Synthesis and Properties of Oligonucleotides that Contain a Triazole-Linked Nucleic Acid Dimer

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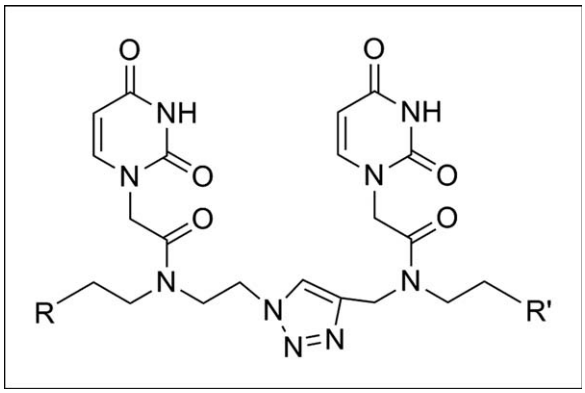

New chemically modified oligonucleotides at the site of the backbone are needed to improve the properties of oligonucleotides. A practical synthesis for a triazole-linked nucleoside dimer based on a PNA-like structure has been developed. This involves synthesizing two uracil-based monomers that contain either an azide or an alkyne functionality, followed by copper-catalyzed 1,3-dipolar cycloaddition. This dimer was incorporated within an oligonucleotide via phosphoramidite chemistry and UV-monitored thermal denaturation data illustrates slight destabilization relative to its target complementary sequence. This chemically modified dimer will allow for a future investigation of its properties within DNA and RNA-based applications.

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INTRODUCTION

Oligonucleotides have found tremendous use as biological tools and as agents with therapeutic promise. One promising example involves RNA interference (RNAi), and exploiting this pathway has become a major tool in elucidating gene function [1]. Furthermore, utilization of the RNAi pathway by administering short interfering RNAs (siRNAs) to cells [2] has offered therapeutic hope in combating diseased cells with aberrant gene expression profiles [3]. Despite awareness of this potential, improvements in enzymatic stability, biodistribution, cell-membrane permeability, and reducing off-target effects are still needed [4,5]. One way to potentially overcome these limitations involves altering the stability and specificity profile of the siRNA through chemical modification.

Within the areas of siRNA, the most common type of chemical modification involves alterations within the ribose moiety. Extensive studies involving 2′-*O*-Me, fluorinated sugars and locked nucleic acids (LNA) have shown favorable siRNA knockdown results depending on its position and sequence context [6–9]. Studies involving chemical modification of the backbone within the field of siRNA are less prevalent than sugar modifications. Some key examples include the negatively charged backbone modification involving a phosphorothioate [10] and boranophosphates [11]. Backbone-altering modifications such as a morpholino analogue [12] and neutral amide bonds at 3′ overhangs of siRNA duplexes, have shown favorable results when utilized for gene-knockdown studies [13,14]. Therefore, it is possible that the RNAi pathway will adopt less conventional backbone modifications at specific positions of the oligonucleotide. Given that siRNA duplexes exhibit thermodynamic asymmetry, being able to potentially destabilize the 5′ end of the guide strand with novel backbone modifications may offer favorable properties. Outside of the RNAi field, modified backbone constructs could have many other potential applications such as in antisense technology, artificial aptamer design or template driven enzymatic reactions. To explore this prospective, we are interested in pursuing alternative backbone modifications such as nonionic hydrophobic backbone mimics.

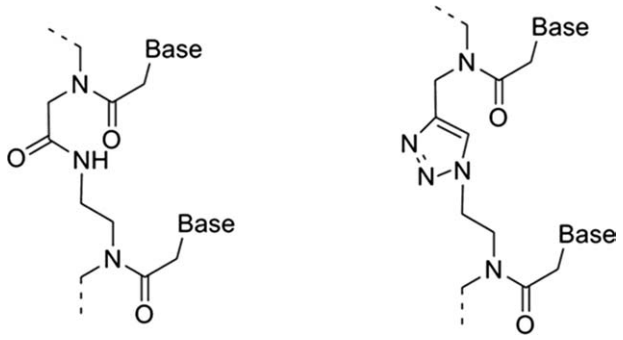


Figure 1. Chemical differences between peptide nucleic acid (left) and triazole-linked nucleic acid (right).

