Solid-Phase Synthesis of Oligopurine Deoxynucleic Guanidine (DNG) and Analysis of Binding with DNA Oligomers.

Studies towards a new antisense therapeutic agent.

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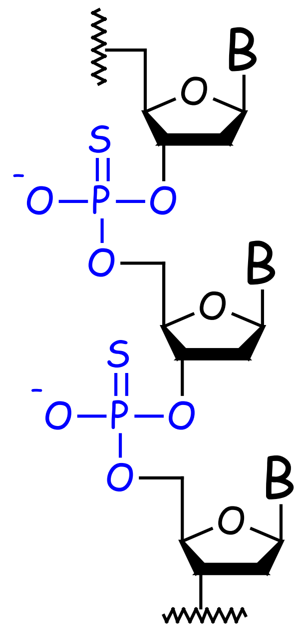
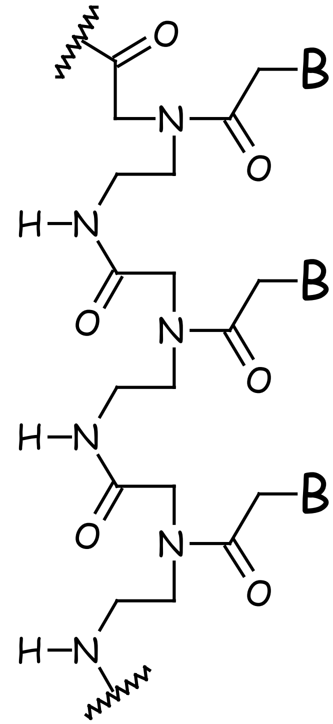
DNA Backbone Analogues

- Amides
- Phosphonates
- Carbamates
- Methylene-methylamino
- Heterocycles
- Acetals
- Peptide nucleic acids



Thiophosphate



PNA

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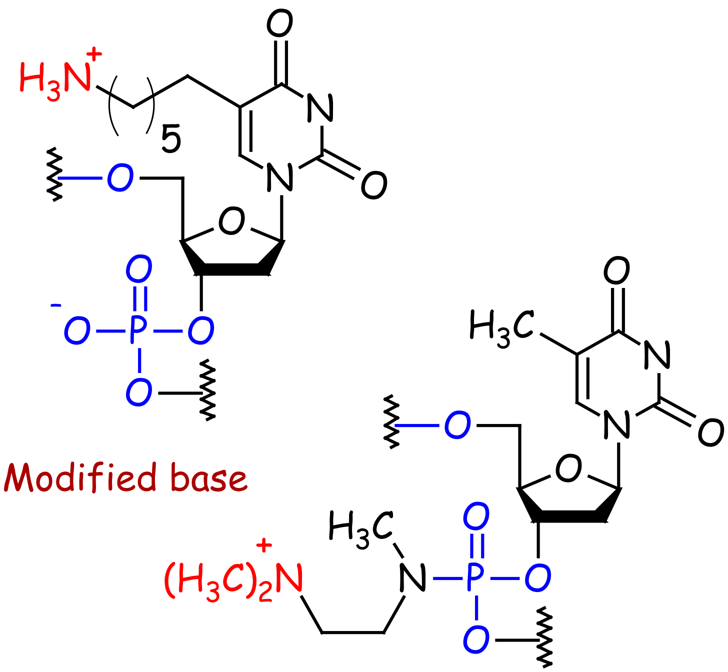
Positively Charged Oligonucleotides

- Modified bases (zwitterionic DNA) [1]
- Modified phosphate backbone [2]
- Positively charged backbone
  - DNmT [3]
  - DNG



Modified base

Modified backbone

(1) Hashimoto, H., Nelson, M.G. and Switzer, C. (1993) *J. Am. Chem. Soc.*, **115**, 7128-7134
(2) Letsinger, R. L., Singman, C. N., *et. al* (1988) *J. Am. Chem. Soc.*, **110**, 4470-4471
(3) Arya, D.P. and Bruice, T.C. (1999) *J. Am. Chem. Soc.*, **121**, 10680-10684.

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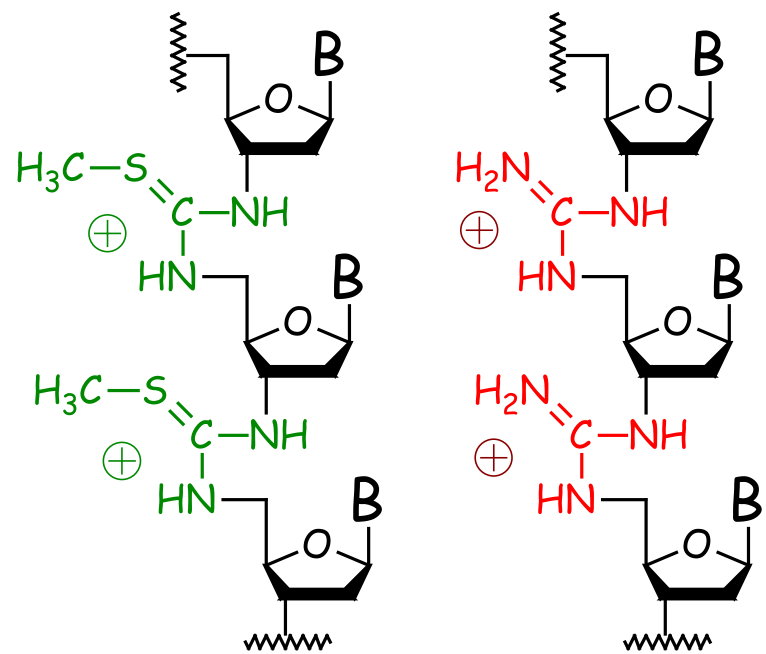
Positively Charged Backbones

- DNmT
  - Deoxyribonucleic Methylated Thioureas
- DNG
  - Deoxyribonuceic Guanidinium



DNmT DNG

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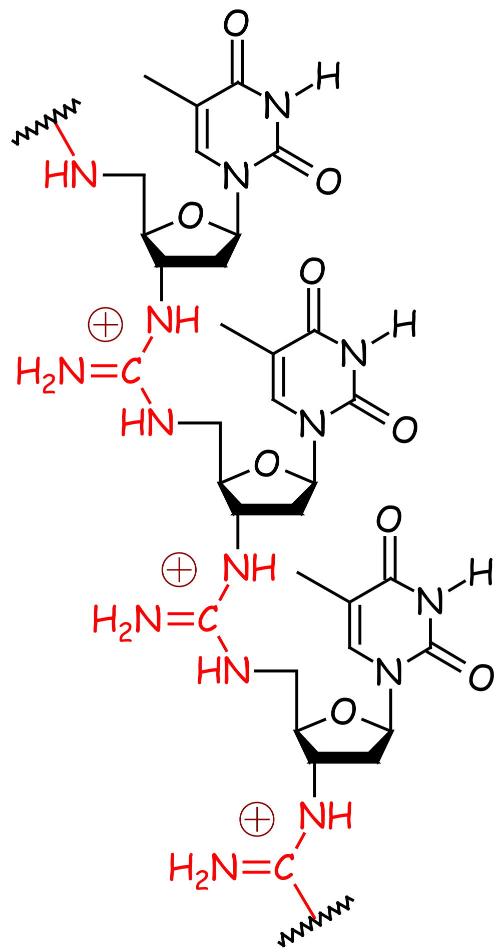
Deoxyribonucleic Guanidinium

