Clickable peptide nucleic acids (cPNA) with tunable affinity†

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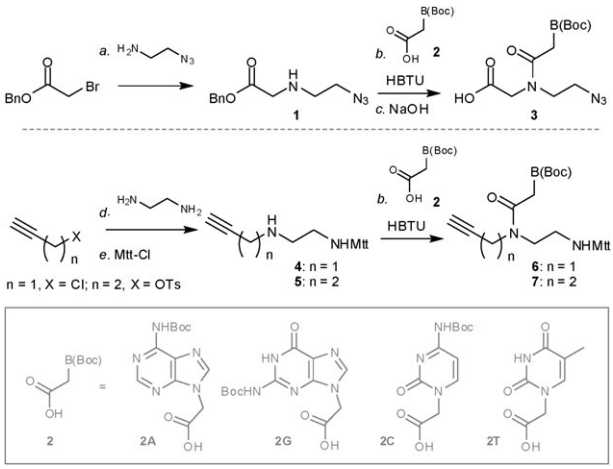
Peptide nucleic acids (PNAs) are functional analogues of natural oligonucleotides. Herein, we report the synthesis of PNAs bearing a triazole *in lieu* of the amide bond assembled using a "click" cycloaddition, their hybridization properties as well as the DNA-templated coupling of the azide and alkyne PNA fragments.

Compared to natural oligonucleotides, PNAs[1,2] are endowed with higher melting temperature (T_m), greater sensitivity to mismatches, and are more resistant to chemical and enzymatic degradation. These features have stimulated a number of applications ranging from biotechnology to medicine.[3,4] We have been particularly interested in PNAs as an encoding tag for supramolecular assemblies.[5,6] Herein, we report the use of an azide–alkyne "click" coupling to assemble PNA fragments and the hybridization properties of these new modified PNA.

The copper-catalyzed coupling of azides with alkynes[7,8] has emerged as one of the most robust (bio)conjugation technologies.[9,10] Additionally, the resulting 1,4-triazole has been shown to be a good mimic of a *trans*-amide bond.[11–14] Furthermore, DNA bearing a triazole linkage *in lieu* of the phosphate have already been reported.[15,16] Based on these precedents, we hypothesized that a triazole substitution of the amide within the PNA backbone should be tolerated and would provide access to PNA *via* click cycloadditions. To investigate the impact of this substitution, the azido-PNA monomers **3** (Scheme 1) were prepared from compound **1** as previously described[17] but with nucleobase-substituted acids **2** bearing Boc groups[18,19] which are compatible with Mtt deprotection conditions.[20] Two alkyne bearing PNA monomers (**6** and **7**, Scheme 1) with one or two carbon atoms between the alkyne and the amide linkage to the nucleobase were prepared. Thus reaction of propargyl chloride or tosylated homopropargylic alcohol with ethylene diamine[21] followed by selective Mtt protection of the primary amine afforded compounds **4** and **5** respectively. Standard HBTU coupling with nucleobase-substituted acids **2** afforded the desired PNA monomers **6** and **7**. This chemistry is readily scalable to obtain gram-quantities of all four nucleic acid monomers. PNA oligomers bearing the triazole modifications were prepared by standard Fmoc-based PNA synthesis, using Boc-protected nucleobases, on a PEG-based resin (NovaPEG) with a Rink linker (Scheme 2). Oligomers were terminated with an azido



Scheme 1 Synthesis of azido PNA **3** and alkynic PNA **6–7**. (a) 2-Aminoethylbromide (1.0 equiv.), NaN_3 (1.1 equiv.), DMF, 60 °C, 3 h; then, benzyl 2-bromoacetate (0.8 equiv.), Et_3N (2.0 equiv.), 0 °C, 2 h, 64%; (b) **2** (1.0 equiv.), HBTU (1.0 equiv.), Et_3N (2.0 equiv.), DMF, 23 °C, 12 h, 40–90%; (c) NaOH (4.0 equiv.), dioxane/H_2O, 23 °C, 1 h, 45–75%; (d) ethylene diamine (10.0 equiv.), DBU (1.0 equiv.), toluene, 23 °C, 4 h, 52–53%; (e) Mtt-Cl (1.3 equiv.), Et_3N (2.0 equiv.), CH_2Cl_2, 23 °C, 5 h, 60%.

