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A NOVEL METHOD TO HIGHLY VERSATILE MONOMERIC PNA BUILDING BLOCKS BY MULTI COMPONENT REACTIONS

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Abstract: A novel approach to monomeric PNA building blocks by a solution phase Ugi multi component reaction (MCR) is described. The reaction is easily performed in 96 well plates. The products precipitate from the reaction solution and are thus obtained in high yields and purity. Those products are not amenable by another multi step synthesis in such a diversity.

Keywords: PNA, MCR, combinatorial chemistry, Ugi reaction.

Peptid Nucleic Acid (PNA), a term coined by Nielsen *et al.* in 1991, describes a polymer being able to pair sequence specifically with natural occuring DNA or RNA.[1] PNA have a different backbone resembling a peptid and thereby replacing the ribose phosphate backbone of DNA and RNA.[2] It has several advantages over natural oligonucleotides *e.g.* greater stability towards degrading enzymes or a better recognition of mismatches.

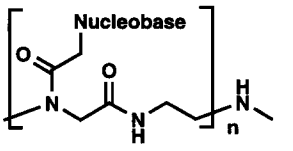

Fig 1.: A sketch of the PNA polymere.

Therefore many practical applications of PNA in diagnostics and possibly anti-sense therapeutics are in use or considered.[3]

For certain applications the classical PNA also has severe drawbacks as the poor water solubility and transfection capability. Consequently there is a need for PNA modifications with improved properties.[4] A recent publication on the use of multi component reactions (MCRs) to PNA monomers prompted us to present our results on PNA monomer synthesis by MCR.[5] It is a continuation of our previous work towards novel PNA chimeras.[6,7,8]

MCRs are very popular reactions in creating molecular diversity.[9] No other reactions are able to give access to potentially millions of small organic molecules. The most famous and versatile reaction under MCRs is the Ugi four component reaction (U-4CR) and related reactions. Because of the

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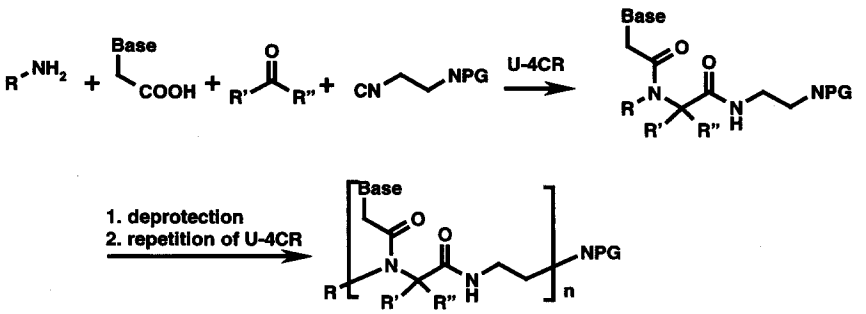
simple reaction conditions and the many different scaffolds which can be created by Ugi-type MCRs, they are often used in an automated way in combinatorial chemistry.[10]

Inspection of the PNA backbone reveals the repeating α-amino amide unit. α-Amino amides on the other hand can be conveniently synthesized by U-4CR. Thereby nucleobase acetic acids react with primary amines, oxocomponents and *N*-mono protected ethylenediamine derived mono isocyanides synthesized by the Ugi MCR. We therefore considered the U-4CR as a possible alternative to the classical coupling route (Scheme 1). Cleavage of the protecting group results in a primary amine which can be used as the amine component with the above mentioned other components in the U-4CR. Repetition of the cycle gives PNA oligomers.



Scheme 1: U-4CR of oxocomponents, amines, nucleobase acetic acid and mono *N*-protected 1-isocyano ethylamines result in a protected PNA monomer. Several repetition cycles give PNA oligomers.[11]

Herein we report the synthesis of previously unknown PNA monomers by U-4CR.[12] The reactions have been performed in a combinatorial manner in 96 deep well plates. In the rows and columns the amines and oxocomponents are systematically varied. During the reaction the products precipitate and can be filtered of in order to yield the PNA monomers in high purity and fair yield.[13] Since no stereochemical induction occurs the compounds are racemic or even diastereomeric (column 12).

As in the recent publication[5] of *Martens et al.* we also use the U-4CR in order to assemble PNA monomers. In contrary to their work towards PNA monomers, who use isocyano components, which finally are converted to carboxylic acids, we use amino protected isocyano components leading to PNA monomers as well, but giving the possibility to an easy extension towards PNA polymers.

The variability of the reaction is not limited to the amine and the oxo component, but a variety of different isocyanides as well as appropriate protected nucleobases can be introduced.[14] In future publications we will also show the utility of the Ugi MCR to synthesize classical as well as non classical PNA oligomers.