Within the field of backbone modified oligonucleotides, one of the most successful examples involves peptide nucleic acid (PNA) [15] and this has shown remarkable stability and specificity to its complementary targets [16,17]. PNA synthesis occurs by the successful amide-bond formation between an acid and an amine with high-yields. Therefore, we envisioned that a scaffold based on a triazole functional group would serve as a potentially good candidate (Fig. 1). To date, a number of different triazole-based backbone modifications have been reported [18–20] from the one presented herein.

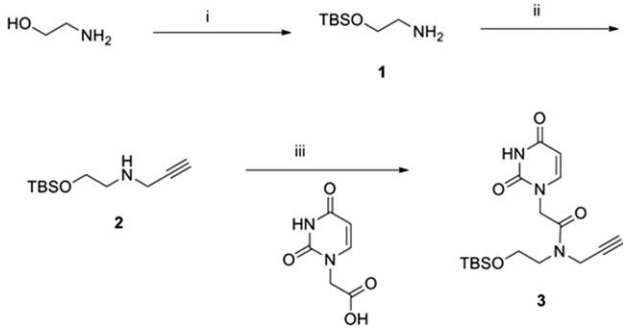
RESULTS AND DISCUSSION

The repetitive backbone unit in most analogues, including PNA, contains six atoms. Although the six-atom spacer unit dominates by choice for a large number of chemically modified nucleic acids, analogues with shorter (five atom linker) [21–23] and longer backbones (seven atom linker) [24,25] have shown varying degrees of specificity to its target, depending on its sequence context and type of backbone. This suggests that site-specific incorporation of unnatural nucleoside backbones of different atom lengths can produce functioning oligonucleotides, capable of binding to their complement strand with varying specificity and stability. As such, chemically modifying oligonucleotides could offer an alternative way to fine-tune their functional effects.

We hypothesized that a copper (I)-catalyzed Huisgen [3 + 2] cycloaddition [26] based on a PNA-type structure would generate an alternative scaffold for bridging adjacent nucleotides together. We report the synthesis of a modified 1,4-triazole-linked uracil dimer through a distance of seven atoms and report its compatibility with hybridization to a complement sequence when appended at the 5′ or 3′ end of a DNA oligonucleotide.

Our strategy involves the synthesis of a uracil-based monomer possessing an alkyne functional group and a

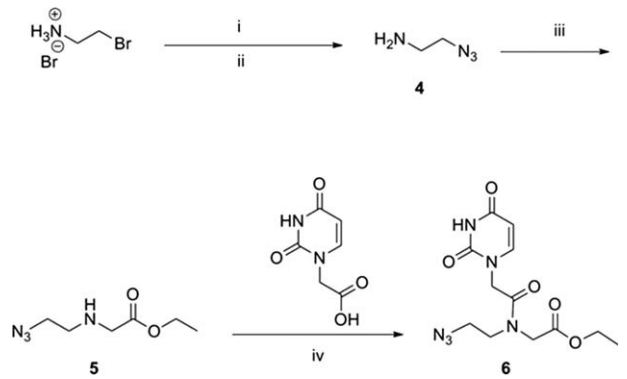
Scheme 1. Reagents and conditions: (i) 1 equiv imidazole, 1 equiv TBS-Cl, DCM, room temperature 96% yield of **1**; (ii) 0.5 equiv DIPEA, 0.5 equiv propargyl bromide, DCM, room temperature, 3 h, 71% yield of **2**; (iii) 1.5 equiv uracil-1-yl acetic acid, 1.5 equiv DIPEA, 1.5 equiv HBTU, DMF, 24 h, 44% yield of **3**.



