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Synthesis of N-Boc-α-Amino Acids with Nucleobase Residues as Building Blocks for the Preparation of Chiral PNA (Peptidic Nucleic Acids).

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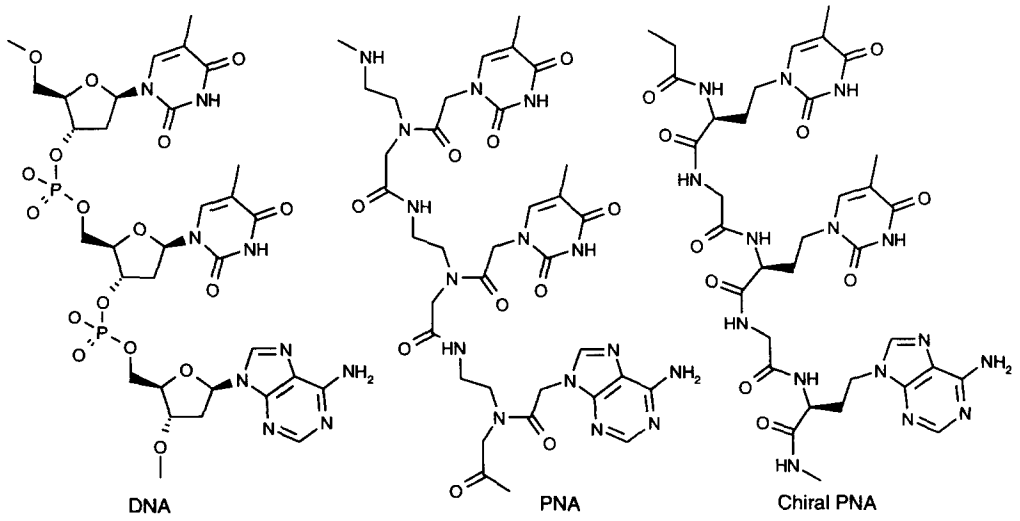
Abstract: *N-Boc-Glutamic acid α-benzyl ester was transformed into 4-bromo-2-(tert-butoxycarbonylamino)-butyric acid benzyl ester using the Barton-Crich protocol. This bromo derivative undergoes nucleophilic substitution with nucleobases to give optically active N-Boc-4-adeninbutyrine (Boc-Aby), N-Boc-4-thyminbutyrine (Boc-Tby), N-Boc-4-guaninbutyrine (Boc-Gby) or N-Boc-4-cytosinbutyrine (Boc-Cby), useful building blocks for the synthesis of chiral peptidic nucleic acids (PNA).*

PNA have been designed as structure having nucleobases linked to achiral polyamidic backbones.[2] The optimal number of bonds between the nucleobases was found to be six, as in DNA, and the optimal number of bonds between the nucleobases and the backbone was found to be two.[3] Such structures resulted homomorphous with the DNA and were able to hybridize to complementary oligonucleotides forming Watson-Crick-Hoogstein triplex, more stable than the corresponding DNA-DNA duplex.[4] Several applications of this class of compounds to "*in vitro*" diagnostic have been recently reported.[5]

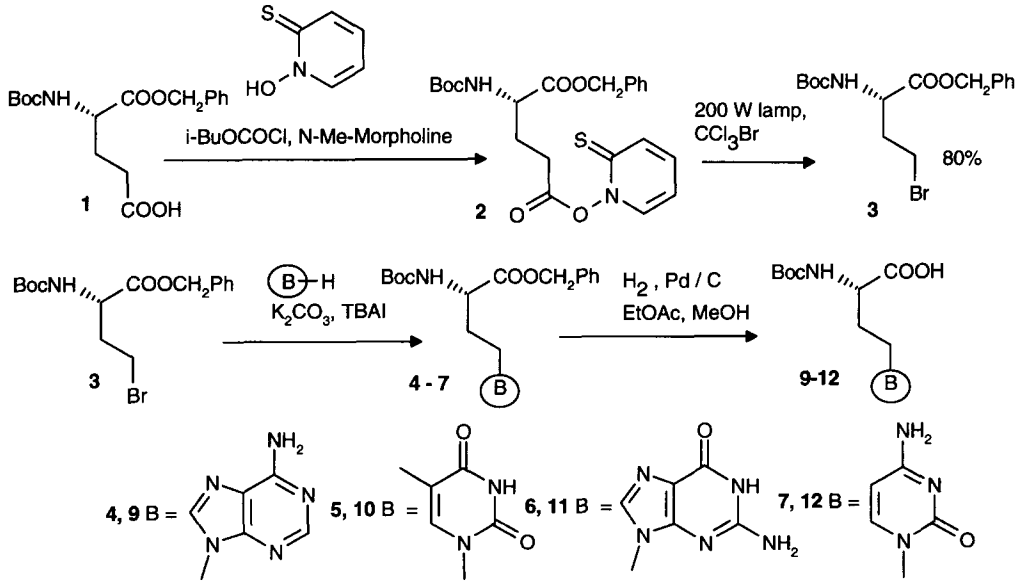


Scheme 1

In order to explore different ways to enhance the rigidity of the backbone, which is supposed to be one of the factors responsible of the binding of PNA to DNA (or RNA),[6] we decided to prepare a "real" polypeptidic structure completely formed by α-amino acids, carrying the nucleobases with the same relative distances that PNA.[7] (Scheme 1)

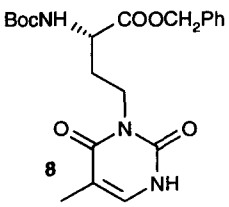
Building blocks for these structures are α-amino acids **9-12**. Therefore we needed an efficient synthesis of these monomers and we decided to use glutamic acid (**1**), protected as Boc on the amino group and as benzyl on the α carboxylic group, as the starting material. We followed the protocol described by Barton and Crich[8] to transform the carboxylic group into the corresponding bromide, the proper substrate for the introduction of the nucleobases through nucleophilic substitution.