monomer **3** yielding oligomers **8** and coupled to an alkyne monomer **6** or **7** to obtain a t1 or t2 junction respectively (Scheme 2). The cycloaddition (click) was carried out in a NMP : water mixture (10 : 1) at room temperature for 12 hours with 7.5 equivalents of alkyne monomer **6** or **7** (70 mM) in the presence of copper sulfate (2.5 mM), TBTA ligand (5 mM) and ascorbic acid (70 mM). The reactions were found to reliably proceed to completion based on the analysis of the cleavage product from an analytical aliquot of resin. The Mtt was removed with hexafluoroisopropanol (HFIP) and the standard PNA synthesis was continued with or without reiteration of cycloaddition (click) couplings. A first set of three 8mers containing no modification (**10**, Scheme 2), a t1 linkage (**11**) and a t2 linkage (**12**) were prepared to assess the impact of these modifications. Hybridization to DNA at 5 μM revealed T_ms of 57 °C, 55 °C and 45 °C for **10**, **11** and **12** respectively. Thus, the t2 junction strongly destabilizes the duplex while t1 has a modest impact. We next asked whether the triazole junction affected the fidelity of hybridization. To this end, we used a microarray bearing 625 oligonucleotides which has been used to decode PNA-libraries.[22] The 625 sequences represent all the combinatorial permutations of four sets of codons encoding five elements of diversity each ($5 \times 5 \times 5 \times 5 = 625$, see Fig. S2, ESI†) for the sequences and their position on the array). It thus follows that for any given spot of the microarray (*i.e.* sequence in the library), there will

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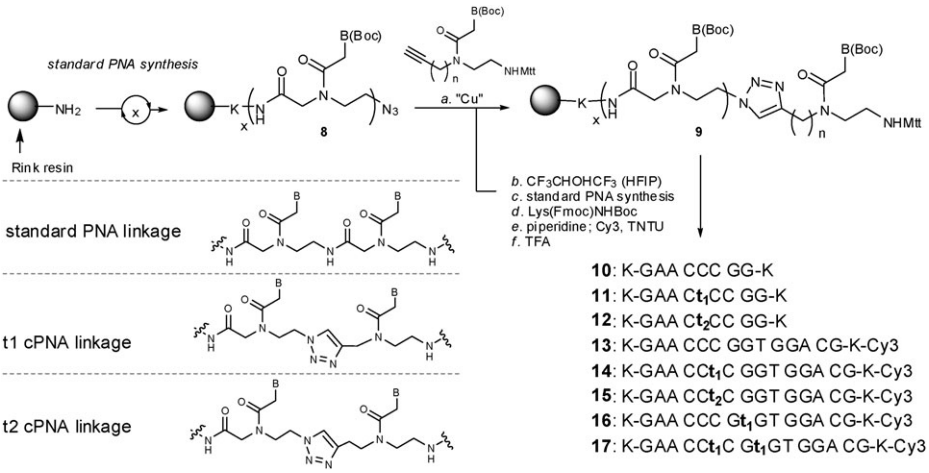

