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Facile access to modified and functionalized PNAs through Ugi-based solid phase oligomerization

Jacques Saarbach[a], Daniela Masi[a] , Claudio Zambaldo[a], and Nicolas Winssinger[a]*

a Faculty of Science, Department of Organic Chemistry, NCCR Chemical Biology, University of Geneva, 30 quai Ernest Ansermet, Geneva, Switzerland

ABSTRACT: Peptide nucleic acids (PNAs) derivatized with functional molecules are increasingly used in diverse biosupramolecular applications. PNAs have proven to be highly tolerant to modifications and different applications benefit from the use of modified PNAs, in particular modifications at the γ position. Herein we report simple protocols to access modified PNAs from iterative Ugi couplings which allow modular modifications at the α, β or γ position of the PNA backbone from simple starting materials. We demonstrate the utility of the method with the synthesis of several bioactive small molecules (a peptide ligand, a kinase inhibitor and a glycan)-PNA conjugates.

1. Introduction

Peptide nucleic acids (PNAs) are artificial oligomers in which the phosphoribose of DNA or RNA has been replaced by a peptidic backbone (Fig. 1).[1-3] PNAs stand out for their ability to form more stable duplex with DNA or RNA than the natural homoduplex, for their high sequence discrimination, as well as for their biological and chemical robustness. These remarkable properties of PNA have attracted tremendous attention for diagnostic and therapeutic applications.[4] Over the past decade the field of applications of PNA has grown significantly with applications of this oligomer as a biosupramolecular tag for programmable assemblies and reactions[5-8], in DNA-based nanotechnologies,[9, 10] to direct folding of protein–like structures,[11-13] and as chloride ion sensor.[14] For these purposes, PNAs bearing modification in the backbone to introduce various functionalities or ligands are frequently used. Several modifications to the N-(2-aminoethyl)glycine backbone have been reported as beneficial or well tolerated in the hybridization.[15-18] Substitution at the α position (replacement of glycine with other amino acids)[19] are tolerated while a gem-dimethyl substitution at the α position provides enhanced duplex stability with DNA.[20] An important modification is the introduction of a chiral γ-substituent ((*S*) stereochemistry defined based on a methyl substituent) which generates a steric bias that favors right-handed helicity with base stacking, resulting in enhanced duplex stability with DNA.[21-25] The enhanced properties of this backbone have recently been used to target genomic DNA.[26] A lysine[22, 27, 28] or cysteine[29, 30] side chain at the γ-position has also proven useful for the conjugation of small molecules. The combination of chirality at the α and γ positions within a single PNA unit provides additive duplex stability with an overriding influence of the γ stereochemistry.[31] More recently, modification at the β position has also been reported to be tolerated (*S*-methyl).[32] Furthermore, using the opposite stereochemistry at the γ positions has been used to create PNA with orthogonal hybridization to DNA or RNA.[33, 34] In addition to modifications of the PNA backbone, many alternative nucleobases beyond the canonical A, C, G, and T have been designed and can provide interesting properties: enhanced hybridization (2,6-diaminopurine,[35] analogs of thymine (7-Cl-bT)[36] and cytosine (G-clamp)[37], promiscuous hybridization (hypoxanthine)[38] or fluorescent probes (thiazole orange).[39]

2. Results and discussion

PNAs are typically synthesized in the C to N direction by iterative cycles of coupling/ deprotection with monomers protected orthogonally on the nucleobases and N-terminus (Fig. 1). However, the exponential synthetic demand to access modified PNA monomers in the various combinations of backbones and nucleobases is limiting their implementation. Herein, we report a straightforward method based on reiterative Ugi reactions[40] to access PNA oligomers with modifications at the α, β, γ positions in combination with any nucleobase. The methodology also lends itself to the synthesis of PNA bearing ligand at defined positions within the backbone. While the Ugi 4-CR reaction has been previously used to synthesize PNA monomers or dimers,[41-45] these previous methods had not been implemented for oligomer synthesis with functionalization of the PNAs.

