Supporting Information

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Supporting Information

for

As Fast and Selective as Enzymatic Ligations:
Unpaired Nucleobases Increase the Selectivity of DNA-Controlled
Native Chemical PNA Ligation

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PNA-Synthesis.
Solid-phase chemistry was carried out in PE syringes, equipped with teflon filters and
teflon piston, purchased from MultiSynTech. If not differently described, all reactions
were carried out at room temperature. Solvents were dried by standard methods and
freshly distilled prior to use. DMF was purchased in peptide synthesis grade and
used without further purification. Commercial reagents were used without further puri-
fication. PNA monomers and PyBOP were purchased from PerSeptive Biosystems.
Resins and protected amino acids were purchased from Novabiochem.

Preloading of PNA monomers onto the MBHA-Linker.
Resin loadings were aimed at approximately 250 µmol/g by adding the resin in ex-
cess. The resin was washed with DCM (4x 2 mL), 5% DIPEA/DCM (3 min, 2 mL),
DCM (4 x 2 mL) and DMF (4 x 2 mL). For preactivation of the PNA-monomer, 1 equiv
of PyBOP and 2 eq. of NMM were added to a solution of the PNA-monomer (0.09 M)
in DMF. After 3 min of preactivation, the mixture was added to the resin. After 12 h
the resin was washed with DCM (4 x 2 mL), 5% DIPEA/DCM (3 min, 2 mL), DCM (4 x
2 mL) and DMF (4 x 2 mL). For capping, the resin was treated with acetic anhydride/py (1:9, 1 mL) for 10 min. After washing with DMF (4 x 2 mL) and DCM (4 x 2 mL), the resin was dried in vacuo.

**Solid-phase Synthesis according to Boc/Z-strategy.**

*Boc-Cleavage:* After treatment with TFA/m-cresol (19:1, 2 x 5 min, 1 mL) the resin was washed with DCM (5 x 2 mL) and with DMF (5 x 2 mL). *Coupling:* After preactivation of 4 eq. building block (final concentration 0.1 M in DMF) for 2 min, using 4 equiv PyBOP and 6 eq. NMM, the solution was added to the resin. After 2 h, the resin was washed with DMF (5 x 2 mL), DCM (5 x 2 mL) and DMF (5 x 2 mL). *Capping:* Acetic anhydride/py (1:9, 1 mL) was added to the resin. After 5 min the resin was washed with DMF (5 x 2 mL), DCM (5 x 2 mL) and DMF (5 x 2 mL). *Terminal capping:* Acetic anhydride/py (1:9, 1 mL) was added to the resin. After 10 min, the resin was washed with DMF (10 x 2 mL) and DCM (10 x 2 mL). *Resin Cleavage:* Method A: A mixture of 800 µL TFA/m-cresol (19:1, 2 x 5 min, 1 mL) was added to the resin. After washing with DCM (5 x 2 mL), 800 µL TFMSA/TFA/m-cresol (2:8:1) were added to the resin for 2 h. Method B: A mixture of 800 µL TFMSA/TFA/m-cresol (2:8:1) was added to the resin for 2 h. *Work-up:* The resin was washed with TFA (2 x 500 µL). The combined solutions were concentrated *in vacuo* before addition of cold diethylether. The precipitated crude product was dissolved in water, analyzed by HPLC, MALDI-TOF/MS (matrix: DHB or sinapinic acid) and purified by preparative HPLC.

The PNA conjugates *NiL-Nu, AbL-Nu1, AbL-Nu2, NiL-PNA* and *AbL-PNA* were synthesized manually on MBHA-resin following the Boc/Z-strategy. In the last elongation cycle, 4 eq. Boc/Trt-protected cysteine or Boc/Trt/Z-protected 9 were preactivated by using an equimolar amount of PyBOP and 6 eq. of NMM, added to the resin and shaken for 2 h. Subsequent capping and cleavage (method A) was accomplished by following the standard procedure. Yields were determined by photometrical analysis.

\[ \text{H}^2\text{N}(\text{HS})-\text{a-c-c-t-a-c-a-Gly-Gly}^{\text{CONH}}\text{NiL-Nu}: \text{Yield: } \text{OD}_{260} = 19.4; 274 \text{ nmol, 5.5\%}; \]
\[ \text{MALDI-TOF/MS (m/z)}: 2023 ([M+H]^+, \text{theor. 2024}); \text{HPLC: } t_R: 11.8 \text{ min}; \]
\[ \text{C}_{79}\text{H}_{103}\text{N}_{43}\text{O}_{21}\text{S} (2023.02). \]
**Preloading of MBHA-resin for solid-phase synthesis of PNA thioesters.**

3-(Tritylthio)propanoyl-methyl polystyrene: A solution of S-trityl protected mercapto-propionic acid (17.5 mg, 50 µmol), PyBOP (26 mg, 50 µmol) and NMM (8.25 µL, 75 µmol) in 500 µL DMF was stirred for 5 min and added to 160 mg prewashed MBHA-resin (0.8 mmol/g). Washing procedure: DCM (4x 2 mL), 5% DIPEA/DCM (3 min, 1 mL), DCM (4x 2 mL) and DMF (4x 2 mL). After 2 h, the resin was washed with DMF (5x 2 mL) and capped with acetic anhydride/py (1:9, 1 mL) for 10 min. After washing with DMF (4x 2 mL) and DCM (4x 2 mL), the resin was dried in vacuo.

PNA-thioester conjugates **NiL-EI** and **AbL-EI** were synthesized manually starting from the preloaded 3-(tritylthio)propanoyl-methyl polystyrene-resin. Subsequent capping and cleavage (method B) was accomplished by following the standard procedure. The reaction yields were determined by photometrical analysis.
t-c-c-c-a-c-Gly-S(CH\textsubscript{2})\textsubscript{2}CONH\textsubscript{2} AbL-EI: Yield: OD\textsubscript{260} = 151.4; 2.24 µmol, 23%; MALDI-TOF/MS (m/z): 2269 ([M+H]\textsuperscript{+}, theor. 2269); HPLC: t\textsubscript{R}: 13.2; C\textsubscript{90}H\textsubscript{118}N\textsubscript{42}O\textsubscript{28}S (2268.23).

HPLC traces of the abasic PNA native chemical ligation reactions presented in Table 1.

**Figure S1:** HPLC traces of the PNA-native chemical ligations on match template ras2TG (a) and the mismatch forming templates ras2TA (b), ras2TT (c) and ras2TC (d). The corresponding yields are presented in Table 1.
Melting temperature measurements of ternary DNA/PNA complexes.

Figure S2: Melting curves of the corresponding ternary DNA/PNA complexes A) Matched complex: NiLNu-ras3T-NiL-PNA, AbL-Nu-ras3T-AbL-PNA and B) Mismatched complex: NiL-Nu-ras3G-NiL-PNA, AbL-Nu-ras3G-AbL-PNA.