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# The use of Sonogashira coupling for the synthesis of modified uracil peptide nucleic acid

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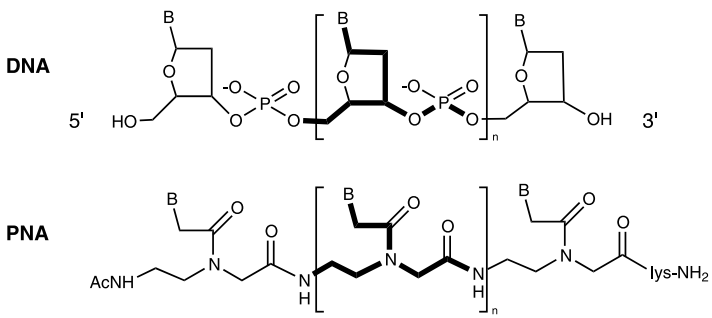
**Abstract**—Palladium-catalyzed Sonogashira coupling has been shown to be compatible with PNA monomers as illustrated by the reaction of 5-iodouracil peptide nucleic acid monomer ($^{I}$U-PNA) with several terminal alkynes. These reactions have been performed in the solution phase and with $^{I}$U-PNA linked to an insoluble polymer support. The results presented herein show that while the isolated yields from the solution phase chemistry are modest (38–53%), the yields of the resin-bound coupling reactions are essentially quantitative, at the monomer level. A selection of alkynes was used to install various additional functionality on the uracil nucleobase. Examples of a hydroxyl, protected thiol and protected amino group are given. Further, an example of derivatization of a resin-bound oligomer with a single $^{I}$U insert is given. 

Peptide nucleic acid (PNA) is an oligonucleotide analog composed of nucleobase derivatives attached to a polyamide backbone. Although PNA is structurally quite different than DNA, the geometric constraint of placing the pendant nucleobases along the polymer backbone is the same '6+3' bond arrangement found in natural DNA, as shown below.[1] It has been determined that PNA may form sequence-specific complexes with either single-stranded DNA or RNA.[2] In addition, PNA is able to bind double-stranded DNA by a strand-displacement mechanism[1,3] or by the more usual triple-helix formation dependent on the sequence context.[4,5]

An important feature in many molecular biological techniques is the recognition of nucleic acid sequences by oligonucleotides. Established techniques such as FISH and quantitative PCR are beginning to make use of PNA due to its improved hybridization characteristics. For such applications, the oligomer is commonly derivatized with a reporter group such as a radioisotope or a fluorophore. It may also be important to use labeled PNA to track cellular compartmentalization or localization in studies that involve antisense-type or ribonucleoprotein inhibition. To date, the radiolabeling of PNA has been done via an appended peptide or oligonucleotide.[6,7] These methods have the potential drawback that the reporter group comprises a substantial portion of the molecule and may modify or govern some of its properties and may be susceptible to degradation by proteases or nucleases. Labeling of PNA with smaller reporter groups has generally been done through the N-terminus or C-terminus that has been limited to only one or two labels thus far.[8] We report herein the development of a method for introducing substituents to uracil peptide nucleic acid monomer that may potentially be used to label or polylabel PNA.

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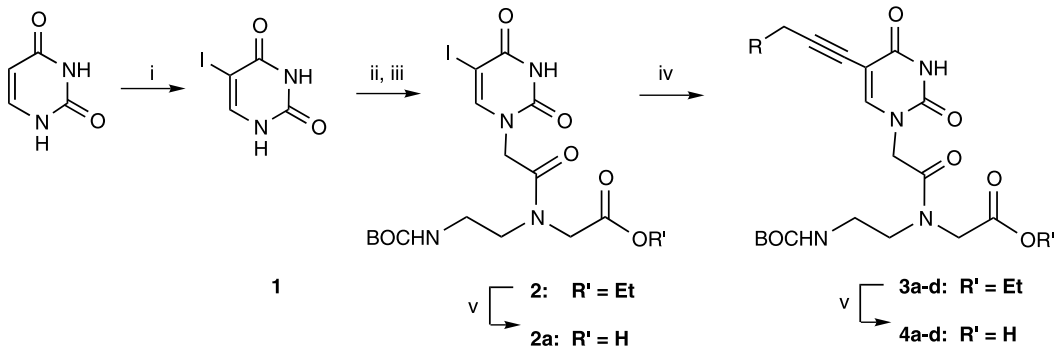
The route is based on well-established coupling of terminal alkynes with aryl iodides and has been employed previously for the synthesis of modified nucleosides.[9,10] Incorporation of a label to the nucleobase avoids the introduction of a stereocenter, as is the case for labeling along the polyamide backbone, for instance by use of an amino acid other than glycine. Importantly, derivatization at the C5 position of uracil does not modify the Watson–Crick base pairing face of the nucleobase and a propynyl-substituent at this position may stabilize duplex formation, as is seen for DNA.[11]

