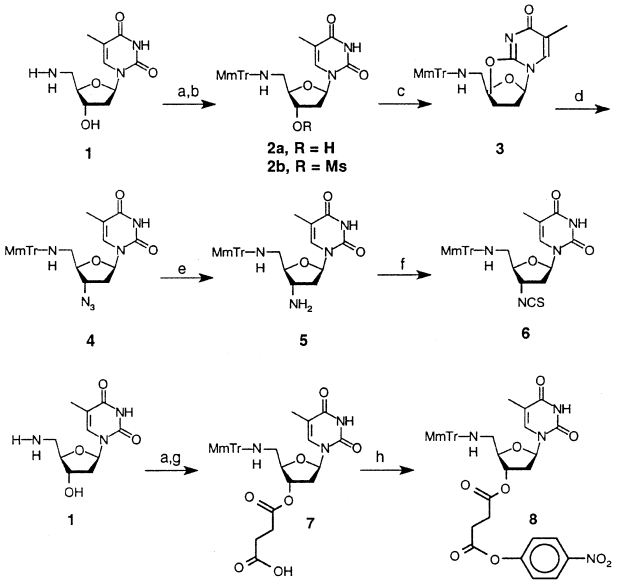
Structures of DNmt, DNT and DNA linkages

Synthesis

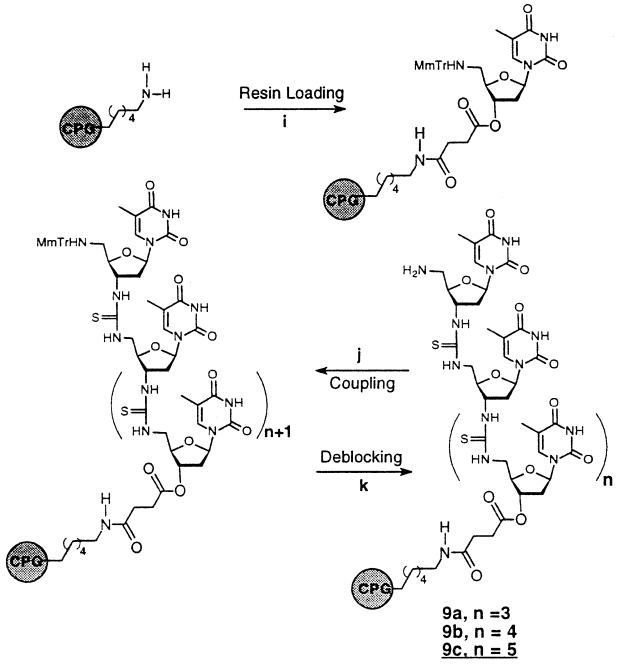
The monomer **6** (Scheme 1) for the SPS of oligothymidyl DNmts is obtained by protecting the 5′-NH_2, group of 5′-amino-5′-deoxythymidine (**1**) with monomethoxytrityl chloride and converting the 3′-OH to 3′-isothiocyanate in five steps.[8,9] The 3′-OH was converted to the mesylate **2b** which upon reaction with Potassium phthalimide in DMF followed by lithium azide gives the azide **3** in excellent yields. Compound **3** was hydrogenated and reacted with thiocarbonylpyridone in CH_2Cl_2 to give monomer **6** in high yields[9–14] (see supplementary material for experimental procedures). ControlPore Glass (CPG) resin with long chain alkylamine was chosen as a convenient commercially available

*Corresponding author. Tel.: +1-805-893-2044; fax: +1-805-893-4120; e-mail: tcbruice@bioorganic.ucsb.edu
†Present address: Department of Chemistry, Pemson University, SC 29634.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00085-8



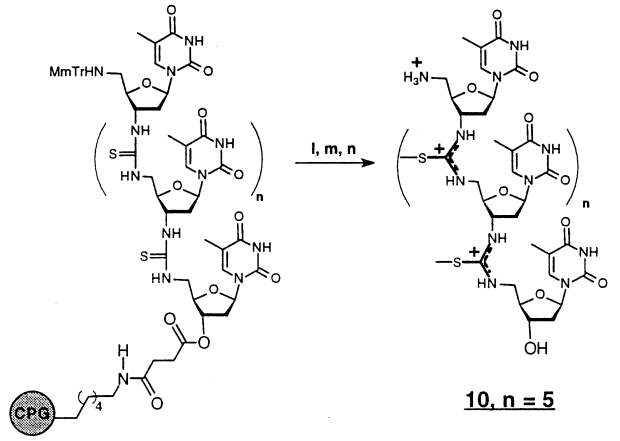
Scheme 1. Synthesis of thymidyl monomer for solid phase synthesis. (a) MmTr-Cl, pyridine. DMAP(2 mol%), 89%; (b) 1.3 equiv MsCl, pyridine, 0 °C to rt, 88%; (c) KPhTh, DMF, H_2O, 100 °C, 20 mm; (d) LiN_3, DMF, 110 °C 90%; (e) H_2S, pyridine, 4 h, rt, 90%; (f) Thiopyridone, CH_2Cl_2, 4 h, 88%; (g) succinic anhydride, DMAP, pyridine; (h) 4-Nitrophenol, DMAP, Et_3N.