Scheme 2 Synthesis of PNA oligomers containing triazole linkages. (a) Alkyne monomer (7.5 equiv.), sodium ascorbate (7.5 equiv.), $CuSO_4$ (0.25 equiv.), TBTA (0.5 equiv.), NMP/H_2O 10 : 1, 23 °C, 12 h; (b) HFIP/DCE (200 μL per 10 mg of resin) 4 × 2 min; (c) monomer (4.0 equiv.), HATU (3.5 equiv.), 2,6-lutidine (6.0 equiv.), DIPEA (4.0 equiv.), NMP, 23 °C, 25 min; (d) Lys(Fmoc)NHBoc (4.0 equiv.), HATU (3.5 equiv.), 2,6-lutidine (6.0 equiv.), DIPEA (4.0 equiv.), NMP, 23 °C, 25 min; (e) 20% piperidine in DMF, 5 × 2 min; then Cy3 (5.0 equiv.), TNTU (4.5 equiv.), DIPEA (10.0 equiv.), 20% DMSO in NMP, 40 °C, 12 h; (f) neat TFA, 23 °C, 3 h.

be 19 other spots on the array which share three of the same codons and will have only two to three mismatches. The array is laid out so that mismatches at the beginning (C-terminus of the PNA) or end (N-terminus of the PNA) appear vertically or horizontally, respectively, while mismatches on the internal portion of the sequence appear on diagonal lines. We had previously optimized hybridization conditions for 14-mer PNAs and found that PNAs could be detected at or below 1 nM with reliable fidelity by hybridizing at 50 °C.[22] We thus prepared five different 14mer PNAs (**13–17**) with a Cy3 fluorophore[18] containing different combinations of triazole linkages to assess their impact. All probes were hybridized at 0.2, 1 and 5 nM at 50 °C on a slide containing sixteen subarrays thus insuring homogeneous conditions for each experiment. Comparing the intensity of the perfect match *vs.* mismatched hybridization at a given concentration reflects the affinity of the PNA and its fidelity. As shown in Fig. 1, hybridization of PNA **13** (1 nM, see Fig. S2, ESI†) for a picture of the whole slide containing the lower and higher concentrations) showed a strong intensity for the perfect match (PM) and weak intensities for hybridization to sequences sharing three out of the four codons. Mismatched hybridizations in the vertical line were most preponderant reflecting the lower impact of a mismatch at the terminal residues relatively to the internal residues. As evident from the comparison of probes **14** and **16** containing a single t1-linkage to unmodified PNA **13**, a single t1 modification has negligible impact on the overall hybridization equilibrium and slightly improves specificity (comparing the ratio of the perfect match [PM] to the most intense mismatch [MM]). Hybridization of the PNA containing the t2 linkage leads to a sharp decrease in intensity (8 fold) reflecting a lower affinity concurring the T_m measurements by hypochromicity. The PNA containing two t1 modifications separated by two pseudo-nucleosides shows a lower intensity (three fold) without a notable compromise in the selectivity. Whether the distance between triazole junctions has a strong impact remains to

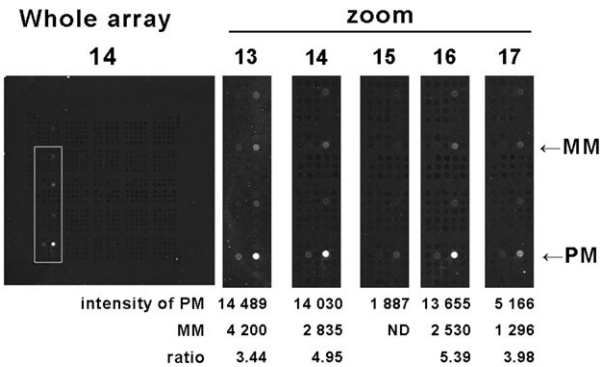

Fig. 1 Fidelity of hybridization for cPNA. Peptide nucleic acids **13–16** were hybridized to a microarray containing 625 different sequences.

be investigated. The mismatched hybridization showing the strongest intensity corresponded to the sequence GCC GGG CCA CCT GC (wherein the underlined residues represent the mismatch) and no signal was detected for mismatches of residues containing the triazole linked nucleotides.

