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Synthesis and studies towards DNA incorporation of the base pair PyDDA-PuAAD.

Synthesis and studies towards the DNA incorporation of a carbocyclic 2'-O-methylated analogue to 6-amino-3-[β-D-ribofuranosyl]-5-methyl-pyrazine-2-one.
Synthesis and DNA incorporation of 2'-deoxy-5-aza-3,7-deaza-guanosine.

A dissertation submitted to the

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V. Summary

The synthesis of both molecules of a new DNA base pair (carbocyclic PyADD and PuDDA) was undertaken in order to explore their chemistry and to assess whether they would be suitable as components of a genetic information system. This included :

- synthesis of the two molecules;
- search for a protection strategy that would allow their DNA incorpotation by DNA automatic synthesis;
- synthesis of DNA oligonucleotides containing both molecules.

Carbocyclic pyrazine analogue

The synthetic route developed for the preparation of the carbocyclic pyrazine **Py1a** is shown in Figure 74 and begins with a Diels-Alder reaction between cyclopentadiene and trans-bromoacrylic acid. The product is then methylated and cis-dihydroxylated to yield a ribofuranose analogue, followed *in situ* by a β-elimination of the bromine group. The reaction must be buffered to hinder direct elimination during cis-hydroxylation.

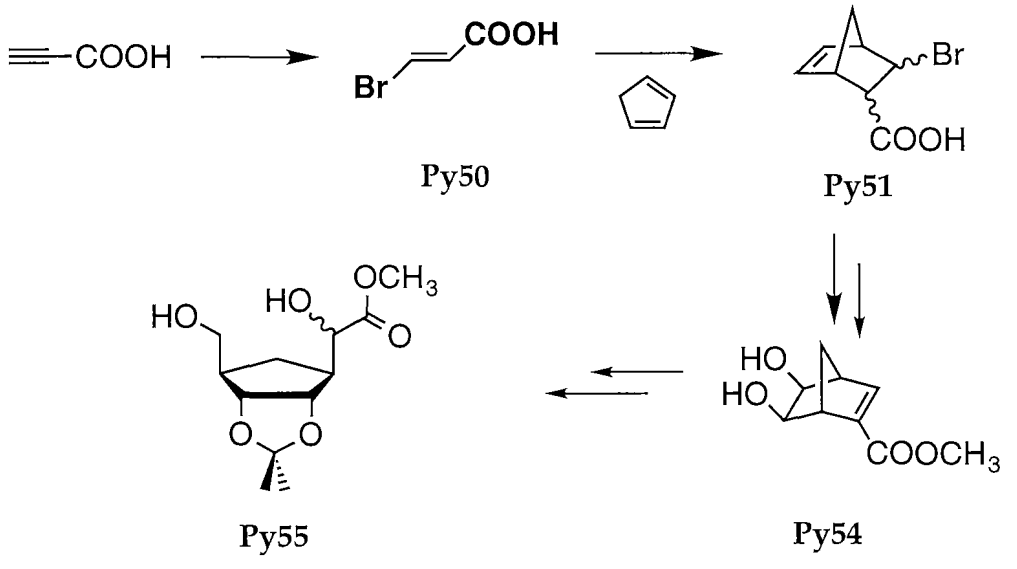

Figure 74. Synthesis of the cyclopentyl derivative **Py55**.

After acetonide protection, the norbornene compound is submitted to an oxidative ring opening by ozonolysis, followed by an *in situ* reduction to yield the racemic cyclopentyl derivative **Py55**.

To gain the key intermediate **Py68a**, a four step synthesis sequence was performed on compound **Py56**, involving treatment of the ester function with methanolic ammonia, mesylation of the alcohol function followed by substitution with NaN_3, and reduction by catalytic hydrogenation. Investigations showed that treatment of the ester functionality must preceed transformation of the alcohol functionality.

The construction of the pyrazine-heterocycle from compound **Py68a** occured in three steps and was based on a route developed by von Krosigk (von Krosigk, 1993). After suitable protection of the pyrazine moiety (with base-labile units) and of the carbocycle, the 2'-position was methylated. The 3',5'-protection was cleaved and the final phosphoramidite derivative **Py109** was made.