- DNA backbone analogue
- Guanidinium internucleoside linkages
  - Positively charged
  - Achiral



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Properties of DNG

- Soluble
- Resistant to nucleases [1]
- High affinity for complementary DNA [2]
- Oligothymidyl DNG will form triple helix with adenyl DNA [3]

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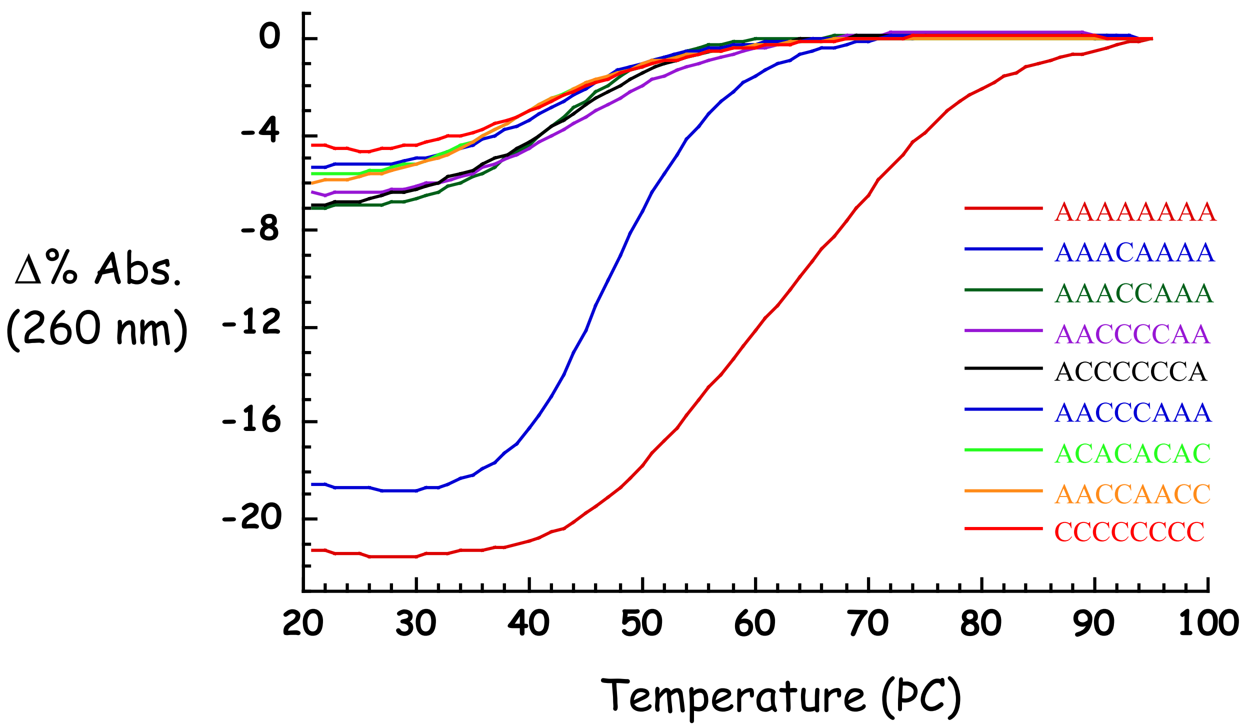
(1) Barawkar, D. A. and Bruice, T. C. (1998), *Proc. Natl. Acad. Sci. USA*, **95,** 11047-11052.
(2) Linkletter, B. A., Szabo, I. E. and Bruice, T. C. (1999) *J. Am. Chem. Soc.*, **121,** 3888-3896.
(3) Blaskó, A., Minyat, E. E., Dempcy, R. O. and Bruice, T. C. (1997) *Biochemistry*, **36,** 1821-1831.

Affinity for Complementary DNA



Thermal melting curves for DNA:DNG association. 1.25 μM DNA oligomer, 2.5 μM **DNG T_8**, 10 mM $KHPO_4$ buffer, pH 7.4, 0.1 M KCl. Rate of heating was 0.2 °C/min. [1]

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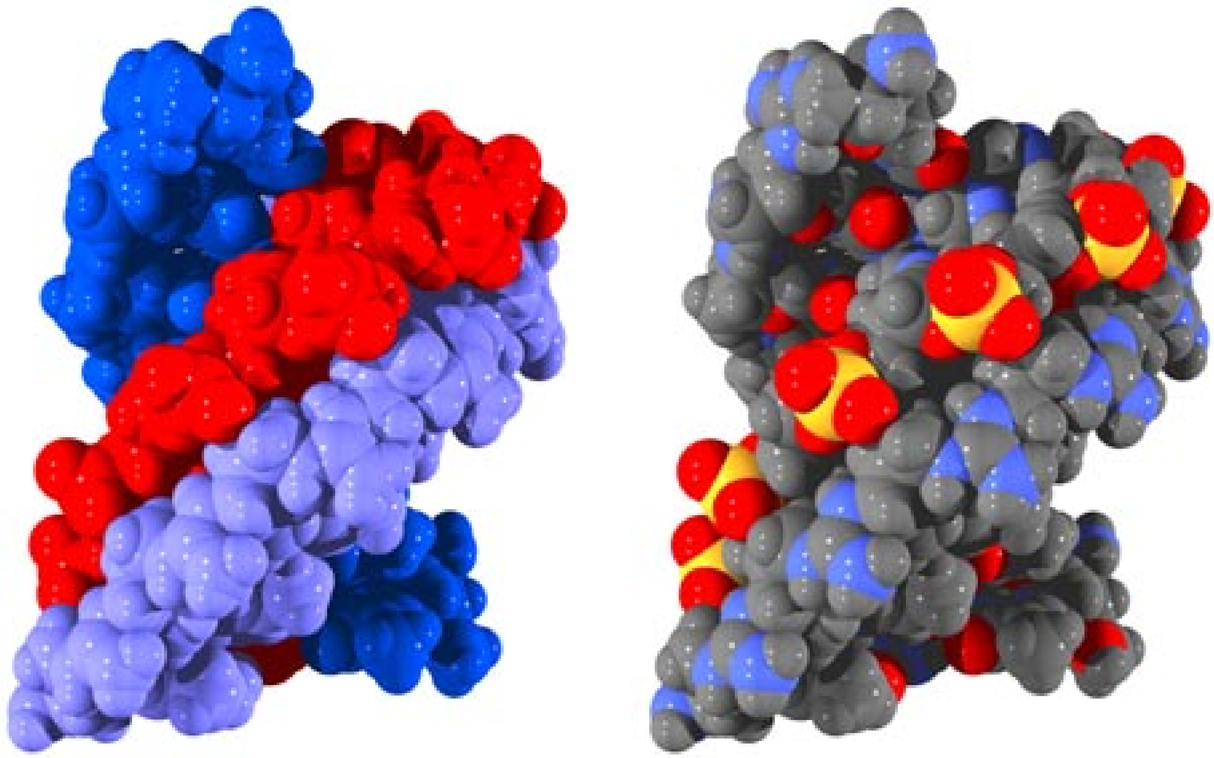
(1) Linkletter, B. A., Szabo, I. E. and Bruice, T. C. (1999) *J. Am. Chem. Soc.*, **121**, 3888-3896.