As with base modified nucleosides, we envision a rich area of PNA chemistry to be accessed by developing this strategy. For instance, internal derivatization of an oligomer is a route to branched or dendrimeric structures,[12] which have not yet been reported for PNA. An internal thiol could be used to form bis-PNA type structures from homo- or hetero-oxidative crosslinking with a second thiol labeled PNA oligomer. PNA functionalized with a thiol group may be used to form an intramolecular crosslink resulting in bent PNA, or to bend PNA:NA hybrid duplexes.[13] The hydroxypropynyl or aminopropynyl PNAs that are possible by extension of this method may have improved solubility[14] or other interesting properties, especially the cationic aminopropynyl derivative.[15] Internal labeling of PNA also leaves both the C- and N-terminus available for other forms of derivatization, most notably the possibility of intramolecular cyclization to form cyclic bisPNA molecules.[16] The incorporation of labels internal to the oligomer or at the terminal residues or in conjunction with appended labels on the C-or N-terminus may be used to construct new types of PNA-based molecular beacons.[17] Internal labeling of PNA oligomers also gives rise to the possibility of placing labels with well-defined spacing along the polymer backbone. Finally, internal modification of PNA oligomers could conceivably modulate their antisense/antigene efficacy and the potency of interruption of nucleic acid processing enzymes based on steric grounds and merits investigation.

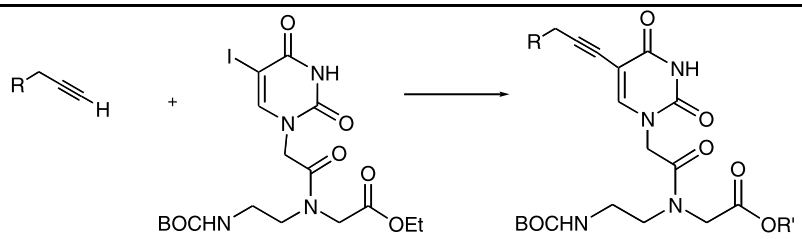
For this work, we started with uracil. Under conditions similar to Robins',[18] 5-iodouracil was easily and reliably prepared in high yield using iodine monochloride in methanol from which the product crystallizes (Scheme 1). The choice of solvent is crucial for the success of this reaction, other common solvents for this type of chemistry such as acetonitrile result in an emulsion from which the product is isolated only with difficulty and in lower yield. A one-step alkylation is then accomplished using chloroacetic acid in aqueous alkali. The moderate yield may be due to the instability of the iodouracil in aqueous base[19] or dialkylation,[20] but it is rapid, convenient and is as efficient as the traditional approach of alkylation with ethyl bromoacetate followed by hydrolysis.[21] The (5-iodouracil-1-yl)acetic acid was then condensed with ethyl (*N*-Boc-aminoethyl)glycinate[22] under standard conditions to give cross-coupling substrate **2**.[23]

The cross-coupling with all of the alkynes was accomplished under standard conditions with only a variation in the reaction times.[24] The alkynes, conditions and yields are illustrated in Table 1. Alkyne **a**, propargyl alcohol, is compatible with the reaction conditions and the hydroxyl group need not be protected. It is envisaged that this alkyne may be used to prepare a hydroxylated PNA oligo that may have improved solubility characteristics. 2-Propynylmercaptan was masked as the *p*-methoxybenzylthioether (alkyne **b**), a standard protecting group compatible with a graded acidolysis deprotection scheme.[25] Incorporation of a thiol group into a PNA represents a unique chemically addressable group and may be used as a site for conjugation to thiol-reactive probes or dyes. Alkynes **c** and **d** are both derivatives of propargylamine in which an electroactive reporter group[26] or photolabile protecting group were used, respectively. The coupling reactions (Scheme 1) and subsequent hydrolysis proceeded with chemical yields given in Table 1. The modest overall chemical yields are due in part to difficulties encountered in the isolation and chromatographic purification of these compounds. However, NMR and HPLC analysis of the purified products indicated that they were homogeneous and they gave satisfactory spectroscopic characterization.[27–30]