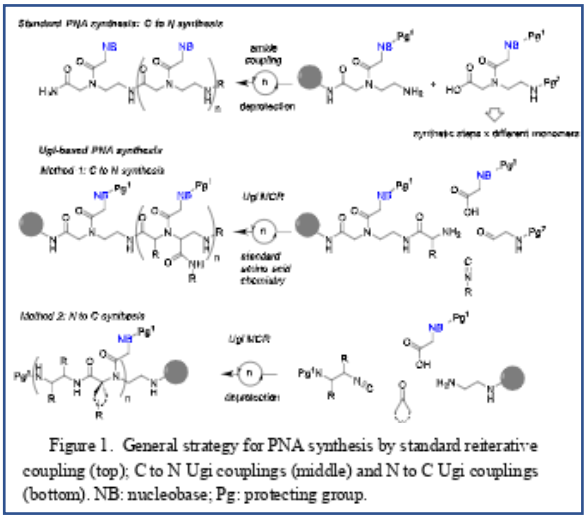

Figure 1. General strategy for PNA synthesis by standard reiterative coupling (top); C to N Ugi couplings (middle) and N to C Ugi couplings (bottom). NB: nucleobase; Pg: protecting group.

We began our investigation with the evaluation of the Ugi reaction to prepare PNA in the C to N direction (Method 1, Fig. 1). To facilitate the LC-MS analysis of the reactions, the synthesis was initiated with a resin loaded with a lysine naphthoate prior to the introduction of the first residue by standard Fmoc-based chemistry (**1**, Fig. 1). The naphthyl group has a UV absorbtion at 283 nm, which provides a distinct window to identify the product and any side product arising from subsequent reactions. This resin loaded with a first amino acid was treated with 4 equivalents of nucleobase (**2**, thymine acetic acid), aldehyde **3** and benzyl isocyanide in EtOH. The reaction proceeded to completion for all 19 natural primary amino acids after 1 h. While there are a few examples of chiral induction in Ugi-4CR,[46, 47] they are performed at low temperature under anhydrous conditions and we did not expect notable diastereoselectivity. Performing the reaction with resin **1** loaded with 1-Aminocyclopropanecarboxylic acid afforded a 1:1 mixture of diastereoisomers resolvable by HPLC. To further ascertain the diastereoselectivity, the reaction was performed in solution with the methyl ester of valine instead of resin **1** followed by Fmoc deprotection with concomitant cyclization to the ketopiperazine clearly indicating a mixture of diastereoisomers as judged by LC-MS and NMR. Based on the efficient Ugi-4CR, we synthesized an 8-mer by reiteration of the cycle (amino acid coupling, Fmoc deprotection, Ugi reaction, Fmoc deprotection) using glycine and the four canonical nucleobases and benzyl isocyanide to obtain the PNA as a single product thus establishing the viability of this Ugi-based oligomerization albeit without control of the stereochemistry at the β position.

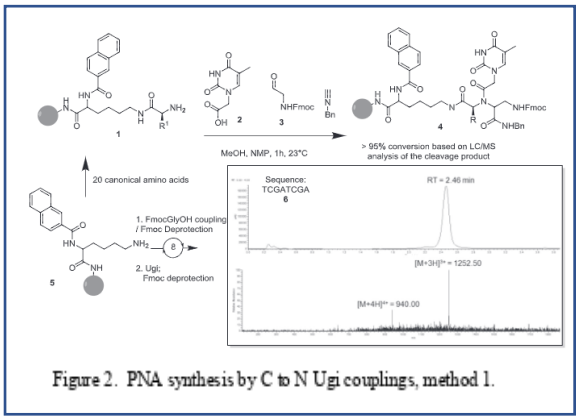

Figure 2. PNA synthesis by C to N Ugi couplings, method 1.