DNG_2:DNA Triple Helix



DNG T_8 with DNA A_8 computer simulation [1]

(1) Luo, J. and Bruice, T. C. (1998) *J. Chem. Soc.*, **120**, 1115-1123.

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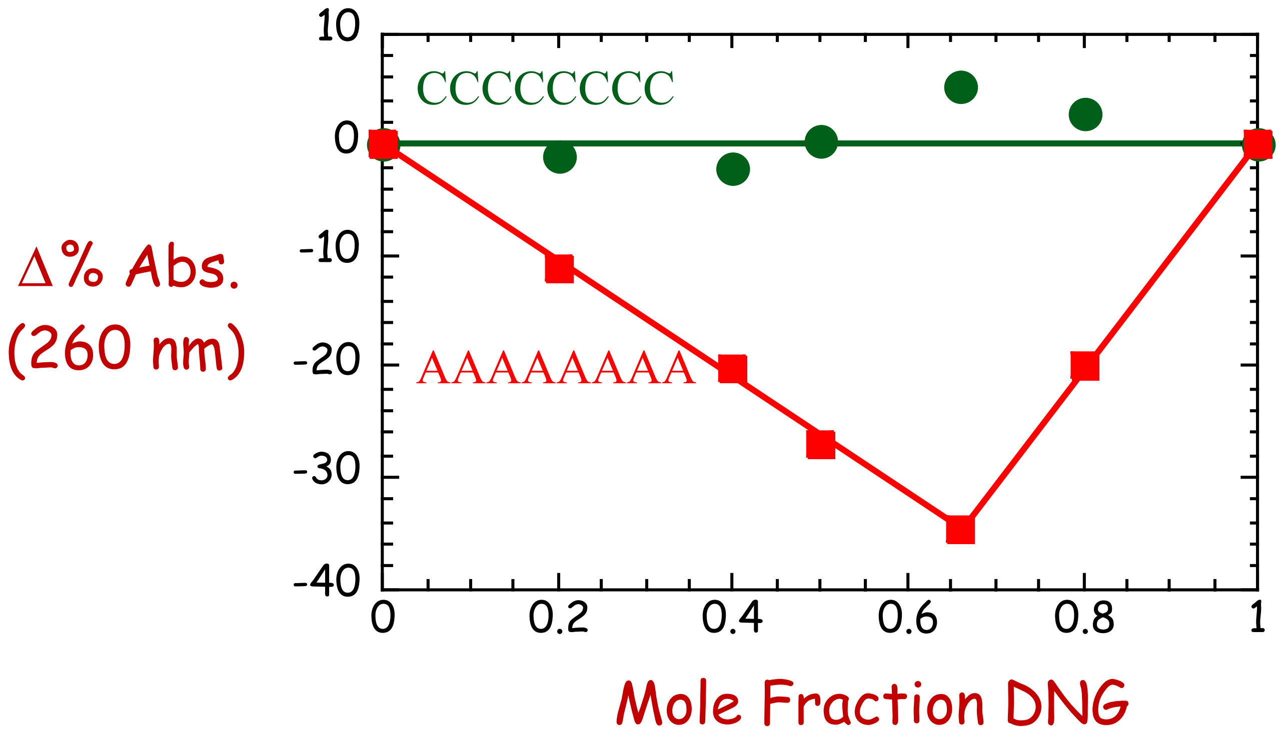
DNG_2:DNA Triple Helix



Job plots for **DNG T_8** with **A_8** and **C_8** DNAs. $[\text{oligomer}]_T$ = 2.0 uM, $[KHPO_4]$ = 10 mM, [KCl] = 0.1 M, pH 7.4, λ = 260 nm. [1]

(1) Linkletter, B. A., Szabo, I. E. and Bruice, T. C. (1999) *J. Am. Chem. Soc.*, **121**, 3888-3896.

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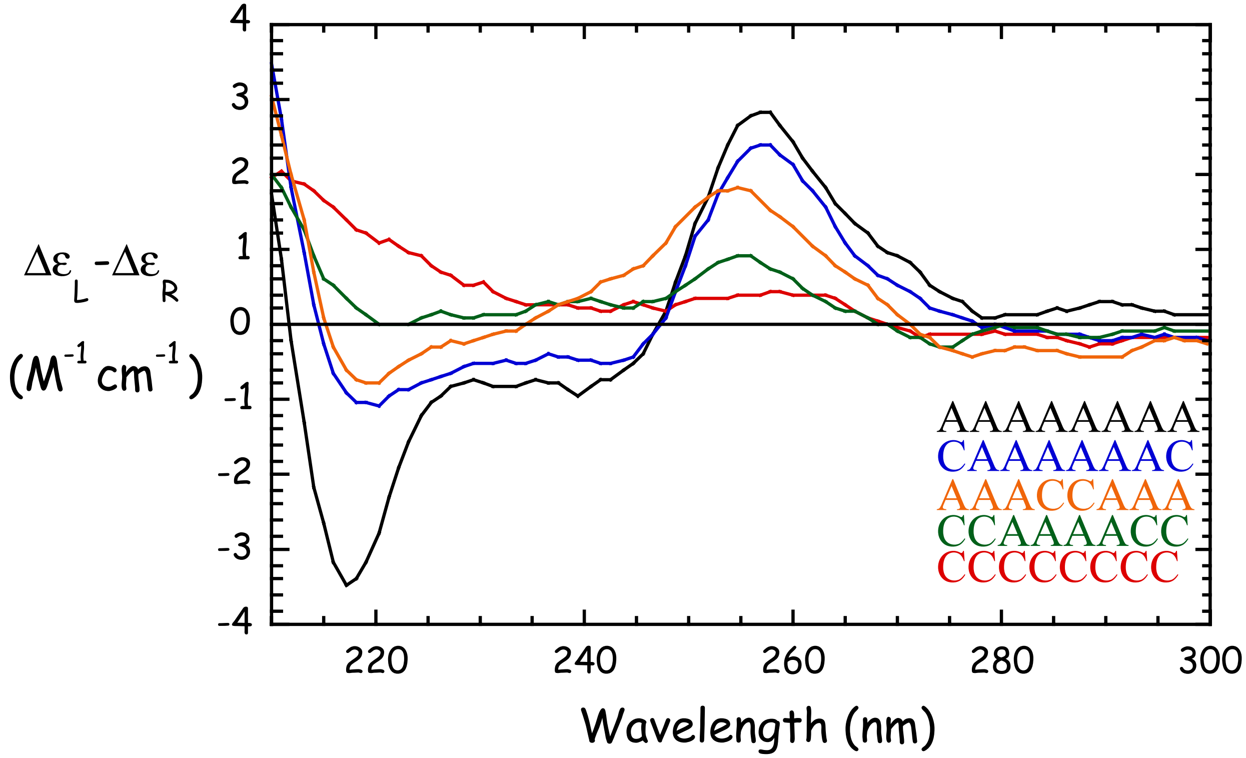
Affinity for Complementary DNA



CD difference spectra of 2:1 mixture of **DNG T_8** and DNA oligomers. Calculated CD spectra for the combined unassociated oligomers are subtracted from the measured CD spectrum. [1]