**Scheme 1.** General scheme for the synthesis of 5-derivatized uracil peptide nucleic acid monomers. *Reagents and conditions* (i) ICl, MeOH, 50°C, 4 h, 98%; (ii) $ClCH_2CO_2H$, aq. KOH, Δ, 1 h, 62%; (iii) DCC, HOBt, $BOCNH(CH_2)_2NHCH_2CO_2Et$, 1:1 DCM:DMF, 73%; (iv) $R'CH_2CCH$, $Pd(PPh_3)_4$, CuI, $NEt_3$, DMF 3–16 h; (v) 1 M NaOH/THF, 45 min.

**Table 1.** Alkyne derivatives used for coupling to 5-iodouracil PNA monomer




**5a-d** **2** **3a-d: R' = Et** **4a-d: R' = H**

| Entry* | alkyne | Time (hr) | Yield **3** (%) | Yield **4** (%) | Isolated (%) |
|---|---|---|---|---|---|
| **a** | R= $HO^-$ | 3 | 43 | 90 | 38 |
| **b** | R= $H_3CO$–C$_6$H$_4$–$CH_2S$- | 3 | 64 | 83 | 53 |
| **c** | R= Fe(C$_5$H$_4$–HN-)(C$_5$H$_5$) | 16 | 51 | 85 | 43 |
| **d** | R= $H_3CO$–C$_6$H$_3$($NO_2$)–O–NH- | 3 | + | + | 38 |

***Reagents and conditions***: **a**, commercially available; **b**, *i)* propargyl alcohol, MsCl, TEA, $K_2CO_3$, toluene, 0 °C, 2hr, 90% *ii)* sodium 4-methoxy-benzylsulfide, MeCN, Δ, 4hr, 63%; **c**, ferrocene carboxylic acid, DCC, HOBt, progargylamine, $CH_2Cl_2$, 18hr, 91%; **d**, *i)* triphosgene, dioxane, 48hr, *ii)* propargyl amine, $NaHCO_3$.
+ not determined.

Next, we investigated the conversion of the iodouracil PNA whilst attached to solid support. We choose to attach to the 5-iodouracil PNA monomer to HMBA-AM resin,[31] a linker that is cleaved under basic conditions to yield C-terminal acids.[32] The products in the cleavage mixture could then be directly compared to the compounds synthesized by solution methods. Fig. 1 illustrates comparison of the crude product mixtures taken directly from resin treated with CsOH, to the standard compounds. Panel a illustrates that $^{I}$U-PNA can be loaded onto the resin and directly cleaved without modification as shown by coinjection with authentic material. Panels b–d are for the final products whose structures are shown inset. Of note, each of these examples shows the complete absence of $^{I}$U-PNA monomer indicating complete conversion to the cross-coupled product.

It was clear that the coupling conditions were effective for derivatization of a PNA monomer on the support,[33] but arguably a more useful approach is the derivatization in the context of an oligomer. For this experiment, a 7-mer PNA of the sequence $H_2$N-lys-T$^{I}$UTCCTT-lys-$CO_2H$[34] (**5**) containing a single $^{I}$U-PNA insert was prepared by manual solid-phase peptide synthesis.[35] A portion of the $^{I}$U-PNA containing oligomer was cleaved under standard TFMSA conditions and analyzed by RP-HPLC (Fig. 2, panel a: crude oligomer; panel b: purified oligomer). A portion of the same resin was then treated to the usual coupling conditions with propargyl alcohol (PA) as the alkyne component. Panel c and d are the chromatograms for the crude and purified $H_2$N-lys-T$^{PA}$UTCCTT-lys-$CO_2H$[36] (**6**). Although it is difficult to gauge the chemical yield of cross-coupling reaction it is evident that it proceeds to give the major product. Both of these oligomers were examined for their capacity to bind to complementary DNA. Under the given ionic conditions (10 mM $PO_4^{3-}$, 100 mM NaCl, 0.1 mM EDTA, pH 7) the DNA:DNA duplex did not give a measurable $T_m$ (<10°C) and the unmodified PNA:DNA duplex melted at 47.5°C. Examination of the $T_m$ for both the 5-iodouracil-containing PNA oligomer ($T_m$ = 46.0°C) and the propargyl alcohol modified uracil PNA oligomer ($T_m$ = 47.0°C) indicate that these substitutions are well tolerated.