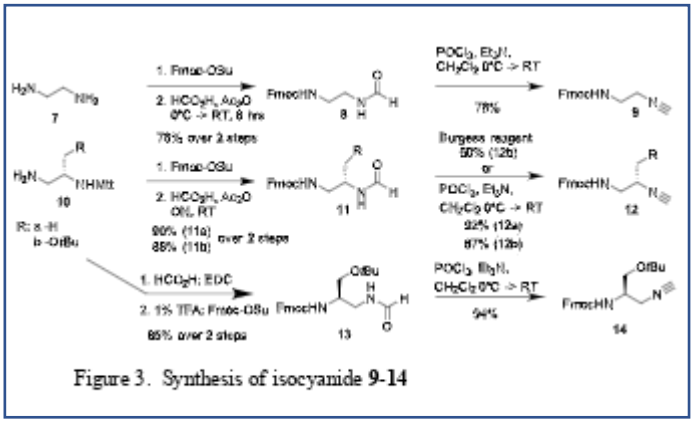

Figure 3. Synthesis of isocyanide **9-14**

We next evaluated the Ugi reaction to prepare PNAs in the N to C direction (Fig. 1). This second Ugi variation provides inherent control over the most important stereochemistry at β or γ positions (either stereochemistry at the α position is tolerated in the hybridization). To this end, three different isocyanides (**9**[48], **12**, **14**) were prepared as shown in Fig. 3 to ultimately yield unmodified PNA, γ or β-modified PNAs respectively.[49, 50] Chiral isocyanides **12** and **14** were obtained in three steps from intermediate **10**.[51] For isocyanide **12**, intermediate **10** was Fmoc protected and the Mtt was removed with formic acid and acetic anhydride to directly obtain **11**. Dehydration of the formamide using Burgess's reagent or $POCl_3$ afforded **12** in excellent yield. A similar sequence starting with the formylation of **10** was used to obtain **14** in equally good yield. Investigation into the Ugi reaction starting from resin **5** with isocyanide **9**, thymine acetic acid **2** and formaldehyde (Fig. 4) led to the formation of two peaks by LC-MS, the expected product **16** and a side product with a mass corresponding to **17**. This side product was assumed to arise from reaction intermediate **15** that engaged in a second imine condensation (reaction path b, Fig. 4) rather than undergo the acyl transfer of the Ugi condensation (reaction path a) to afford **16**. Attempts to optimize the reaction conditions (solvent, equivalents of formaldehyde, temperature) failed to completely shut down this side reaction. This side reaction precludes the use of formaldehyde for reiterative Ugi coupling towards PNA oligomers. However, this side reaction was not observed with other aldehydes and the use of diverse aldehydes afforded the desired product in > 95% yield in < 30 min reaction time at room temperature. The best conditions employed THF as a solvent with, 4 equivalents of isocyanide, 4 equivalents of a nucleobase (added as a NMP solution) and 50 equivalents of hexafluoroisopropanol (HFIP)[52] for 30 minutes. Notably, the reaction could be used to introduce diverse substituent at the α position (PEG **18a** for solubility[53], 2-nitrobenzyl **18b** for photocleavable PNA or azide functionalized side chain **18c** for further conjugation) to obtain PNAs **19a-d**. Performing the reaction with chiral isocyanides **12** or **14** proceeded equally well affording the products as a mixture of diastereosiomers at the α position (ca. 1:1, based on LC-MS integration of compound **19d-2**), however, the lack of stereochemical control at the α position has minimal impact on PNA hybridization based on the overriding influence of the

defined stereochemistry at the γ position, which is defined by the isocyanide.[54] Nonetheless, we sought an alternative to obtain PNAs without substitution at the α position. As an alternative to the use of formaldehyde, we reasoned that performing the Ugi with aldehyde **18e** would afford a product that would readily undergo a decarboxylation, following cleavage and global deprotection. It was found that aldehyde **18e** could be used in the Ugi reaction affording product **20e** which indeed underwent a decarboxylation under classical conditions (10% aq. acetic acid, 90 °C). Thus, the use of aldehyde **18e** affords PNAs unmodified at the α position.

