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Synthesis of an Amide-Based Extended Heterocyclic System Capable of Hydrogen Bonding to Both the Adenine and Uracil in dsRNA for RNA Recognition Using PNA

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Synthesis of an Amide-Based Extended Heterocyclic System Capable of Hydrogen Bonding to Both the Adenine and Uracil in dsRNA for RNA Recognition Using PNA

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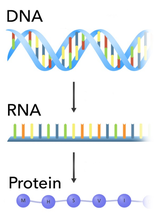
Abstract

The majority of information known about RNA is centered around coding RNA for its role in synthesizing proteins from DNA. However, noncoding RNA is also biologically relevant, showing importance in gene expression and catalyzing reactions. Peptide Nucleic Acid (PNA) is a promising tool that can be used to study noncoding RNA. PNA can bind to double-stranded RNA forming a triple helix and is highly selective for specific sequences of dsRNA. A current limitation of PNA is that traditional nucleobases only bind with high affinity to purine residues on the RNA, as triplex formation relies on the two hydrogen bonding sites offered by purines as opposed to only one offered by pyrimidines. More recent developments in our group and others have shown that synthetic nucleobases may be used to increase both affinity and selectivity. Ab initio computations suggest that a variation of the uracil nucleobase with an added benzamide moiety will bind to both the adenine and the uracil of the A-U base pair. We have synthesized this uracil nucleobase (**T3**) by adding a benzamide moiety to isoorotic acid. This modification is predicted to increase the affinity of binding and make PNA relevant for use in dsRNA sequences containing both purine and pyrimidine bases.

RNA: More Than Messengers

- About 80% of DNA in humans is transcribed into RNA
- Known as the 'central dogma', coding RNA acts as a messenger and translates proteins
- Only ~1.5% of DNA transcribes coding RNA
- RNA that doesn't translate proteins is considered 'non-coding' RNA



Non-coding RNA is also important in cellular function:

Catalyzing chemical reactions	Regulating Gene Expression	Post-Transcription Modification	*Mutations in non-coding RNA could be linked to diseases

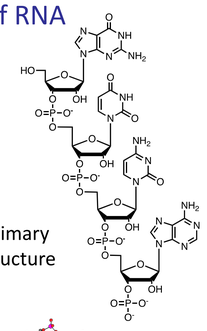
Thus, finding a reliable method for studying RNA is a critical part of understanding its functionality and significance

Your Genome. https://www.yourgenome.org/facts/ what-is-the-central-dogma. Accessed 1 Apr 2019.
Sharp, P. A. Centrality of RNA. *Cell*, **2009**, *136*, 577–580.

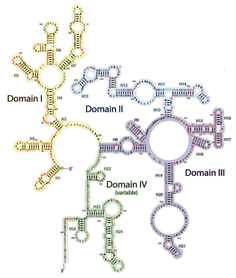
Unique Structural Properties of RNA

Certain characteristics of RNA make it a challenging target for shape and sequence selective recognition with high affinity:

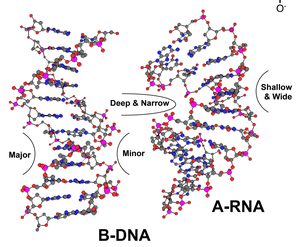
- RNA often folds in on itself, resulting in a variety of shapes and forms such as hairpins, loops, and bulges
- Due to RNA's uniform, negatively charged backbone, double-stranded regions form a deep and narrow groove



Primary Structure

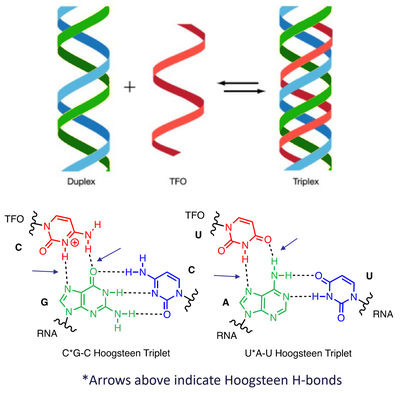


Secondary Structure



Tertiary Structure

Triplex Formation via Hoogsteen Complexes



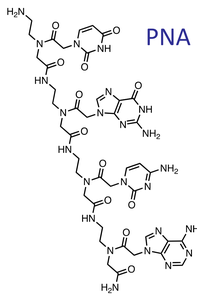
*Arrows above indicate Hoogsteen H-bonds

- dsRNA can form natural triple helices
- Triplex Forming Oligonucleotides (TFOs) were developed for dsDNA recognition
- In a triplex, hydrogen bonds form between bases of the TFO and the Hoogsteen face of Watson-Crick base pairs
- Pi-stacking interactions also stabilize triplex
- Traditional TFOs don't work with dsRNA due to negative electrostatic repulsions

Novikova, I. V.; Hennelly, S. P.; Tung, C.-S.; Sanbonmatsu, K. Y. *J. Mol. Biol.* **2013**, *425*, 3731.

Peptide Nucleic Acid (PNA): An Innovative Solution

The backbone of PNA is comprised of neutral peptide bonds instead of charged phosphodiester bonds

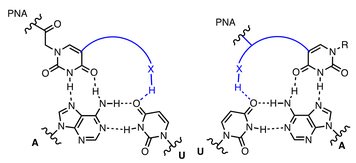


- Neutrality resolves binding concerns
  - Flexibility to conform to variable dsRNA structure
  - No electrostatic repulsion
  - Forms stable triplexes
- PNA can be prepared with either traditional or modified nucleobases

Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science*, **1991**, *254*, 1497.