In summary, we have demonstrated that both solution-phase and solid-phase Sonogashira coupling is a feasible route to 5-derivatized uracil-PNA residues. As expected, this reaction is tolerant to a variety of alkynes. Importantly, the cross-coupling reaction also works in the context of an oligomer. We are currently examining the scope and utility of this method for the synthesis of nucleobase-modified PNA.

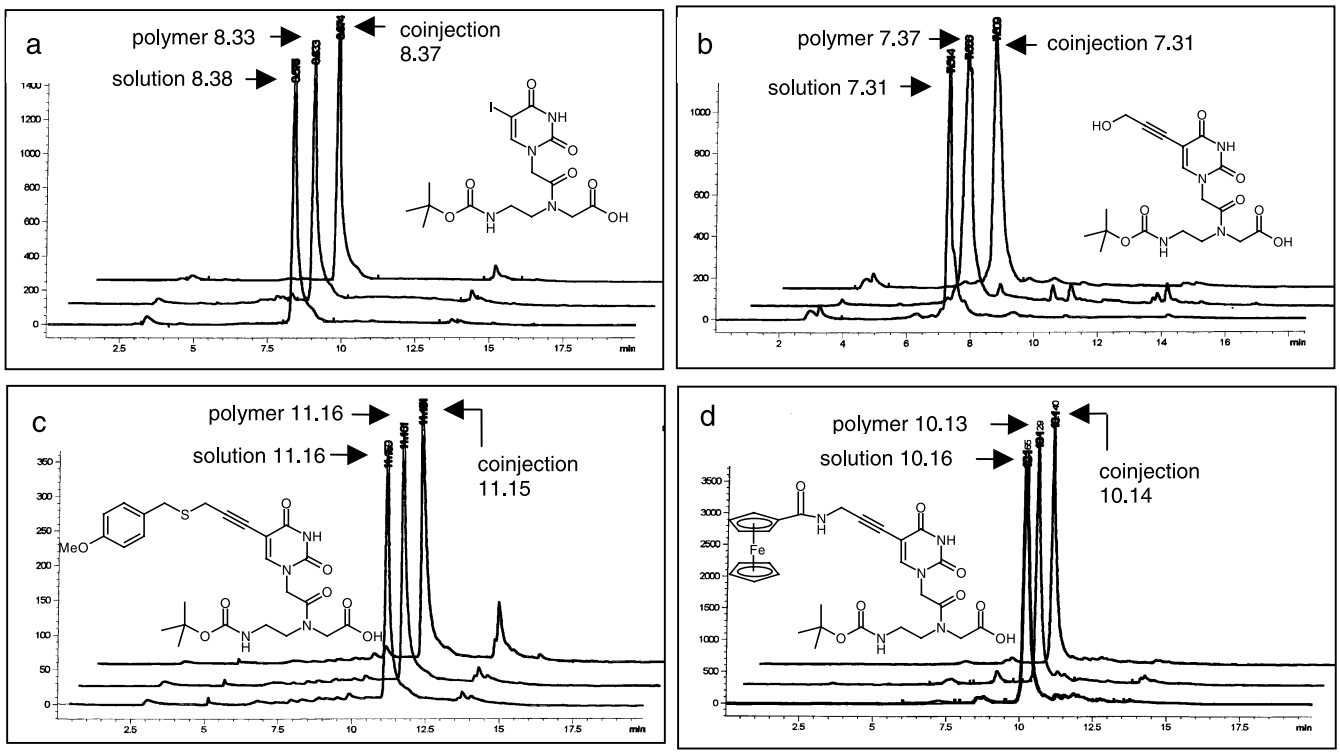



**Figure 1.** RP HPLC analysis of the solid-phase derivatization of 5-iodouracil PNA monomer. (i) solution: authentic sample prepared by solution-phase methods, (ii) polymer: crude mixture direct from cleavage of polymer-supported reaction, (iii) coinjection: approximately an equimolar mixture of (i) and (ii). Panel a: 5-iodouracil PNA monomer. Coupling with alkyne component - Panel b: **4a**; Panel c; **4b**; Panel d: **4c**. Conditions: reversed-phase HPLC (C18, Rainin Microsorb-MV, 100 Å) and a gradient composed of A (aqueous $H_3PO_4$, pH 2.00) and B (acetonitrile) at a flow rate 1.00 mL/min. Gradient: initial conditions - 90% A, 10% B for one minute then a linear gradient to 35% A, 65% B over 9 min followed by a linear gradient to 100% B over the next 3 min.