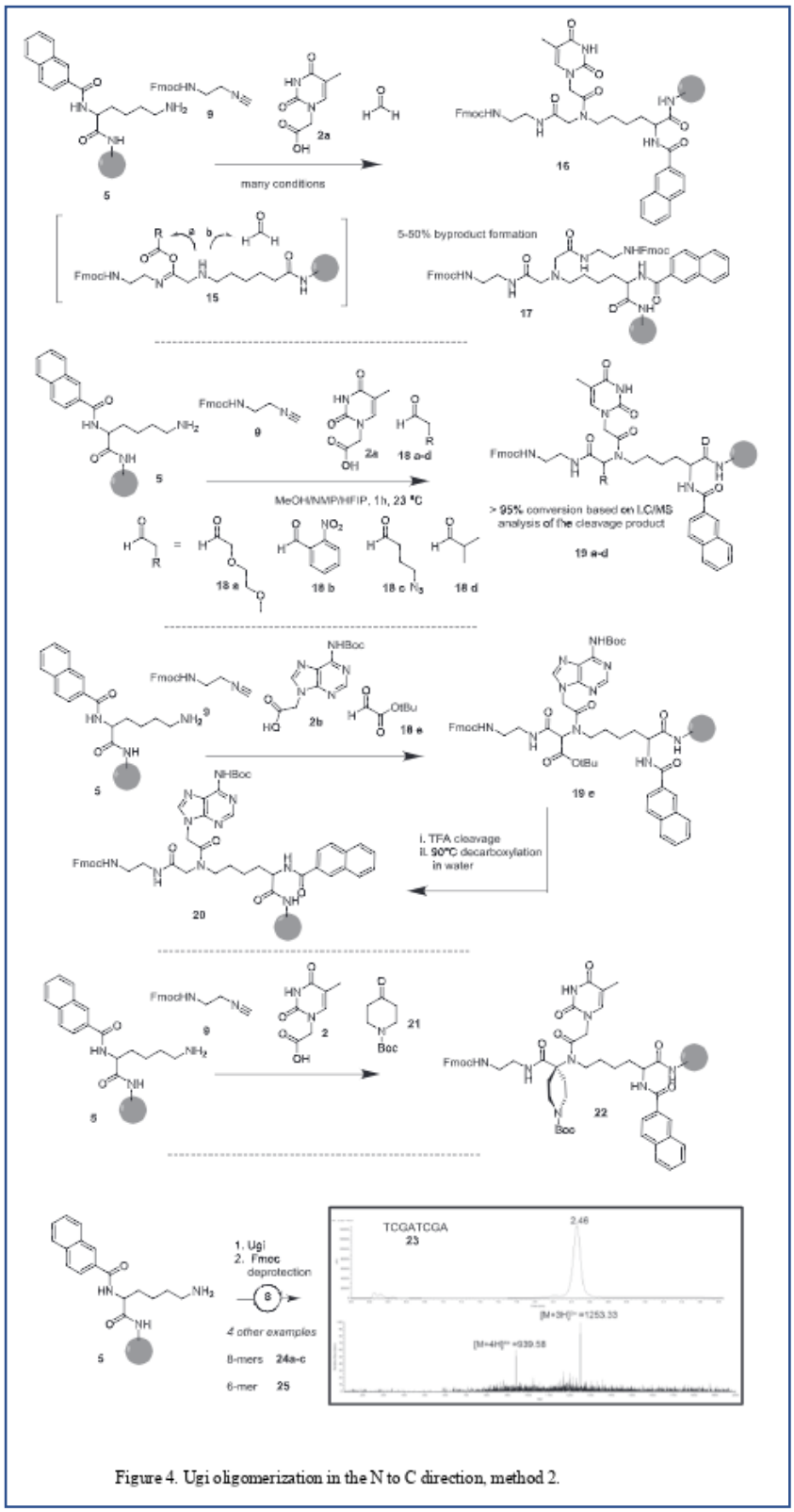

Figure 4. Ugi oligomerization in the N to C direction, method 2.

Reaction with cyclic ketone **21** afforded the desired product with a gem-disubstitution at the α position. The latter two reactions with aldehyde **18e** or ketone **21** where slower and required 50 °C to reach completion within 1 h. To illustrate the utility of this Ugi-based PNA synthesis method, we prepared various examples combining different nucleobases and backbone modifications (four examples of 8mer PNA using isobutyraldehyde, isocyanide **9** or **12a** and five nucleobases; one example of 6mer PNA alternating PEG and isobutyraldehyde with isocyanide **9**). All PNAs were obtained as a single major product showing the expected mass by MALDI and LC-MS (one example shown in Fig. 4, see SI for additional examples).