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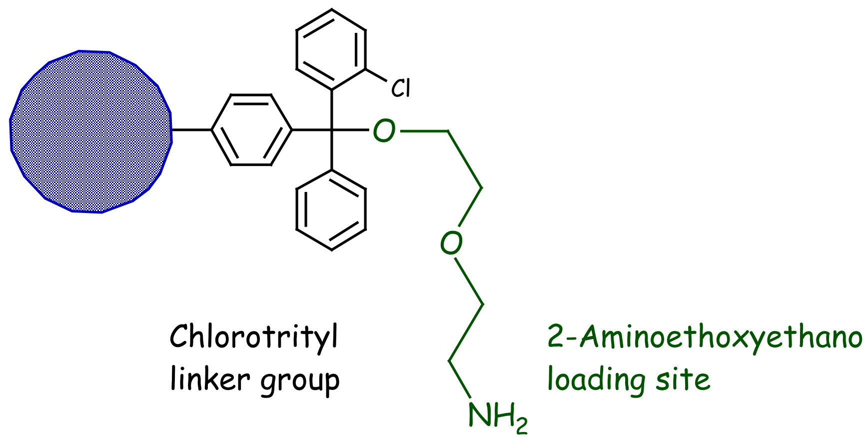
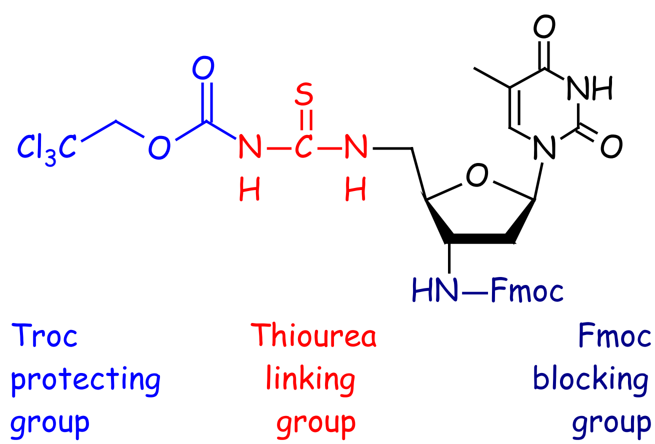
(1) Linkletter, B. A., Szabo, I. E. and Bruice, T. C. (1999) *J. Am. Chem. Soc.*, **121**, 3888-3896.

Peptide Compatible DNG Synthesis

- Polystyrene resin with acid-labile chlorotrityl linker
- Base labile Fmoc blocking group
- Trichloroethoxy-carbonyl (Troc) Protecting groups
- Activated carbodiimide linking chemistry





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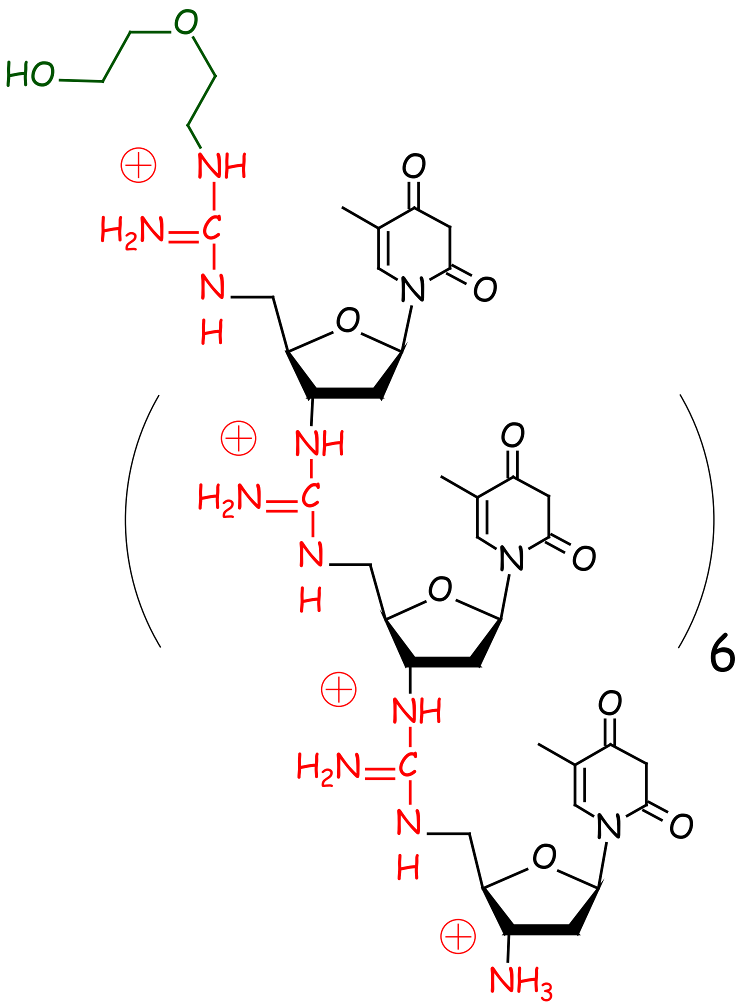
Peptide Compatible DNG Synthesis

- Monomer added to loading group via mercury abstraction of the sulfur.
- Fmoc deblocking group removed by brief treatment with piperidine.
- Mercury ppte removed by Thiophenol.
- Cycle repeated
- Cleaved from resin by 0.5% TFA
- Troc removed by mild Cd/HOAc oxidation



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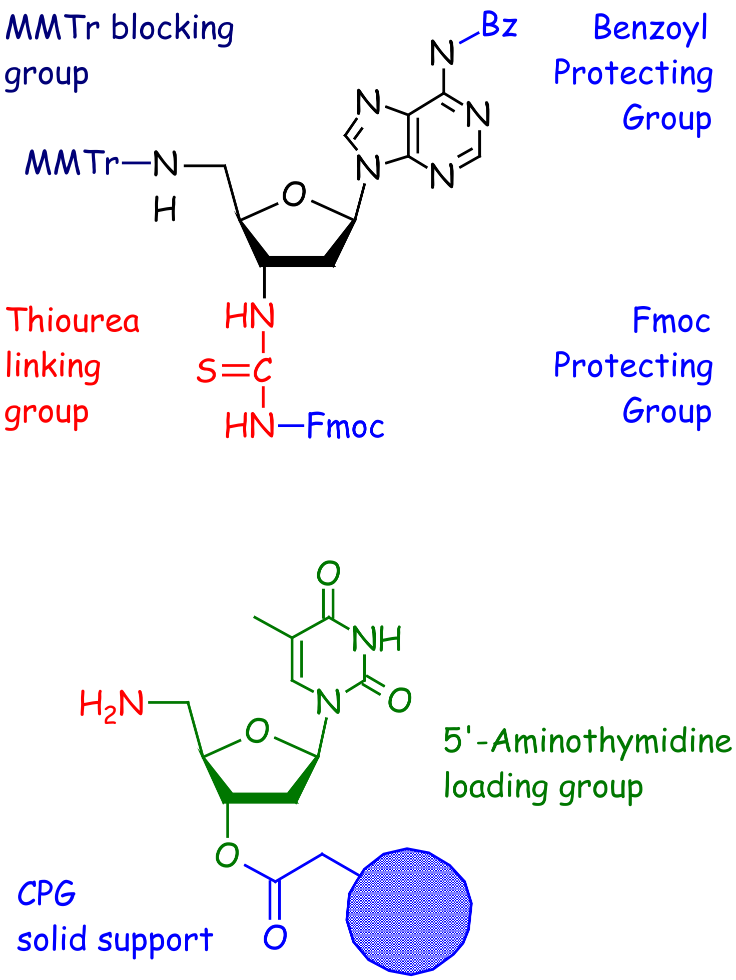
DNA Compatible Synthesis