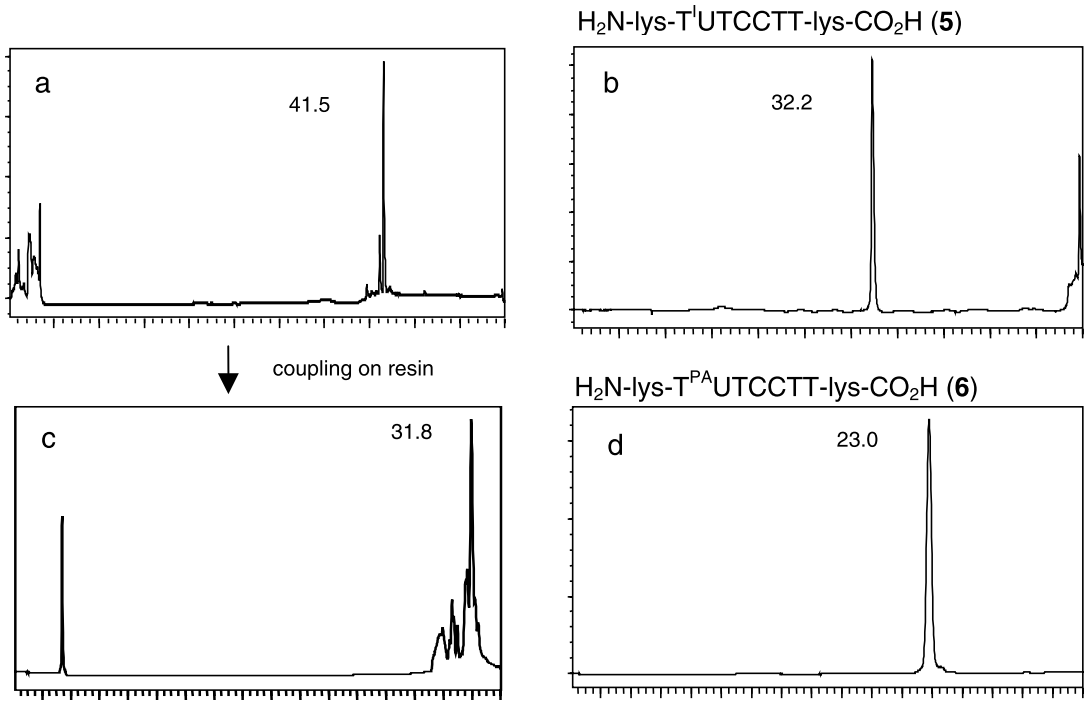



**Figure 2.** HPLC analysis of the solid-phase derivatization of 5-iodouracil within a 7-mer PNA. Panel a, b: oligomer-$H_2N$-lys-$T^{I}$UTCCTT-lys-$CO_2H$ (**5**), crude and purified, respectively. Panel c, d: oligomer-$H_2N$-lys-$T^{PA}$UTCCTT-lys-$CO_2H$ (**6**), crude and purified, respectively. Note: different elution conditions were used, thus retention times, shown inset, cannot be reliably compared. Analytical RP HPLC (C18, Rainin Microsorb-MV, 100 Å, solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in acetonitrile) conditions for the analysis of oligomer **5**: 1 min. 100% A, linear gradient over 45 min to 30% B followed by a gradient to 100% B over the next 2 min. Analysis of **6**: 1 minute 100% A, linear gradient over 30 min to 35% B followed by a gradient to 100% B over the next 2 min, at 50°C.