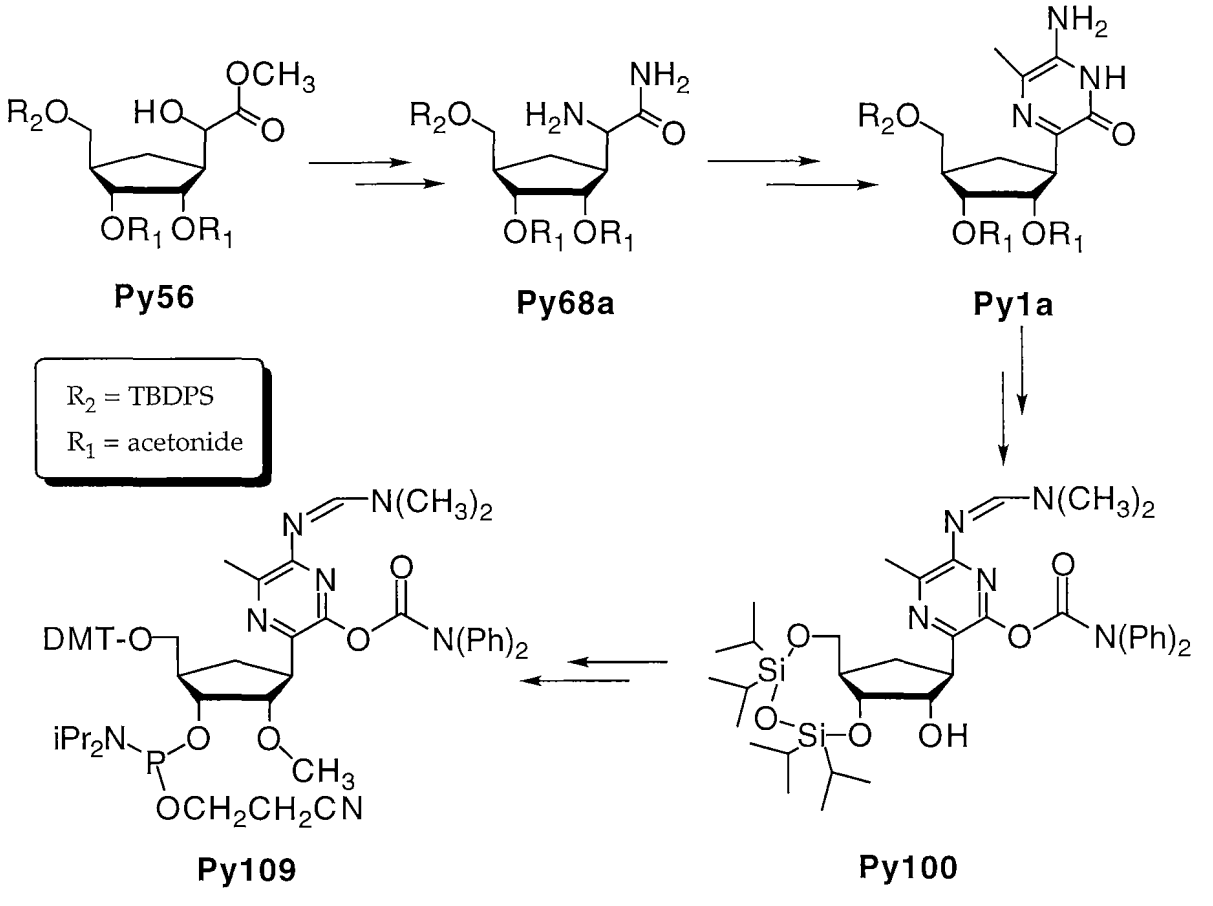

Figure 75. Synthesis of the carbocyclic pyrazine analogue **Py1a**. The latter is transformed into the methylated phosphoramidite building block **Py109**.