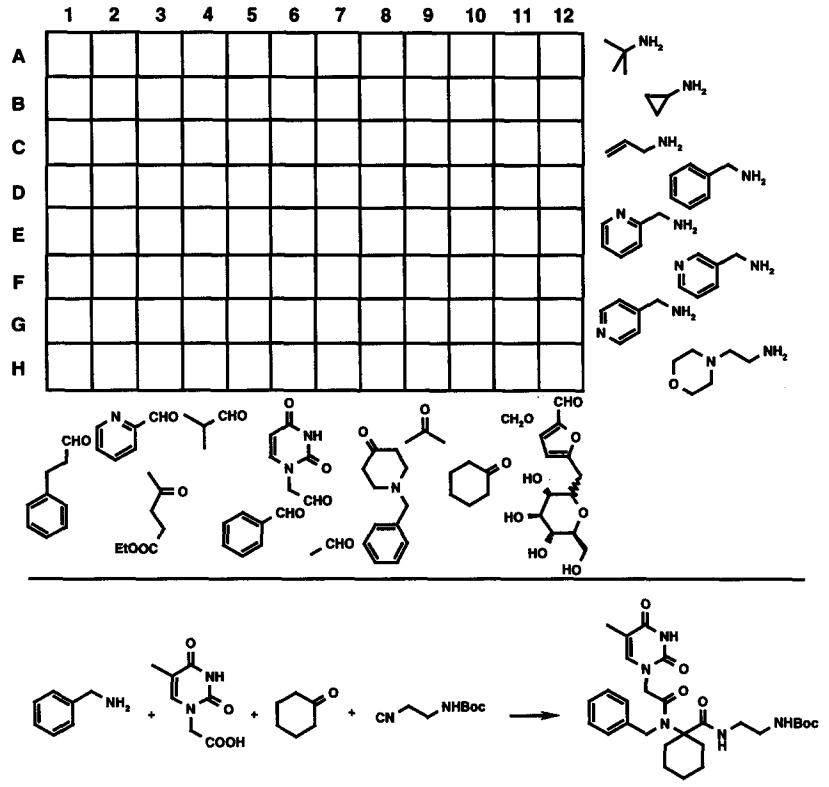


Fig 2.: Sketch of the filling of a 96-well plate by different starting materials in order to obtain PNA monomers. The rows are filled by eight different primary amines, the columns are filled by twelve different aldehydes and ketones and each well is filled by the isocyanide and thyminyl acetic acid. Similar reactions resulting in the other nucleobase monomers can be performed by the use of appropriately protected nucleobase acetic acid derivatives. Each of the wells have been analysed by hplc-ms and occasionally nmrs were taken.[13] According to the analytics the precepitates are pure products.

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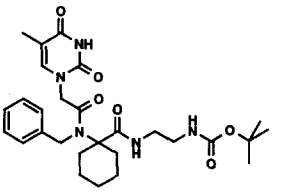
[13] **Typical procedure:**
A mixture of 0.5 mmol of each of the compounds isocyanide, keton or aldehyde, primary amine and thyminyl acetic acid are stirred in 0.5 ml of methanol for 24 h. The resulting precipitate is filtered and washed with 10 ml of cold methanol. The resulting white powder is pure according to HPLC-MS and NMR.

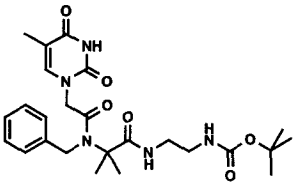
e.g.:



D10:
$C_{28}H_{39}N_5O_6$: 541.65
yield: 80 %

^{1}H-NMR (360 MHz / d_6-DMSO): 0.83 - 1.54 (8H, m), 1.37 (9H, s), 1.75 (3H, s), 2.24 (2H, m), 2.95 - 3.17 (4H, m), 4.56 (2H, s), 4.73 (2H, s), 6.67 (1H, tr), 7.27 - 7.48 (6H, m), 11.28 (1H, s, thymine-NH).

^{13}C-NMR (62.9 MHz): 11.6, 22.0, 24.7, 28.0, 32.2, 46.6, 49.7, 65.2, 77.4, 107.8, 126.5, 126.9, 128.4, 138.1, 136.1, 142.1, 142.4, 151.0, 155.5, 157.1, 164.3, 167.7, 172.7.



D9:
$C_{25}H_{35}N_5O_6$: 501.56
yield: 68 %

^{1}H-NMR (250 MHz / d_6-DMSO): 1.23 (6H, s), 1.37 (9H, s), 1.75 (3H, s), 2.95 - 3.17 (4H, m), 4.58 (2H, s), 4.73 (2H, s), 6.70 (1H, tr), 7.29 - 7.50 (6H, m), 11.25 (1H, d, thymine-NH).

^{13}C-NMR (62.9 MHz): 12.2, 24.4, 28.5, 43.0, 47.4, 49.7, 62.9, 78.0, 108.0, 126.9, 127.5, 128.9, 128.2, 138.8, 142.6, 151.4, 156.1, 164.8, 169.9, 174.1.

Representative yields of resynthesized compounds on a 0.5 mmol scale: **D11** (85%), **H11** (54%), **F8** (65%), **D6** (50%).

[14] This is behind the scope of this communication and will be published in near future.