- Basic coupling conditions for stability of purine bases.
- Acid labile MMTr blocking groups
- Basic cleavage/ deprotection



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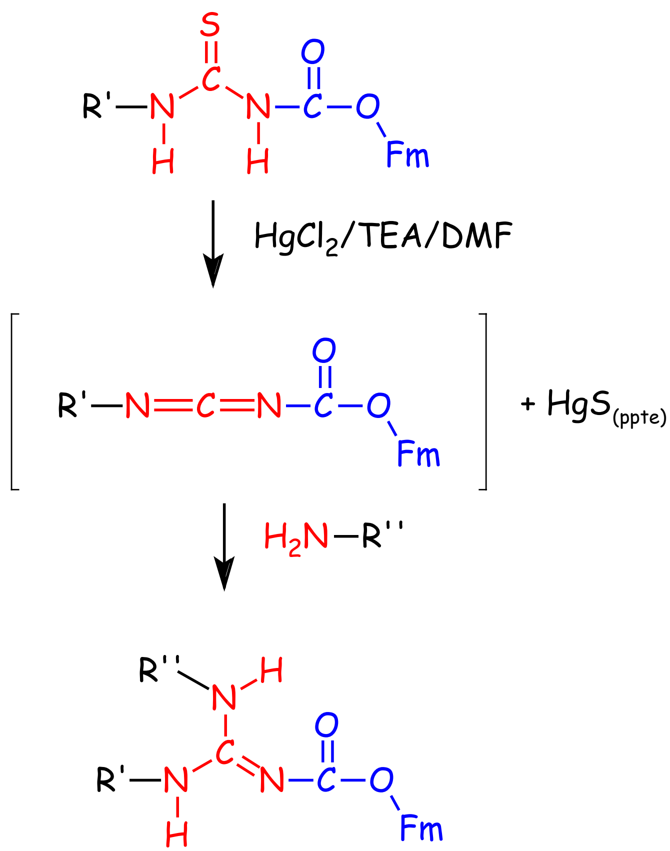
Coupling Chemistry

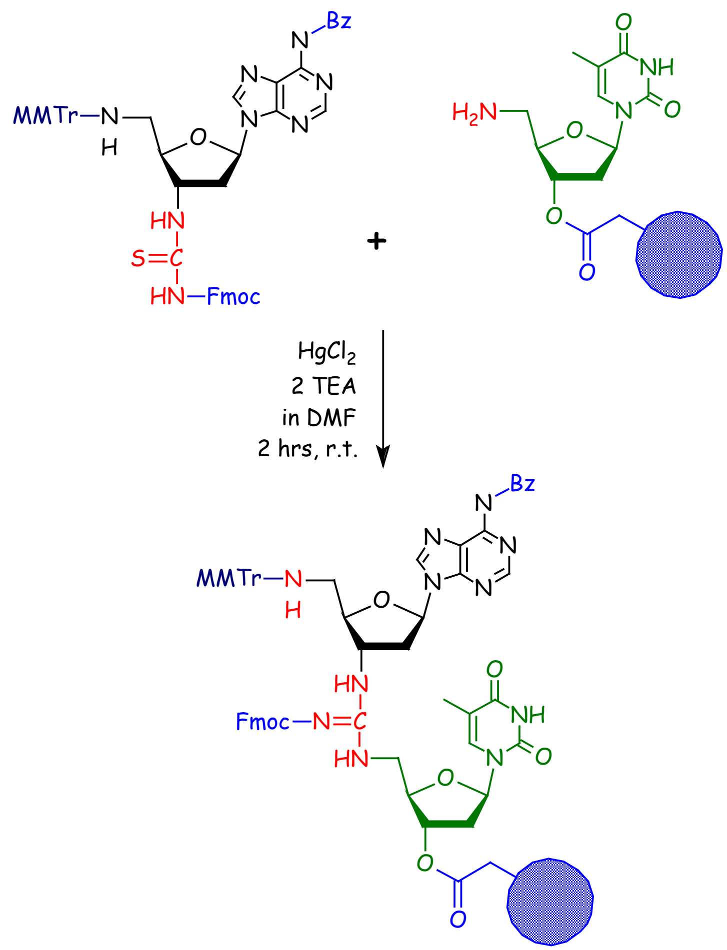
- Abstraction of sulfur from thiourea by mercury (II) gives carbodiimide and $HgCl_2$.[1]
- Electron withdrawing character of carbonyl of Fmoc protecting group activates carbodiimide for nucleophilic attack.
- Terminal amino group of growing oligomer attacks and forms protected guanidinium group.



(1) Robinson, S. and Roskamp, E. J. (1997) *Tetrahedron*, **53**, 6697-6705.
(2) Kim, K. S. and Qian, L. (1993) *Tetrahedron Lett.*, **34**, 7677-7680.

Synthesis Cycle

A. Couple monomer to terminal amine with 1 equiv. $HgCl_2$ and 2 equiv. TEA in DMF.
B. Remove $HgCl_2$ ppte. with 10% thiophenol in DMF
C. Deblock the new terminal amine with 3% DCA in DCM.
D. Repeat, repeat...



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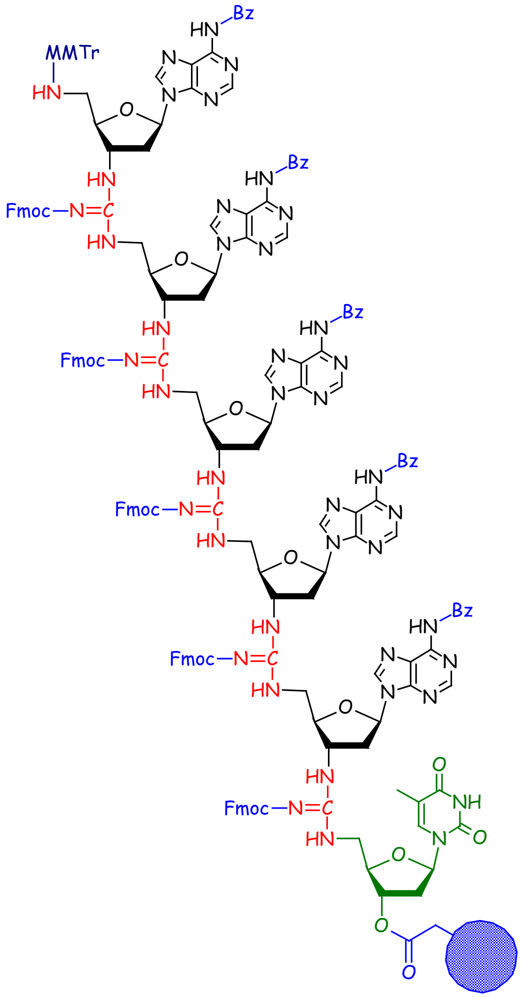
Cleavage and Deprotection

A. Final MMTr group is left on the terminal amino group.
B. Exposure to conc. ammonia/MeOH at 60 °C for 16 hrs
C. Purified by RP HPLC and the fraction containing a trityl group collected (trityl-on purification).
D. MMtr removed with HOAc treatment.