Scheme 2a. Solid phase synthesis of DNT: (i) pyricline, DMF, DMAP, monomer **8**; (j) coupling: pyridine, monomer **6**, DMAP (2 equiv); (k) deblocking: 4% dichloroacetic acid in dichioromethane.

support with a linker that was cleavable by mild base. The synthesis was designed to be compatible with standard DNA synthesis techniques to facilitate future synthesis of DNmt–DNA conjugates.[15] Analogous to DNA synthesis, 3′-OH of **1** was converted to the activated nitrophenyl ester **8**, was loaded as the first base on the resin.[16] The coupling reaction involves the addition of **6** to a 5′-aminothymidyl residue on the resin. In a typical synthesis, 36 mg of resin was placed in 0.5 mL Pyridine in a 3 mL reaction vial. Stock solutions of the isothiocyanate **6** (30 mM, 1.0 mL), DMAP (12 mM) in pyridine were added and the vial was agitated for 4 h. The addition step was repeated twice to insure a complete reaction and then the resin was washed with copious amounts of pyridine, methanol and ether. The resulting 5′-MmTr protected oligomer was deblocked with 4% dichioroacetic acid in CH_2Cl_2 and the cycle began again (Scheme 2a). The addition/deblocking cycle was repeated six more times to produce the seven unit oligomer. The thiourea was methylated with 2 mL methyl iodide for 4 h. The 5′-MmTr group was removed in 4% DCA and the resin washed with methanol, ether. The product was cleaved from the resin by treatment with NH_4OH at room temperature. The oligomer can be cleaved from the resin before methylation to give the corresponding thiourea (**9a**–**c**) as well (DNT). Methylation of the tritylated thiourea can be performed in solution as well as on the solid phase. HPLC analysis of the crude DNmt product showed the desired product in >90% purity with an estimated coupling yield averaged over the seven additions of 87% (determined by UV analysis of absorbance of monomethoxy trityl cation). The deprotected DNmt oligomer **9** [5′-NH_3^+-d(Tmt)$_7$-OH] was purified on a preparative Alltech WCX cation exchange column employing 1.50 M ammonium acetate buffer, pH 6.0, as the mobile phase to give the pure oligomer (Scheme 2b). Mass spectroscopic analysis indicates the expected mass for the singly charged (m/z = 2019, calculated for $(M+H)^+$: 2019) form of the oligomeric DNmt **9**.

We have previously reported[10,11] that Thymidyl DNmt (5′-NH_3^+-d(Tmt)$_4$-T-OH) has much stronger affinity for DNA and RNA, due to electrostatic attractions, than DNA for RNA or vice versa. In order to further evaluate the high stability of DNmt·DNA complexes, binding of neutral DNT (deoxynucleic-thiourea) **9a** to poly (dA) was studied.[17] To investigate the interaction of **9a** {5′-NH_3^+-T_t-(T_t)$_4$-OH} with polynucleotides, we constructed UV continuous variation plots. Mixtures of **9a** with poly(dA) at 10 °C (Fig. 1) reach a minimum



Scheme 2b. Solid phase synthesis of DNmt: (l) MeI, EtOH; (m) NH_4OH, rt; (n) 4% dichloroacetic acid in dichloromethane.

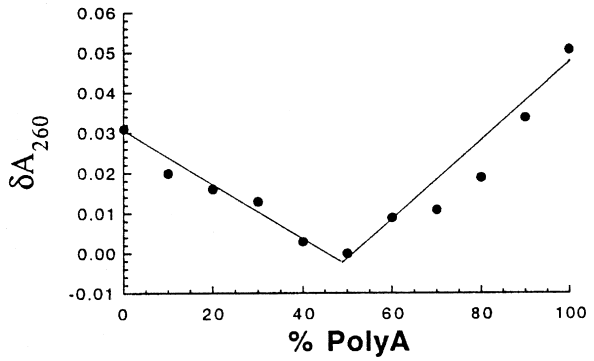


Figure 1. Job plots of poly(dA) with 5′-NH_3^+-d(Tt)$_4$-T-OH in a concentration of 4.0×10^{-5} M/base at 260 nm in 15 mM potassium phosphate (pH 7.5).