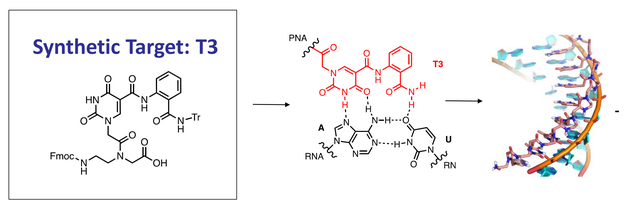
Modification is Key

- Currently, PNA can only be used in sequences that are purine rich - pyrimidines only offer one site for hydrogen bonding
- Our objective is to modify the uracil base to increase binding affinity through a scaffolding of amide moieties
- Scaffolding will allow attachment to PNA from either side



- **T3** will hydrogen bond to both the adenine and uracil of the Watson-Crick base pair
- This will allow for recognition of either A or U in a dsRNA sequence

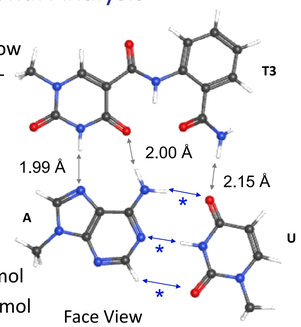
Synthetic Target: T3



Triplex photo: Kotikam, V.; Kennedy, S.; MacKay, J.; Rozners, E. *Chem. Eur. J.* **2019**, *25*, 4367-4372.

Computational Analysis

- Computational evaluation of how **T3** would interact with Watson-Crick A-U base pair
- Geometry Optimization Calculations
- Hartree-Fock Theory
- 6-31G(d) Basis Set
- WebMO Software, Gaussian
- U*A-U Binding Energy: -47 kJ/mol
- T3*A-U Binding Energy: -67 kJ/mol

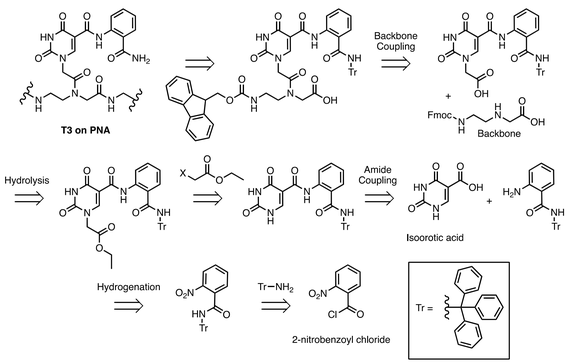


*Stars indicate Watson-Crick H-bonds

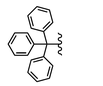
Out-of-Plane View

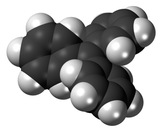
- Planarity indicates likelihood of favorable pi-stacking interactions in triplex

Retrosynthesis



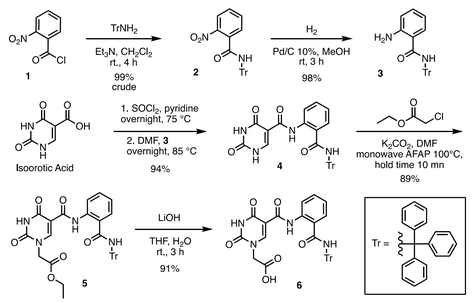
About the Triphenylmethyl (Trityl, Tr) Protecting Group



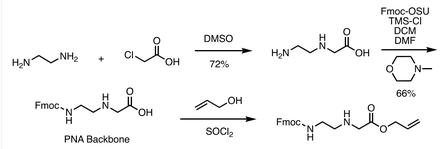


- Used for amide protection
- Polar basic chemistry keeps trityl attached
- Sterically protects amide but doesn't interfere with other chemistry involved
- Removed in acidic conditions – will be taken off in PNA synthesis when PNA is cleaved from resin

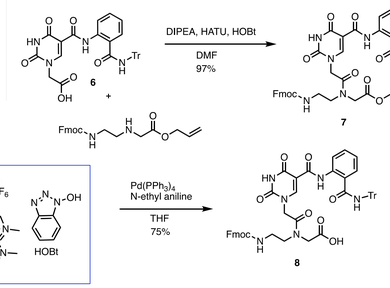
T3 Nucleobase Synthesis



Synthesis and Protection of Backbone

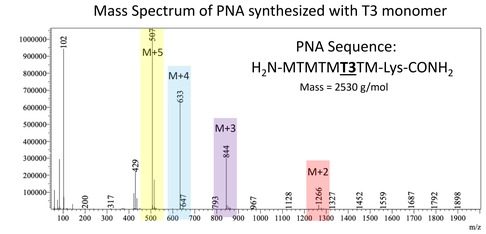


Putting It All Together: Coupling of **T3** Nucleobase to Peptide Backbone



PNA with T3 Nucleobase

Mass Spectrum of PNA synthesized with T3 monomer



Shimadzu LC/MS, Single Quad MS, Electrospray Ionization, Reversed Phase C18 HPLC Column, Water/Acetonitrile mobile phase with 0.1% formic acid buffer

Nikita Brodyagin, *unpublished.*

Results and Future Direction

- **T3** nucleobase has been successfully synthesized in 7 total steps with an overall yield of 54%
- Our collaborators have synthesized PNA with **T3** to test for binding affinity
- Alternative amide nucleobases are being explored

Acknowledgements

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- Binghamton University Department of Chemistry
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