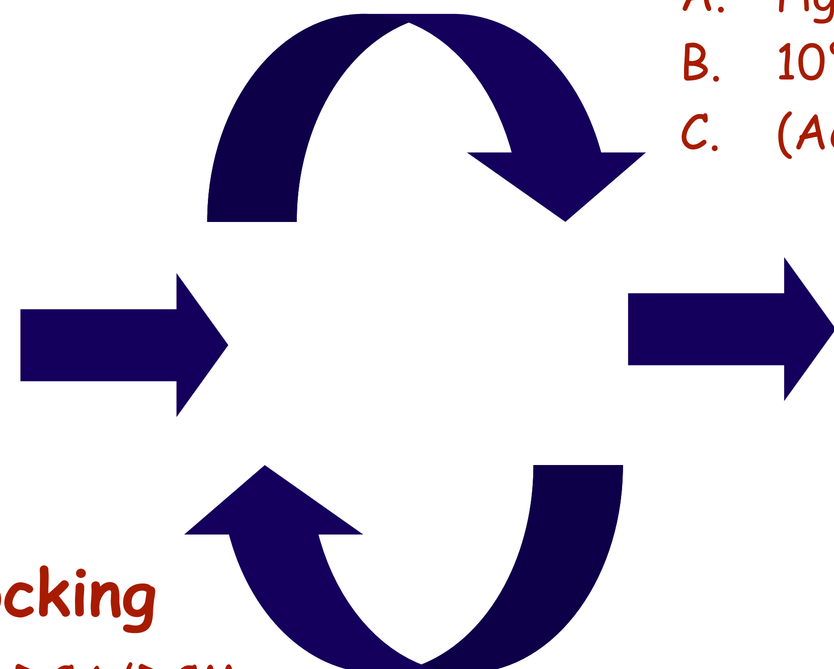


Overview of Synthesis Cycle

Coupling (95-99%)

A. $HgCl_2$/TEA/DMF
B. 10% thiophenol/DMF
C. $(Ac)_2O$/TEA/DMF

Loading



Cleavage/ Deprotection/ Detritylation

Deblocking

3% DCA/DCM

Isolated yield of TAAAA DNG: 27% (optical density)

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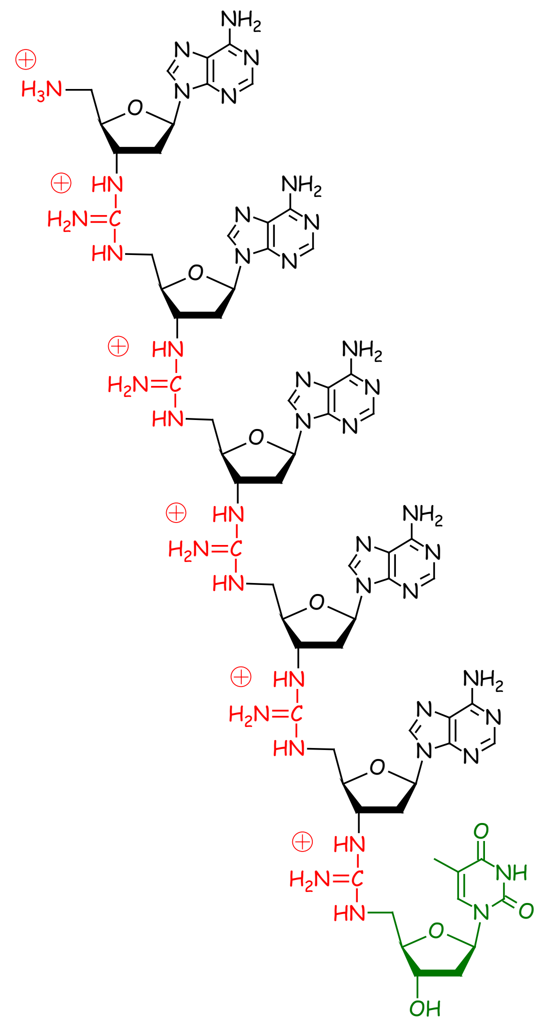
Oligoadenyl DNG

- Pentameric adenyl tract in a positively charged hexameric DNG molecule.
- 3'-terminal hydroxy group
- 5'-terminal amino group.



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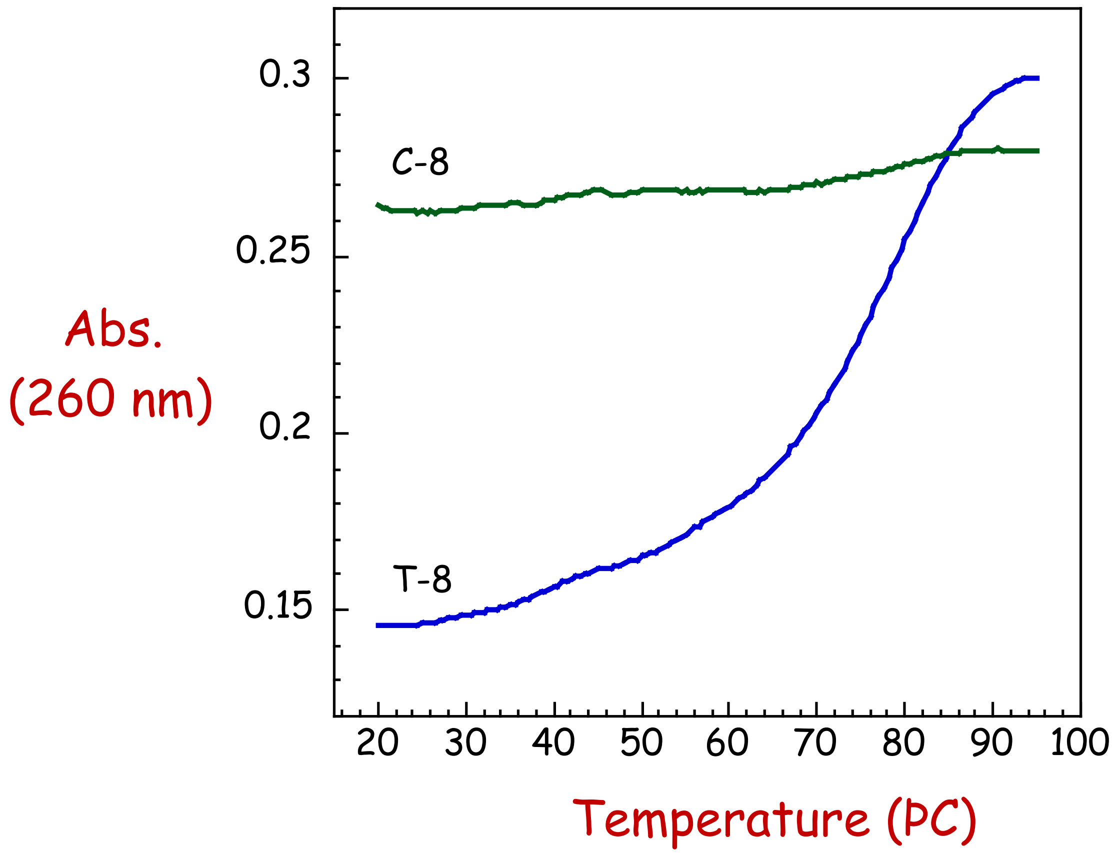
Properties of Oligoadenyl DNG



Thermal denaturation plots for DNA:DNG association. 2.0 μM DNA oligomers **C-8** and **T-8**, 2.0 μM DNG **TAAAAA**, [$KHPO_4$] = 10 mM, [KCl] = 0.1 M, pH 7.0. Observed at λ = 260 nm. Rate of heating was 0.2 °C/min.