## Acknowledgements

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## References

1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
2. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566–568.
3. Nielsen, P. E.; Egholm, M.; Buchardt, O. *J. Mol. Recognit*. **1994**, *7*, 165–170.
4. Wittung, P.; Nielsen, P. E.; Norden, B. *Biochemistry* **1997**, *36*, 7973–7979.
5. The chemistry and properties of PNA has been reviewed recently and appears in *Peptide Nucleic Acids Protocols and Applications*; Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific Press: Wymnodham, England, 1999.
6. Kozlov, I. A.; Nielsen, P. E.; Orgel, L. E. *Bioconjugate Chem*. **1998**, *9*, 415–417.
7. Uhlmann, E. *Biol. Chem*. **1998**, *379*, 1045–1052.
8. A recent example: Hess, A.; Metzler-Nolte, N. *Chem. Commun*. **1999**, 885–886 and Ref. 5.
9. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett*. **1975**, 4467–4470.
10. Hobbs, F. W., Jr. *J. Org. Chem*. **1989**, *54*, 3420–3422.
11. Froehler, B. C.; Wadwani, S.; Trehorst, T. J.; Gerrard, S. R. *Tetrahedron Lett*. **1992**, *33*, 5307–5310.
12. Comb-type branched nucleics have been synthesized by branching from the nucleobase, for example: Horn, T.; Urdea, M. S. *Nucleic Acids Res*. **1989**, *17*, 6959–6967.
13. Glick, G. D. *Biopolymers* **1988**, *48*, 83–96 and references cited therein.
14. An experimental observation is that the C-5 (3-hydroxypropynyl) derivative PNA monomer is a deliquescent solid, unlike thymine PNA monomer and is easily dissolved in water.
15. (a) Strauss, J. K.; Roberts, C.; Nelson, M. G.; Switzer, C.; Maher, L. J., III. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9515–9520; (b) Dande, P.; Liang, G.; Chen, F.-X.; Roberts, C.; Nelson, M. G.; Hashimoto, H.; Switzer, C.; Gold, B. *Biochemistry* **1997**, *36*, 6024–6032.
16. Cyclic oligonucleotides have been extensively studied and reviewed by: Kool, E. J. *Chem. Rev*. **1997**, 1473–1487.
17. Tyagi, S.; Kramer, F. R. *Nature Biotech*. **1996**, *14*, 303–308.
18. Robins, M. J.; Barr, P. J.; Giziewicz, J. *Can. J. Chem*. **1982**, *60*, 554–557.
19. We have not attempted to identify any by-products. Ueda, T. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, USA, 1988; Vol. 1, pp. 33–37.
20. Dallaire, C.; Arya, P. *Tetrahedron Lett*. **1998**, *39*, 5129–5132.
21. In our hands, the alkylation of 5-iodouracil with ethyl bromoacetate and $K_2CO_3$ in DMF and subsequent hydrolysis gave the free acid in approximately 60% yield (isolated).