The phosphoramidite **Py109** was incorporated into a DNA strand by automatic DNA synthesis. The coupling yield of the carbocyclic pyrazine building block reached 80%. The DNA oligonucleotide was deprotected in AMA at 60°C for one hour. Standard characterization procedures (UV-spectroscopy, enzymatic digestion and MALDI-TOF MS) all showed that no pyrazine moiety was present in the oligonucleotide. Investigations showed that the heterocycle was most probably degraded during the treatment with AMA.

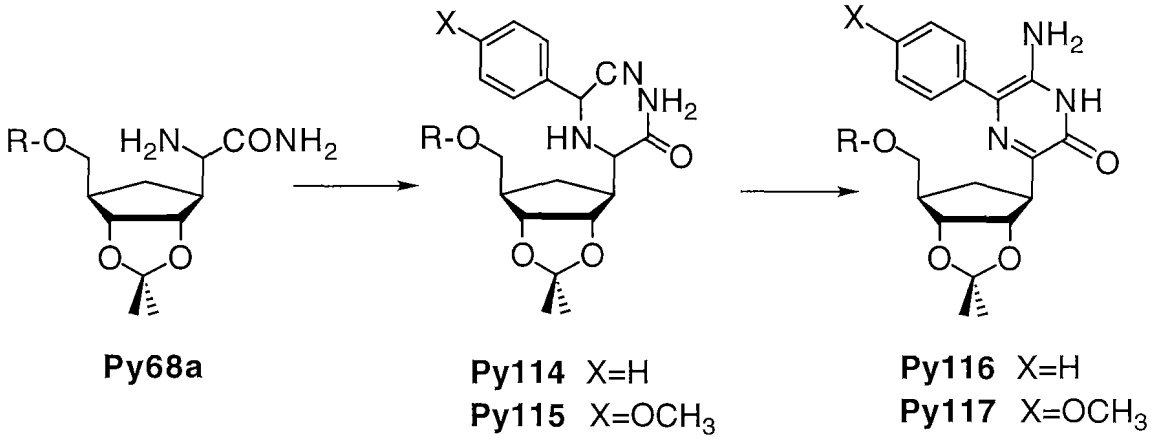

Figure 76. Synthesis of the 5-phenyl-pyrazine analogues **Py116** and **Py117**.

In order to improve the stability of the pyrazine moiety, the analogues **Py116** and **Py117** were synthesized as shown in Figure 76. The precursor **Py68a** could be reacted with the corresponding aldehydes to produce, after treatment with a strong base, the desired compounds. Compounds **Py116**, **Py117** and **Py1a** were incubated over days in a 1:1 solution of aqueous buffers at pH 4.0, 7.0 and 10.0, and methanol. The half-life at each pH was determined by UV-spectroscopy as a measurement of the degradation rate. This confirmed the sensitivity of the pyrazine moiety towards aqueous alkaline treatments.

Purine analogue

Once silylated, the chloropurine **Pu5** is reacted with 1-acetyl-2,3,5-tribenzoylribose **Pu9** and with a Lewis acid to yield 82% of the protected ribonucleoside **Pu10**. The latter was reacted with an excess of natrium azide in hot DMF, reduced to **Pu14**, followed by an almost quantitative deprotection in methanolic ammonia.