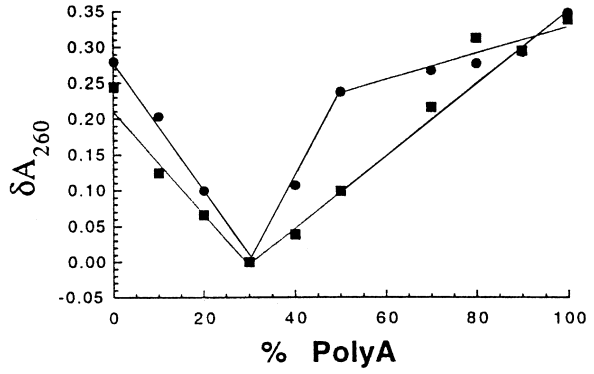


Figure 2. Job plots of poly(dA) with 5′-NH_3^+-d(Tmt)$_4$-T-OH in a concentration of 4.0×10^{-5} M/base at 260 nm in water (●) and 15 mM potassium phosphate (■).

absorbance at a mol fraction of ~0.5 d(Tmt) to 0.5 d(Ap) (single phosphate-linked adenosyl unit).

These numbers indicate that double stranded complexes are formed containing one d(T_t) for every d(Ap). This is in contrast to the 2:1 binding observed between DNmt·DNA complexes (Fig. 2) where triple helices are always observed. DNmt·DNA complexes always show a minimum at 2:1 and a break at 1:1 is observed only when mixing plots are carried out in pure water (Fig. 2). This is in agreement with the fact that DNmt·DNA complexes are destabilized upon addition of salt, as previously reported.[9,10] Melting curves for the duplex **9a**·poly(dA) gave a melting point less than 15 °C, showing much weaker binding compared to the DNmt·DNA triplexes. Changing the ionic strength (up to 0.2 M KCl) had no effect on the stability of the DNT·DNA duplex. As reflected in the δA_{260} values in Figures 1 and 2, the % hypochrornicity is considerably less for DNT·DNA (Fig. 1) implying weaker binding for DNT·DNA duplex. Attempts to study binding of longer DNT (**9b–c**) were unsuccessful due to the lack of solubility of thiourea oligomers in aqueous solution.

In summary, an efficient and rapid solid-phase method for the synthesis of thiourea and *S*-methylthiourea linked DNA analogues has been successfully demonstrated. This solid-phase synthesis technique opens the door for the rapid synthesis of DNmt/DNT oligomers for further binding studies, for combinatorial libraries and the synthesis of DNmt-peptide conjugates on solid phase. If the nucleotide bases are protected with base-labile phenoxyacetyl groups, the synthesis can be accomplished as detailed above and the base protecting groups removed by subsequent ammonia treatment after removal of the MmTr protecting groups. Attachment of charged functional groups to the 5′-amine of DNT (**9a–c**) should give them increased solubility and enable comparative studies of neutral thiourea with DNmt and DNG oligos. The synthesis and binding studies of such mixed sequences should be very interesting and are currently the focus of our continuing investigations.

Acknowledgement

This project was supported by the National Institute of Health (3 R37 DKO9171-3451).

References and Notes

1. Bennett, C. F. *Biochem. Pharmacol.* **1998**, *55*, 9.
2. Hawkins, J. W. *Antisense and Nucleic Acid Drug Development* **1995**, *5*, 1.
3. Cohen, J. S. *Antiviral Res.* **1991**, *16*, 121.
4. Cook, P. D. *Medicinal Chemistry Strategies for Antisense Research*; CRC Press: Boca Raton: 1993.
5. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.
6. Dempcy, R. O.; Almarsson, O.; Bruice, T. C. *Proc. Natl. Acad. Sci. USA*. **1994**, *91*, 7864.
7. Dempcy, R. O.; Browne, K.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 6140.
8. Dempcy, R. O.; Browne, K.; Bruice, T. C. *Proc. Natl. Acad. Sci. USA* **1995**, *117*, 92.
9. Arya, D. P.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 12.
10. Arya, D. P.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 6619.
11. Arya, D. P.; Bruice, T. C. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4384.
12. Blasko, A.; Dempcy, R. O.; Minyat, E. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1996**, *118*, 7892.
13. Blasko, A.; Dempcy, R. O.; Minyat, E. E.; Bruice, T. C. *Biochemistry* **1997**, *36*, 7821.
14. Kim, S.; Yi, K. Y. *J. Org. Chem.* **1986**, *51*, 2613.
15. Barawkar, D. A.; Bruice, T. C. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1104.
16. Atkinson, T.; Smith, M. In *Solid-Phase Synthesis of Oligodeoxvribonucleotides by the Phosphitetriester Method*; Gait, M. J. Ed.; Oxford University Press: New York, 1990; pp 35.
17. **9a** (after removal from CPG) was dissolved in DMSO and diluted with 15 mM phosphate buffer.