We then turned our attention to the application of this methodology to prepare PNAs modified at defined position with ligands. We anticipated that peptides or other ligands could be derivatized with a Fmoc-*D*-Dap(Mtt)OH residue that would result in the desired stereochemistry at the γ position, while providing orthogonally protected amino terminus to synthesize the PNA via regular Fmoc chemistry then deprotect the Mtt[51] and continue with Ugi-based PNA synthesis. As shown in Fig. 5, this strategy was used to synthesize an Abl kinase ligand[55] (11 mer peptide, **26**) which was derivatized with a PEG followed Fmoc-*D*-Dap(Mtt)OH to obtain **27**. The Fmoc was removed and 7 cycles of regular Fmoc-based PNA synthesis were performed. The Mtt was then deprotected and 4 cycles of N to C Ugi oligomerization were performed. Cleavage of the product from the resin afford the desired compound **28** as the major product. Alternately, the trifunctional linker **29** can be used to achieve ligand coupling after completion of PNA synthesis with CuAAC conjugation of the ligand.

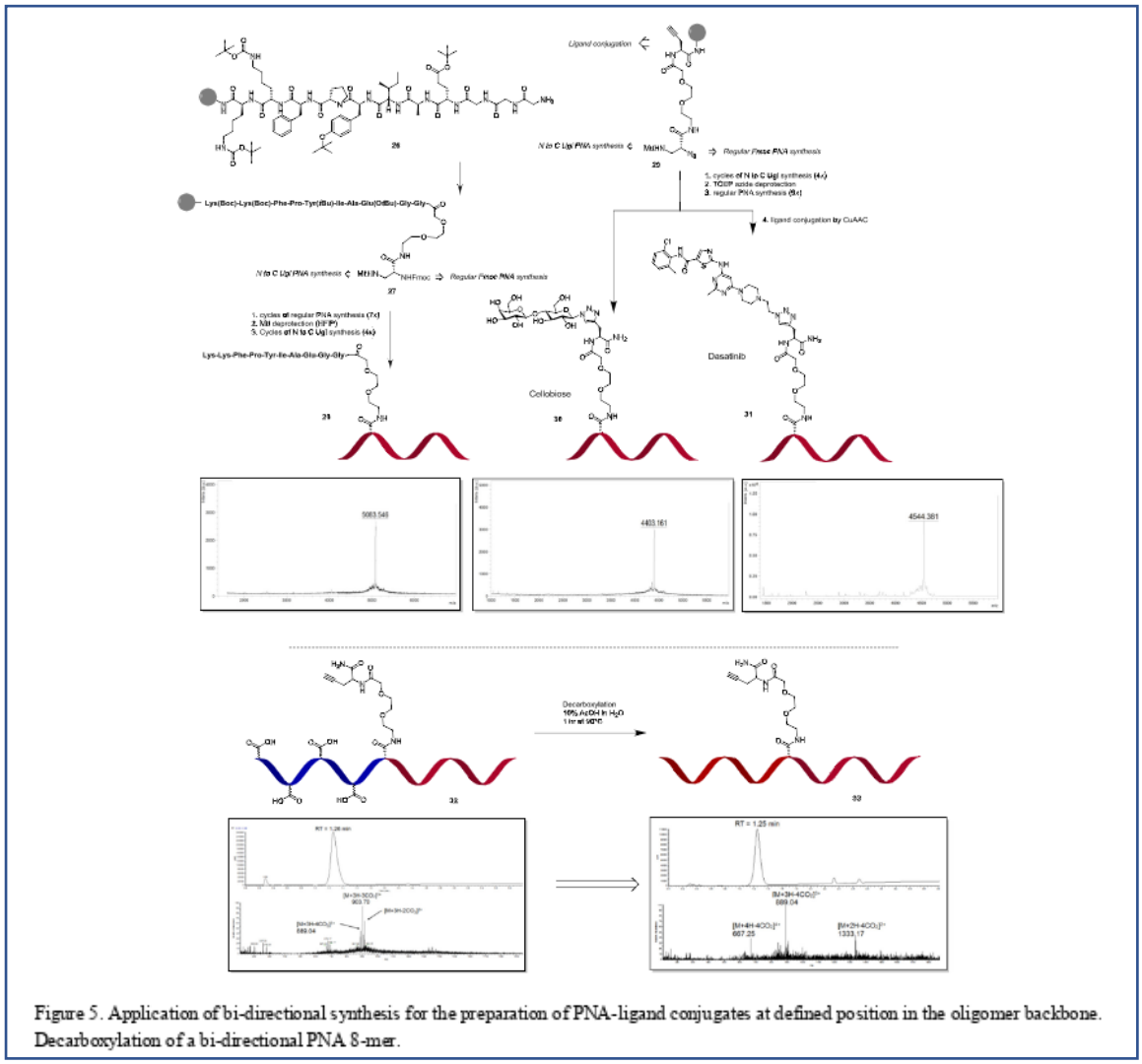

Figure 5. Application of bi-directional synthesis for the preparation of PNA-ligand conjugates at defined position in the oligomer backbone. Decarboxylation of a bi-directional PNA 8-mer.