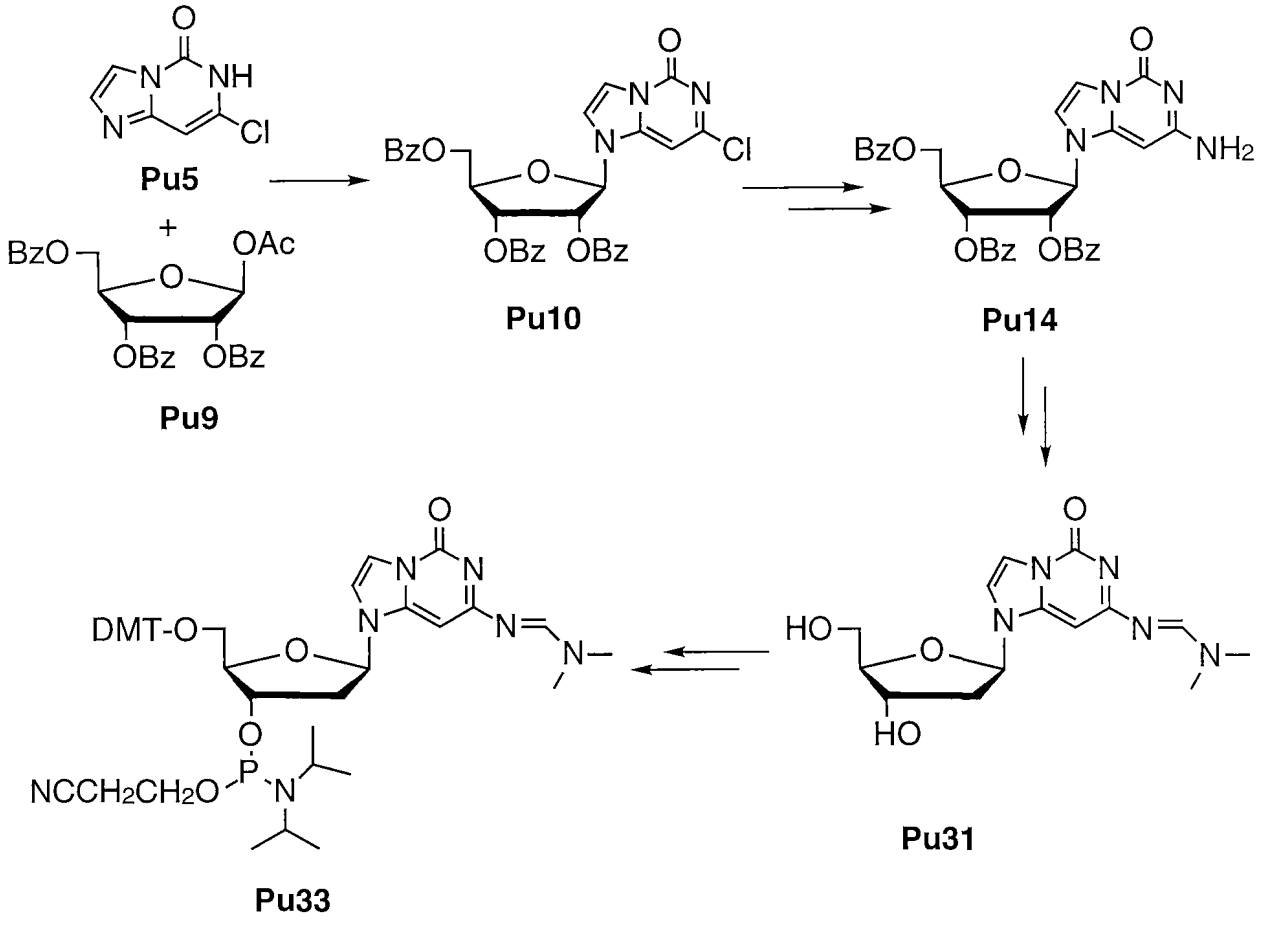

Figure 77. Synthesis of the 2'-deoxynucleoside phosphoramidite building block **Pu33**.

Suitable protection of the ribonucleoside allowed the deoxygenation of the 2'-OH functionality under standard conditions. The deprotected 2'-deoxynucleoside **Pu31** was then obtained and converted to the 5'-dimethoxytrityl-2'-deoxyphosphoramidite **Pu33**.

The phosphoramidite **Pu33** was incorporated in four DNA oligonucleotides (**Ob1-4** listed below) by automatic DNA synthesis. Once incorpotated into DNA, the nucleoside corresponding to **Pu33** was called **Ob**.

The DNA oligonucleotides were deprotected under standard conditions, purified by RP-HPLC, and characterized by enzymatic digestion and MALDI-TOF MS.

Ob1 5'-GGA CCG G**Ob**A A GG TAC GAG
Ob2 5'-GGA CCG G**Ob**A **Ob**GG TAC GAG
Ob3 5'-G ATG CGG **Ob**CA CCT GGA
Ob4 5'-G ATG CGG **Ob**C**Ob** CCT GGA