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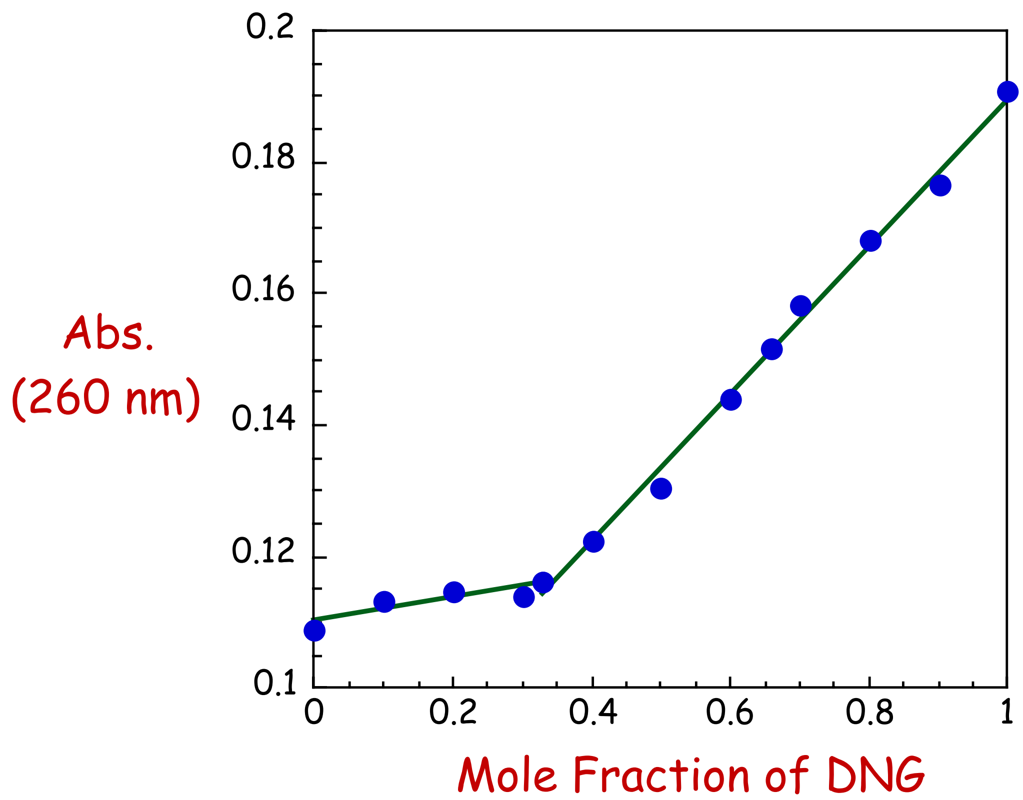
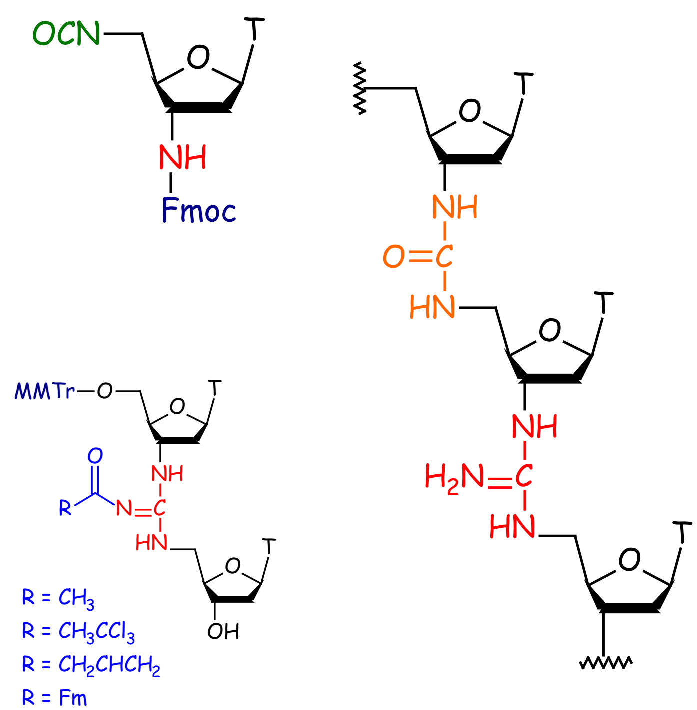
Properties of Oligoadenyl DNG



Job plots for DNG **TAAAAA** with DNA **T-5** plotted by %hyperchromicity. [oligomer] = 2.0 uM, [$KHPO_4$] = 10 mM, [KCl] = 0.1 M, pH 7.0, 25 °C, observed at 260 nm.

Mixing and Matching

- Urea linkages can be included by using isocyanate monomers
- DNG can be included into DNA using DNG dimer in standard DNA SP synthesis.



(1) Linkletter, B. and Bruice, T. C. (2000), *Bioorg. Med. Chem.* 1893-1901.

(2) Barawkar, D. A., Linkletter, B. and Bruice, T. C. (1998) *Bioorg. Med. Chem. Lett.*, **8**, 1517-1520.

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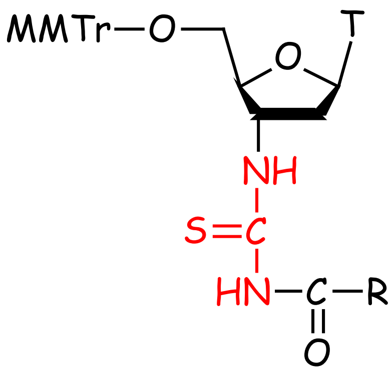
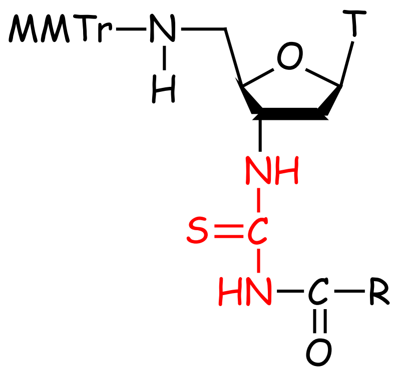
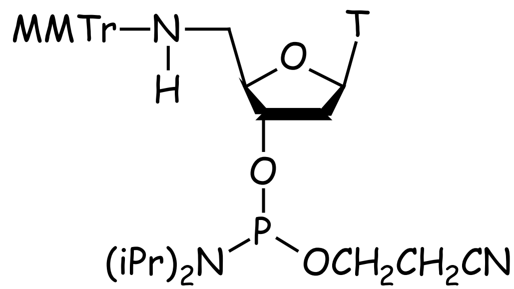
Stepwise Synthesis of DNG/DNA