To illustrates this strategy, resin **29** was Mtt-deprotected and engaged in 4 cycles of Ugi-based PNA synthesis. Then, the azide was reduced (TCEP) [56] and the 8 cycles of regular Fmoc-based PNA synthesis were performed. Ultimately, two different ligands were coupled, a kinase inhibitor **30** (dasatinib)[57] or glycan **31** (cellobiose).[58] This strategy provided the desired conjugate with high purity. Finally, to check the efficiency of the decarboxylation of PNAs prepared with aldehyde **18e**, a bidirectional 8-mer PNA **32** (4 cycles of Ugi-based synthesis and 4 cycles of regular Fmoc-based PNA synthesis) was prepared and decarboxylated to

yield **33** (10% acetic acid in water, 90 °C, 1h). The decarboxylated PNA **33** was lyophilized and LC-MS analysis indicated a complete decarboxylation without any degradation, which establishes that Ugi-based strategy can be used in combination with classical PNA synthesis.

3. Experimental Section

Experimental procedures for reagents and resins are detailed in the supplemental information

3.1. General procedure for C to N Ugi reaction

Representative example with aldehyde 3 and benzyl isocyanide (other reactions performed under the same conditions with different aldehydes or isocyanides): To a solution of nucleobase acetic acid (8.8 μmol, 4.0 equiv) in 5 μL of NMP was added Fmoc-acetaldehyde **3** (2.4 mg, 8.8 μmol, 4.0 equiv) and benzyl isocyanide (1.2 μL, 8.8 μmol, 4.0 equiv) followed by 25 μL of MeOH. The solution was first sonicated in an ultra-sound bath then added to resin **1** (5 mg, 2.5 μmol, 1.0 equiv) which was shaken for 1h at room temperature. Subsequently the resin was first washed with DMF (6x) and CH_2Cl_2 (6x).

3.2. *General procedure for N to C Ugi reaction*

Representative example with isocyanide **9** and thymine acetic acid **2** and aldehyde **18** (other reactions performed under the same conditions with different aldehydes or isocyanides): Isocyanide **9** (4.0 equiv, 10.0 μmol, 2.8 mg) was dissolved in 10.0 μL of a 1 M solution of thymine acetic acid **2** in NMP (4.0 equiv., 10.0 μmol), the suspension was sonicated until the solution became clear, then HFIP (50.0 equiv, 110.0 μmol, 12.0 μL) and 80 μL of THF were added. Aldehyde **18** (4.0 equiv, 10.0 μmol, 1.2 μL) was added to the previous solution and the mixture was transferred to the resin. The reaction was shaken for 30 min at room temperature. Subsequently the resin was first washed with DMF (6x) and CH_2Cl_2 (6x).

3.3. *General decarboxylation procedure*

The PNA was cleaved from resin using 100% TFA (1 h, room temperature) and precipitated in diethylether (10x volume of TFA). The solution was centrifuged to obtain a pellet, which was decanted and taken up in 10% acetic acid in water. The solution was heated to 90°C for 1 hour. The decarboxylation can be observed by formation of bubbles inside the vial and monitored by LC-MS. The final PNA derivative was obtained by lyophilizing the solution.

4. Conclusion

The methodologies reported herein greatly facilitates the synthesis of functionalized PNAs. The N to C Ugi oligomerization enable bi-directional PNA synthesis with conjugation of ligands to the PNA at a given position in the oligomer. The current interest in the use of ODNs to display small molecules with defined topology or use of PNA as sensor and foldamers should greatly benefit from the synthetic methods reported herein. The fact that any combination of backbone and nucleobase modifications can be accessed without lengthy synthesis of monomers should open new horizons in PNA applications.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, athttp://dx.doi.org/10.1016/j.bmc.2017.05.064.

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