We then turned our attention to the DNA-templated copper catalyzed cycloaddition of alkynic PNA with azido PNA. There is significant interest in these types of reactions for nucleic acid sensing[23–25] and to translate DNA into synthetic polymers.[26–28] While a number of copper catalyzed "click" cycloadditions have been reported in the presence of DNA, our first efforts were unfruitful and led to decomposition of the DNA.[29,30] We found it essential to use a large excess of ligand (THPTA[31]) relatively to copper to avoid DNA degradation. Using 7.0 equiv. of THPTA relatively to copper sulfate (which is reduced *in situ* with ascorbic acid), we observed a complete coupling for the PNA matching the DNA template (**21** + **20**) at 21 °C (Fig. 2) whereas negligible reaction was observed if the azide component was not complementary to the DNA template (**23** + **20**) when performing the reaction at or below 10 μM. A single base pair mismatch in the azide component

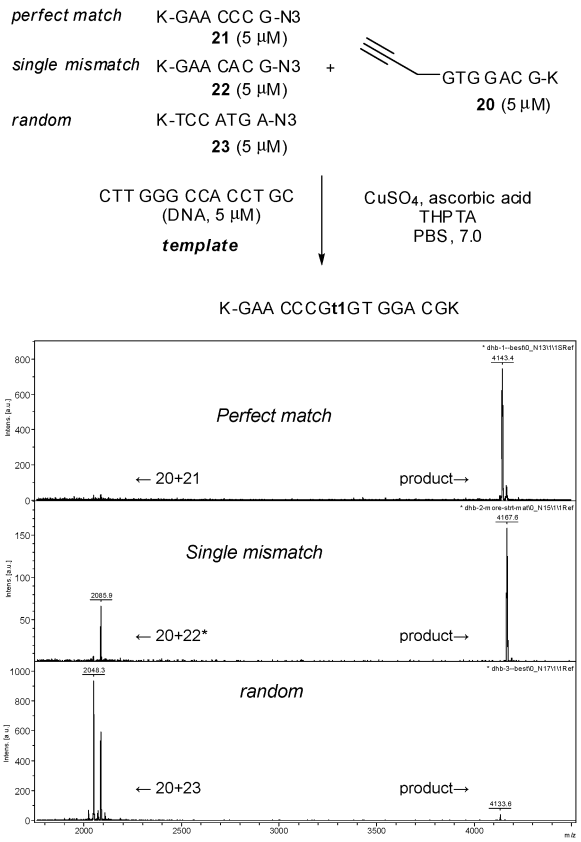

Fig. 2 DNA-templated ligation of azido PNA with alkynic PNA. MALDI analysis of the reaction under positive mode. *Compounds **20** and **22** have similar molecular weight and are not resolved.

(**22** + **20**) afforded intermediate results but never proceeded to completion as for the perfect match. Heating the reaction to 40 °C afforded lower conversion concurring the fact that the azide and alkynic PNA must be hybridized to the DNA template for the reaction to proceed. In order to semi-quantitatively assess the yield of the reaction, the relative intensity of **21** and the product of the cycloaddition (**20** + **21**) in mixtures of known proportion (100 : 1 to 1 : 100) were analyzed by MALDI (Fig. S5, ESI†). Based on these references, the yield of the perfect match reaction (**20** + **21**) can be extrapolated to be between 90%–100% while the reaction with the non-complementary sequence (**20** + **23**) has below 10% conversion. While these results are not of a quantitative nature, they clearly highlight the potential to translate DNA instructions into synthetic nucleic acid polymers.

These results demonstrate the suitability of a triazole *in lieu* of the amide linkage in PNA and enable the coupling of PNA fragments by templated reactions. The versatility of the "click" cycloaddition coupled to the comparable affinity and sequence fidelity of t1 modified PNA for DNA should make these clickable PNAs an important addition in nucleic acid applications and broadens the scope of PNA-encoding technologies. The difference in T_m obtained between t1 and t2 junction in click PNA (cPNA) should be of interest in supramolecular systems requiring different hybridization dynamics.

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