DNG to DNA



DNG to DNG



DNA to DNG

R = CH_2CCl_3
R = Fm

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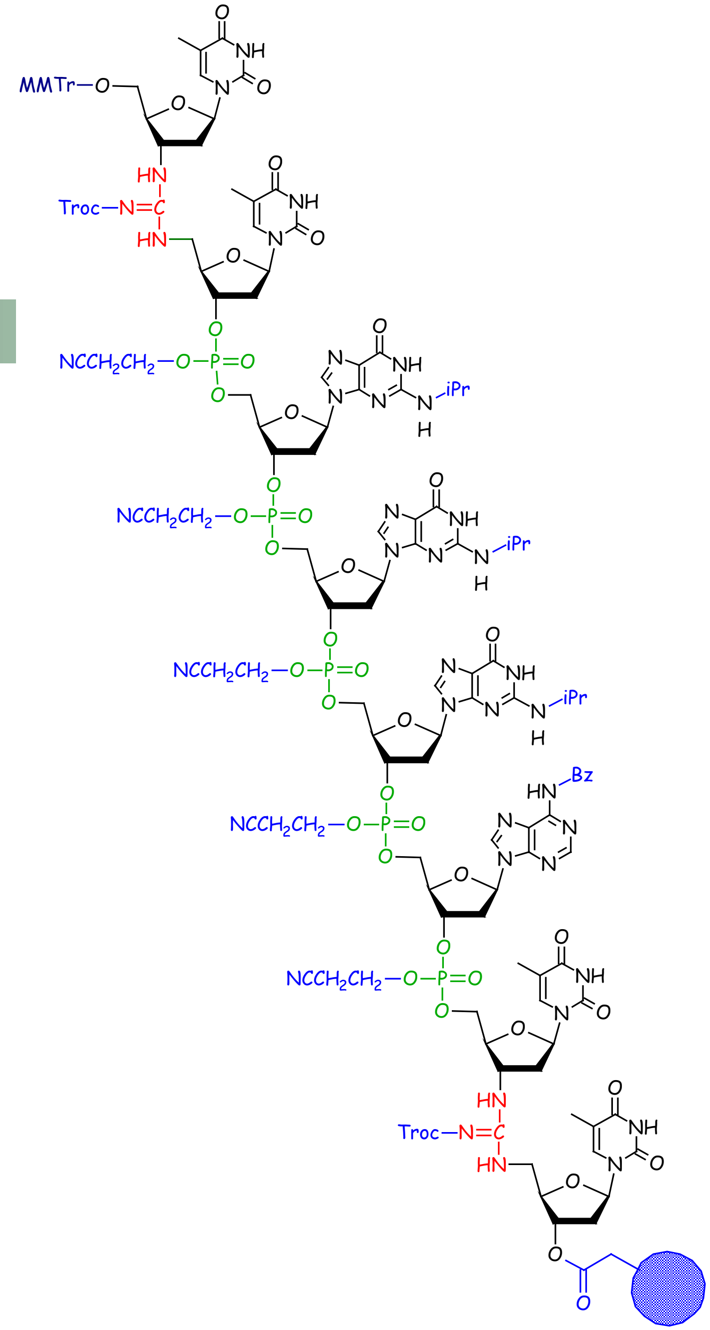
DNA/DNG

- TgTAGGGTgT (complementary to human telomerase template sequence AATCCC
- Coupling yields
  - 1 (DNG-A) 94%
  - 2-5 (DNA) 96% Ave.
  - 6 (DNA-G) 85%
  - 7 (DNG) 95%



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Current Capabilities and Goals

- Synthesis of DNG/peptide and DNG/DNA oligomers now possible
- Mixed sequence DNG oligomers
  - A, C, and T currently available
  - G on the way
- Self complementary DNG oligomers
  - Will DNG form a duplex with itself?
  - $(gAgT)_n$ being synthesized
  - is the DNG world a happier place?

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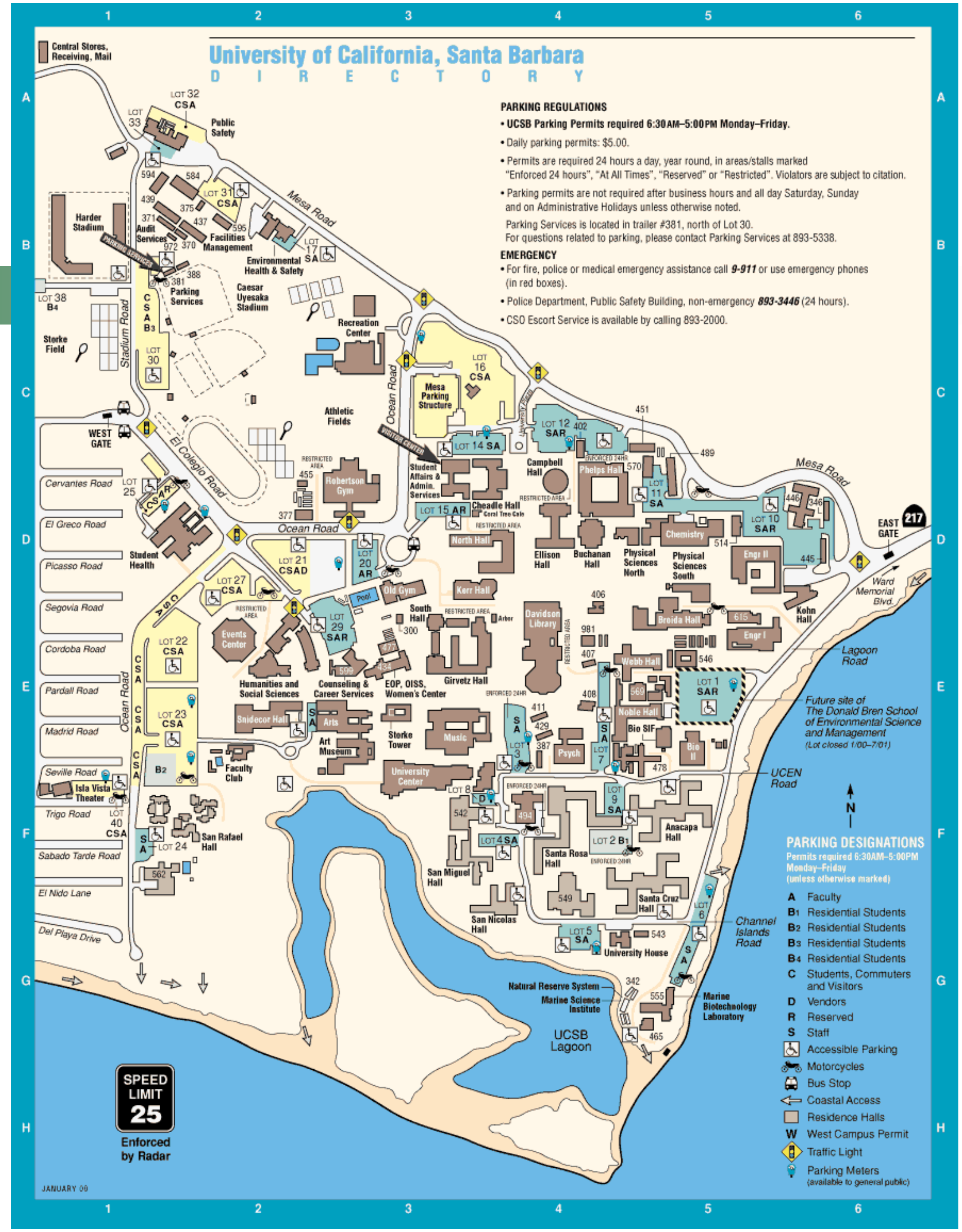

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Thank You

- Prof. Thomas C. Bruice
- Istvan Szabo
- Jia Luo, Helgi Adalsteinson
- NIH, UC Biostar, Genelabs Technologies

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