22. (a) Ethyl (*N*-Boc-aminoethyl)glycinate was prepared by the alkylation of ethyl bromoacetate with 2-*N*-*tert*-butoxycarbonylaminoethylamine as reported: Meltzer, P. C.; Liang, A. Y.; Matsuidaira, P. *J. Org. Chem*. **1995**, *60*, 4305–4308; (b) Preparation of 2-*N*-*tert*-butoxycarbonylaminoethylamine followed the method of: Krapcho, A. P.; Kuell, C. S. *Synth. Commun*. **1990**, *20*, 2559–2564.
23. Ethyl *N*-(2-Boc-aminoethyl)-*N*-(5-iodouracil-1-yl) glycinate (**2**): $^1$H NMR (400 MHz, $(CD_3)_2CO$, major (ma.) and minor (mi.) rotamer signals) $\delta$ 7.96 (ma.) and 7.94 (mi.) (s, 1H), 6.25 (ma.) and 5.97 (mi.) (s, 1H), 4.86 (ma.) and 4.68 (mi.) (s, 2H), 4.37 (mi.) and 4.12 (ma.) (s, 2H), 4.12 (q, 2H, $J$=7.7 Hz), 3.58 (ma.) and 3.50 (mi.) (t, 2H, $J$=6.0 Hz), 3.36 (ma. and 3.21 (mi.) (m, 2H), 1.41 (ma.) and 1.39 (mi.) (s, 9H), 1.22 (t, 3H, $J$=7.0 Hz). HR-MS (FAB) calcd for $C_{17}H_{25}IN_4O_7$, 524.0768, found: 523.9984.
24. A typical procedure for the cross-coupling reaction and subsequent purification follows: To a solution of compound ethyl *N*-(2-Boc-aminoethyl)-*N*-(5-iodouracil-1-yl) glycinate **2** (1 equiv.), alkyne (3 equiv.), dry triethylamine (2 equiv.) in 5 mL of dry DMF were added tetrakis(triphenylphosphine) palladium (0) (0.1 equiv.) and copper(I) iodide (0.3 equiv.). The resulting mixture was stirred at room temperature for the indicated time and the reaction was quenched by the addition of 7 mL of cold water. The crude product was isolated by extraction into EtOAc (3×10 mL). The combined organic layers were washed with water (2×10 mL), (brine 10 mL) and then dried over $Na_2SO_4$. The desired product was isolated by silica gel column chromatography (typically 5–10% MeOH in DCM). The alkynes used were: propragyl alcohol (Aldrich, used as received); methyl 4-[(2-propynylthio)methyl] phenyl ether, $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.27 (d, 1H, $J$=8.8 Hz), 6.88 (d, 1H, $J$=8.4 Hz), 3.83 (s, 2H), 3.76 (s, 3H), 3.14 (d , 2H, $J$=2.8 Hz), 2.74 (t, 1H, $J$=2.4 Hz), HRMS calcd for $C_{11}H_{12}OS$: 192.0609, found: 192.0615; 3-(ferrocenyl carbonyl)amino-1-propyne, $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 5.80 (br s, 1H), 4.70 (s, 2H), 4.38 (s, 2H), 4.23 (s, 5H), 4.17 (s, 2H), 2.27 (t, 1H, $J$=2.0 Hz) HRMS calcd for $C_{14}H_{13}ONFe$: 267.0346, found: 267.0347; 4, 5-dimethoxy-2-nitrobenzyl 2-propynylcarbamate, $^1$H NMR (400 MHz, $CDCl_3$) $\delta$7.65 (s, 1H), 6.92 (s, 1H), 5.47 (s, 2H), 5.05 (b, 1H), 3.96 (q, 2H, $J$=1.6 Hz), 3.92 (s, 3H), 3.89 (s, 3H), 2.20 (t, 1H, $J$=2.4 Hz). MS (EI) $m/z$ 294 ($M^+$).
25. Barany, G.; Merrifield, R. B. In *The Peptides Analysis, Synthesis and Biology*; Gross, E.; Meienhofer, J., Eds.; Academic Press: New York, USA, 1980; Vol. 2, pp. 233–240.
26. A recent report highlighting an unexpected modification to a uridine base under cross-coupling conditions with

