

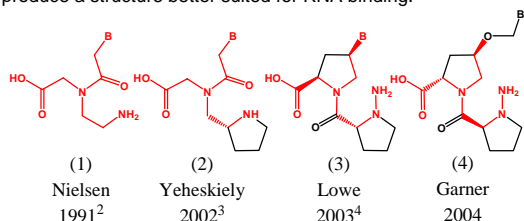
# A New Twist on Antisense: Proline-Proline Nucleic Acids (ProNAs)

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## Introduction – Pyrrolidine Based PNAs

The advent of peptide nucleic acids has triggered much research activity towards the development of PNA based antisense therapeutics. Ideally, a well designed PNA should exhibit selective binding to complementary mRNA sequences and be amenable to chemical modification. We now report the design and synthesis of a novel PNA building block with a proline-proline core structure. This new platform, termed ProNA, merges Lowe's backbone with our hydroxymethyl linker<sup>1</sup> to produce a structure better suited for RNA binding.



## Tuning the ProNA – Energy studies

Modeling studies indicate that ProNAs are geometrically tailored to bind RNA. Using Spartan<sup>®</sup>, in silico models of **3** and **4** were assembled. Their nucleobases were constrained in the correct positions for Watson-Crick base pairing with A-helical RNA and B-helical DNA. The equilibrium geometries and heats of formation (kcal/mol) were determined using Spartan's<sup>®</sup> Semi-Empirical modeling environment. By varying the relative configurations of the chiral centers, we were able to arrive at an optimal design. Table 1 compares our current design (**4**) with that of Lowe (**3**).

Lowe's PNA is reported to not bind to RNA. Because the heat of formation of Lowe's PNA-RNA complex is higher than our ProNA-RNA complex, we believe that our ProNA is more likely to bind to RNA experimentally. The energy difference may reflect the inability of Lowe's scaffold to accommodate the more stable *trans*-amide bond (Figure 1).

Table 1 – Calculated Heats of Formation (Kcal/mol) after energy minimization

Backbone	A – Helical RNA Binding	B – Helical DNA Binding
ProNA	-187.00 kcal/mol	-139.00 kcal/mol
Lowe's PNA	-110.54 kcal/mol	-79.08 kcal/mol

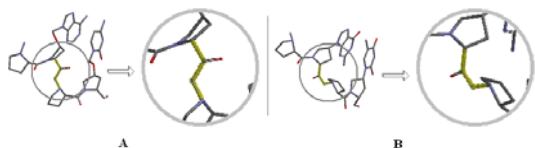
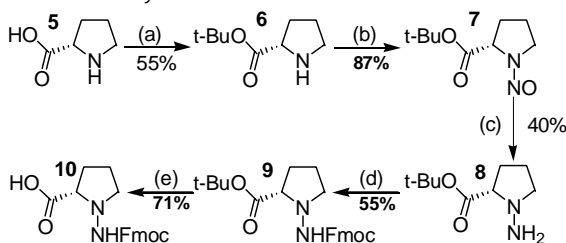


Figure 1 - A) *trans*-amide bond in ProNA-RNA complex  
B) *cis*-amide bond in Lowe's PNA-RNA complex

## Synthesis of ProNA Building Blocks

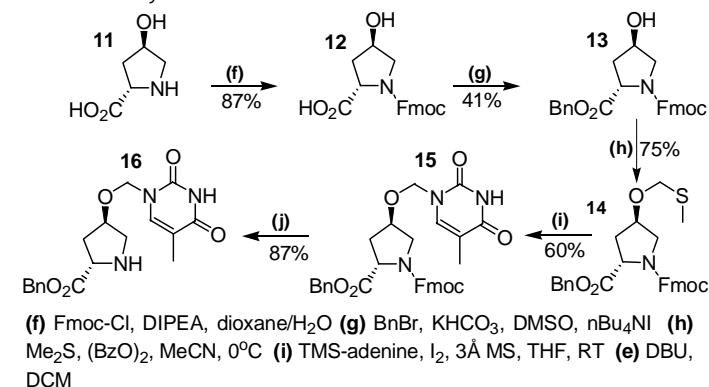
The ProNA building block (**4**) is considered to be a condensation between two unnatural amino acids (**10**, **15**). The syntheses of these building blocks is displayed in Schemes 1 and 2 and their union in Scheme 3. The thymine regiochemistry was established by a Heteronuclear Multiple Bond Correlation (HMBC) NMR experiment (Figure 2).

### Scheme 1 – Synthesis of 10

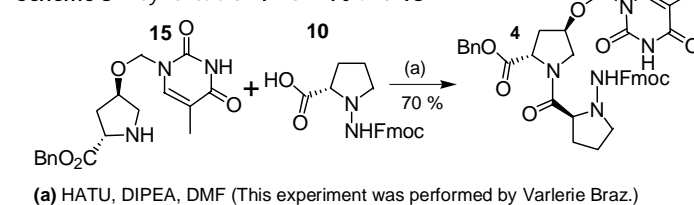


(a) 2-methyl propene, H<sub>2</sub>SO<sub>4</sub>, dioxane (b) SiO<sub>2</sub>-OSO<sub>3</sub>H, wet SiO<sub>2</sub>, NaNO<sub>2</sub>, DCM (c) HCl, Zn, MeOH -78°C (d) Fmoc-Cl, DIPEA, dioxane/H<sub>2</sub>O (e) TFA, DCM

### Scheme 2 – Synthesis of 15



### Scheme 3 – Synthesis of 4 from 10 and 15



## Confirmation of Thymine Regiochemistry in 16

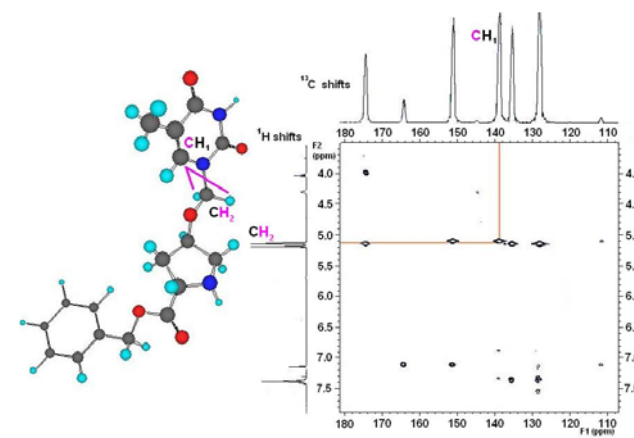


Figure 2 – HMBC plot for **16**. The diagnostic interaction for the thymine regiochemistry is indicated by the pink lines.

## Summary

We report the design and synthesis of a novel PNA building block with a proline-proline core structure. Modeling studies of the chirally optimized ProNAs indicate that they are geometrically tailored to bind RNA.

## Acknowledgements

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