Synthesis of a Modified Amide-based Extended Heterocyclic Nucleobase Capable of Hydrogen Bonding to the U-A Base Pair in Double Stranded RNA

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Background

Increased interest in non-coding RNA and its involvement in a multitude of cellular processes has led to a need for a technique to selectively track RNA sequences. Many non-coding RNAs fold into double helices which allows for a triplex forming oligonucleotide (TFO) approach using Peptide Nucleic Acids (PNA) which can be synthetically modified.

Nucleobases to discern purines have been previously developed. However, there has been difficulty discovering nucleobases that selectively bind to pyrimidines

Triplex Formation via Hoogsteen Complexes

- dsRNA can form natural triple helices
- Triplex Forming Oligonucleotides (TFOs) are used 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

Tertiary Structure of RNA

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

Peptide Nucleic Acids (PNA): A Solution

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

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

Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, 254, 1497.
Li, M.; Zengaya, T.; Rozners, E. *J. Am. Chem. Soc.* **2010**, 132, 8676.

Modified PNA and Sequence Selectivity

Zengaya, T.; Gupta, P.; Rozners, E. *Angew. Chem., Int. Ed.* **2012**, 51, 12593.
Hnedzko, D.; Rozners, E. *Methods in Enzymology RNA Recognition* **2019**, 401.

Extended Nucleobase Design

- An extended nucleobase design can be utilized to afford three hydrogen bonds

Kumpina, I.; Brodyagin, N.; Mackay, J. A.; Kennedy, S. D.; Katkevics, M. *J. Org. Chem.* **2019**, 84, 13267.

- The same scaffold can be used for the recognition of both nucleobases
- T3 will bond across adenine and uracil

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

T3 Retrosynthesis

Synthesis and Protection of PNA backbone

Wojciechowski, F. and Hudson, R.H.E. *J. Org. Chem.* **2008**, 73, 3807.
Coull, J. D.; Elghorm, M.; Hodge, R.P.; Ismail, M.; Rajur, S. B. U.S. Patent 6,133,444, Oct. 17, 2000.

Synthesis of T3 and Backbone Coupling

PNA Stand with T3 Nucleobase

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

ITC Binding studies HRP1 and PNA containing T3

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N-phenylisoorotamide Pi Stacking or Hydrogen Bonding?

ITC Data from the T-series (T1, T2, & T3) revealed that the third hydrogen bond in the extended system may be less important to the overall thermodynamics than the pi stacking interactions between the levels of nucleobases.

Emily Kagarise
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- Thus, N-phenylisoorotamide is being synthesized to explore this possibility.

N-phenylisoorotamide Synthesis and Backbone Coupling

Results and Future Directions

- T3 has been synthesized and incorporated into a PNA strand
- Initial Binding studies of T3 show strong affinity for U-A base pair
- Synthesize N-phenylisoorotamide control to determine importance of third hydrogen bond relative to pi stacking interaction
- Synthesis of monomers with various hydrogen bond donor functionalization