ferrocenylacetylene (Yu, C. J. et al. *J. Am. Chem. Soc.* **2000**, *122*, 6767–6768.) was not found to occur with alkyne **c**, nor **a**, **b** and **d**.

27. *N*-(2-Boc-aminoethyl)-*N*-{[5-(3-hydroxy-1-propynyl)-uracil-1-yl]acetyl} glycine (**4a**).
$^1$H NMR (400 MHz, basic $D_2O$, major (ma.) and minor (mi.) rotamer signals): $\delta$ 7.78 (ma.) and 7.77 (mi.) (s, 1H), 4.75 (ma.) and 4.59 (mi.) (s, 2H), 4.32 (s, 2H), 3.93 (ma.) and 3.86 (mi.) (s, 2H), 3.63 (mi.) and 3.40 (ma.) (m, 2H), 3.27 (mi.) and 3.14 (ma.) (m, 2H), 1.34 (s, 9H). MS (MALDI TOF) calcd for $C_{18}H_{24}N_4O_3$ 424.405, found: 424.5.
28. *N*-(2-Boc-aminoethyl)-*N*-[5-{3-[(4-methoxybenzyl)thio]-1-propynyl}uracil-1-yl]acetyl glycine (**4b**).
$^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 10.25 (s, 1H), 7.64 (ma.) and 7.63 (mi.) (s, 1H), 7.21 (d, 2H, $J=8.8$ Hz), 6.75 (d, 2H, $J=8.8$ Hz), 6.15 (ma.) and 5.88 (mi.) (br s, 1H), 4.73 (ma.) and 4.58 (mi.) (s, 2H), 4.25 (mi.) and 4.03 (ma.) (s, 2H), 3.76 (s, 2H), 3.65 (s, 3H), 3.47 (ma.) and 3.40 (mi.) (t, 2H, $J=6.4$ Hz), 3.26 (ma.) and 3.11 (mi.) (m, 2H), 3.17 (s, 2H), 1.29 (ma.) and 1.27 (mi.) (s, 9H). MS (MALDI TOF) calcd for $C_{26}H_{32}N_4O_8S$ 560.620, found: 562.4.
29. *N*-(2-Boc-aminoethyl)-*N*-{5-[3-(ferrocenecarbonyl)amino-1-propynyl]uracil-1-yl}-acetyl glycine (**4c**).
$^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 10.32 (s, 1H), 7.74 (ma.) and 7.71 (mi.) (s, 1H), 7.56 (br s, 1H), 6.28 (ma.) and 6.00 (mi.) (br s, 1H), 4.84 (s, 2H), 4.84 (ma.) and 4.74 (mi.) (s, 2H), 4.35 (d, 2H, $J=1.8$ Hz), 4.44 (mi.) and 4.22 (ma.) (s, 2H), 4.27 (d, 2H, $J=2.3$ Hz), 4.21 (s, 5H), 4.15 (ma.) and 4.27 (mi.) (s, 2H), 3.58 (ma.) and 3.51 (mi.) (t, 2H, $J=5.8$ Hz), 3.38 (ma.) and 3.22 (mi.) (m, 2H), 1.42 (ma.) and 1.40 (mi.) (s, 9H). MS (MALDI TOF) calcd for $C_{29}H_{33}FeN_5O_8$ 635.446, found: 635.3.
30. *N*-(2-Boc-aminoethyl)-*N*-{5-[3-([(4,5-dimethoxy-2-nitrobenzyl)oxycarbonyl]amino)-1-propynyl]-uracil-1-yl}acetyl glycine (**4d**).
$^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 10.39 (s, 1H), 7.78 (s, 1H), 7.74 (s, 1H), 7.22 (s, 1H), 7.12 (br s, 1H), 6.28 (br s, 1H), 5.48 (s, 2H), 4.88 (ma.) and 4.73 (mi.) (s, 2H), 4.39 (mi.) and 4.08 (ma.) (s, 2H), 4.08 (s, 2H), 3.98 (s, 2H), 3.97 (s, 6H), 3.59 (ma.) and 3.54 (mi.) (m, 2H), 3.29 (ma.) and 3.24 (mi.) (m, 2H), 1.41 (s, 9H). MS (MALDI TOF) calcd for $C_{28}H_{34}N_6O_{13}$ 662.602, found: 662.6.
31. HMBA-AM resin (HMBA-AM: p-hydroxymethylbenzoic acid aminomethyl Merrifield resin) was obtained from NovaBiochem (La Jolla, CA, USA). Conditions used for loading $^1$U-PNA: HMBA-AM resin (421 mg, 0.43 mmol) was swollen with 2 mL of dry DMF for 30 min. After draining the solvent, a solution containing compound **2a** (645 mg, 1.30 mmol), HBTU (411 mg, 1.08 mmol), DIPEA (153 mg, 1.30 mmol) and catalytic amount of DMAP dissolved in 2 mL of dry DMF was added to the resin. The resulting mixture was stirred at room temperature for 24 h. The excess reagents were drained off and the resin was washed alternating with 3×2 mL of DCM, 3×2 mL of MeOH.
32. Cleavage was effected by a brief treatment of the resin (50 mg) with 1 mL of a solution of the composition: 1 mL of 50% aqueous CsOH solution, 1 mL MeOH, 10 mL water and 11 mL THF.
33. 50 mg of the $^1$U-PNA derivatized HMBA-AM resin was swollen with 0.5 mL of dry DMF for 30 min. After draining the solvent, a solution of copper(I) iodide (0.2 equiv.) and dry triethylamine (2 equiv.), alkyne (3 equiv.), tetrakis(triphenylphosphine) palladium (0.1 equiv.) in 0.5 mL of dry DMF were added in that order. The mixture was then agitated for 16 h after which the excess reagents were washed off with 3×1 mL of DCM, 3×1 mL of MeOH alternatively. The resin was then dried in air.
34. MALDI-TOF, $C_{86}H_{119}N_{34}O_{29}I$ , calcd: 2220 amu, found: 2224 amu.
35. Boc-lys 2-Cl-Z PAM resin is available from NovaBiochem. The oligomerization chemistry follows that of: Koch, T. In *Peptide Nucleic Acids: Protocols and Applications*; Nielsen, P. E.; Egholm, M., Eds., Horizon Scientific Press: UK, 1999; Chapter 2.1.
36. MALDI-TOF, $C_{89}H_{122}N_{34}O_{30}$ , calcd: 2148 amu, found: 2189 amu (K+ adduct).