Acid **1** was transformed into the ester **2** using i-BuOCOCl, N-methylmorpholine and 1-hydroxy-2-thiopyridone in THF. After filtration of the N-methylmorpholinium chloride and evaporation of the solvent, sheltering the flask from the light, the crude ester **2** was dissolved in CCl_3Br and irradiated with a 200 W lamp, maintaining the temperature of the irradiated solution below 20°C. When the production of CO_2 stopped (generally after 1-2 h), we evaporated the solvent under vacuum and purified the bromoderivative **3** by column chromatography on silica gel.[9] (Scheme 2)



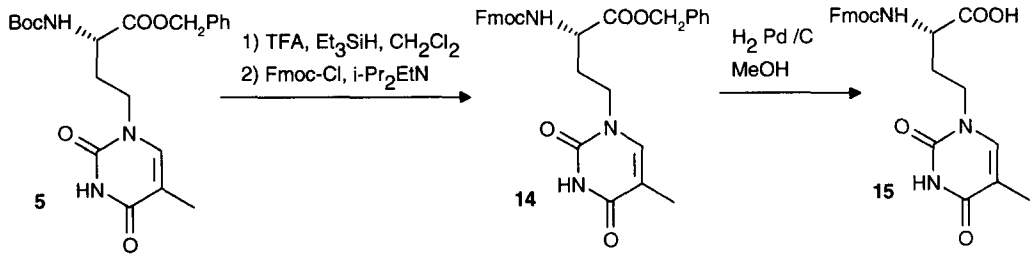
Scheme 2

Compound **3** reacted with 3 equiv excess of the free base (adenine, thymine, cytosine or guanine) at 80°C in DMF in the presence of 3 equiv of K_2CO_3 and 0.1 eq of TBAI to give products **4-7**, isolated after column chromatography on silica gel in 55-65% yields. The reaction proceeded without the formation of the possible elimination products. During the preparation of compound **5** we



observed the formation of 25% of its regioisomer **8**, alkylated at position 3 of the thymine ring, which could be separated from **4** only by column chromatography on neutral alumina (eluent $CHCl_3$ / MeOH 20 / 1, yield of **5** after the separation, 42%). Compounds **4-7** were finally deprotected at the nitrogen function with TFA / Et_3SiH in CH_2Cl_2 to the corresponding amines.[10] The NMR analysis of the corresponding (R)-MTPA amides showed that no racemisation occurred during the introduction of the bases. Finally deprotection of the carboxylic group of products **4-7** was achieved using H_2 in the presence of Pd / C in ethyl acetate (8 h, rt, 1 Atm of H_2), followed by filtration on celite and continuos extraction of the filter cake with hot methanol in a Soxhlet apparatus for 12 h. Evaporation of the solvent under vacuum gave pure products **9-12** in 45-65% yields. Following the above reaction sequence protection of the nitrogen atoms of the nucleobases was not necessary and compounds **9-12** could be prepared in a multi-gram scale.

Acids **9-12** [**9**: Boc-adeninbutyrrine (Boc-Aby), **10**: Boc-thyminbutyrine (Boc-Tby), **11**: Boc-guaninbutyrine (Boc-Gby), **12**: Boc-citosynbutyrine (Boc-Cby)][11] are in a suitable form to be used in solid phase peptide synthesis using the Boc protocol. Nevertheless we found that it is possible to change the protecting group of the α nitrogen as described in scheme 3 for compound **5**.



Scheme 3

Product **5** was quantitatively deprotected using TFA / Et_3SiH in CH_2Cl_2 and the crude product was reacted with Fmoc-Cl in the presence of diisopropylethyl amine in dry THF (3 h at 0°C and 20 min at rt) to give product **14** in 75% yield, after column chromatography on silica gel. The following deprotection of the carboxylic group was carried out with H_2 on Pd /C in MeOH and product **15** isolated after continuos extraction of the charcoal (collected on celite) with methanol in a Soxhlet apparatus (52% yield).

Compounds **9-12** and **15** are new not natural amino acids with nucleobase residues and their main uses can be the preparation of new chiral peptidic nucleic acids (see the following communication). Furthermore they can be employed for the solid phase preparation of encoded combinational libraries.

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9. Product **3** was isolated in 80% yield, after column chromatography on silica gel, mixed with 2,2'-dipyridyl disulphide (40% of the global weight extimated by ^{1}H NMR). Further chromatography gave an analytical sample of **3** in 50% yield. Nevertheless the impure bromide **3** can be used in the next step without any problem since the sulphurated by-products can be easily separated from compouds **4-7** during the final chromatography.
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