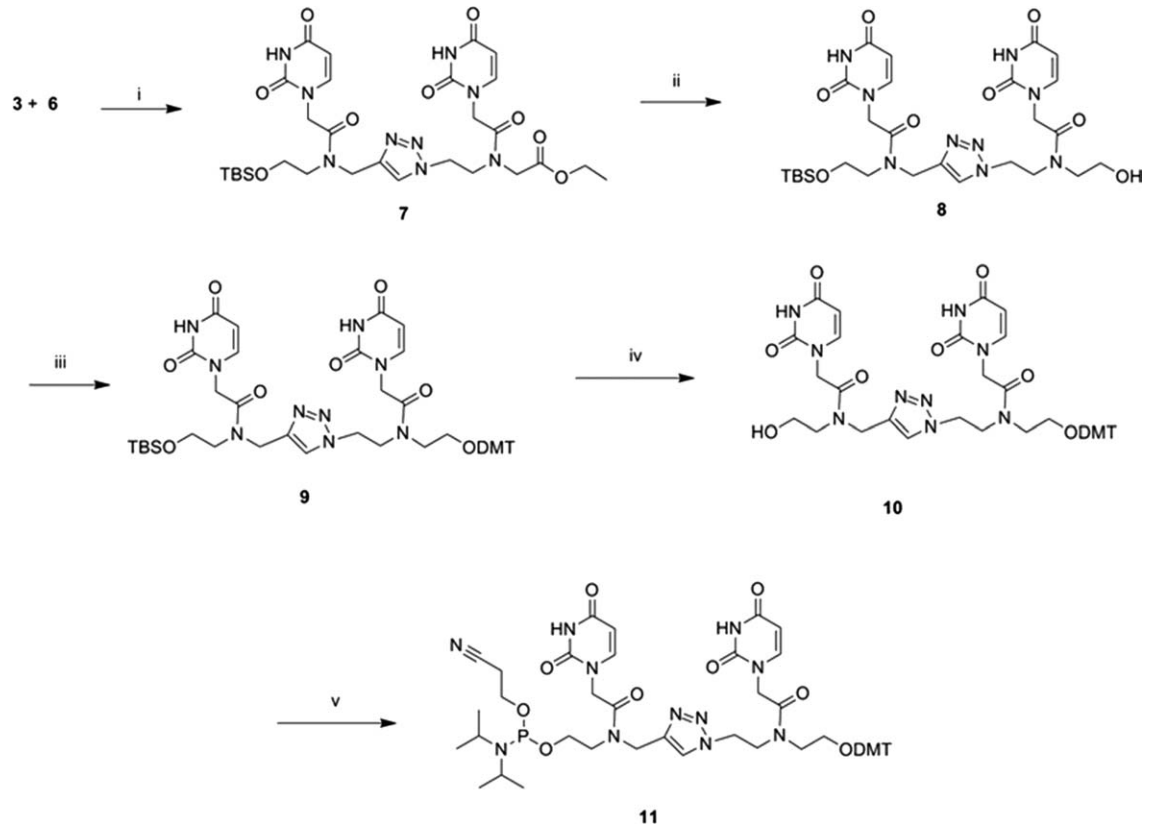
uracil-based monomer with an azide. For the synthesis of the uracil-based alkyne monomer, the first step involved *tert*-butyldimethylsilyl (TBS) protection of ethanolamine and this was conducted using TBSCl under standard basic conditions to afford **1** in 96% yield. Alkylation of **1** with propargyl bromide generated the alkyne **2** as a liquid in 71% yield. Utilizing peptide bond coupling conditions involving HBTU, the alkyne **2** was amide-bond coupled to uracil-1-yl acetic acid [27] to afford compound **3** as a white solid in 44% yield (Scheme 1).

For the synthesis of the azide-based uracil monomer **6**, 2-azidoethylamine **4** was prepared by the addition of 2-bromoethylamine to an aqueous solution of sodium azide. Alkylation of **4** with a limiting amount of ethyl 2-bromoacetate afforded compound **5** as an off-colored liquid in 83% yield. This liquid **5** was amide-bond coupled with uracil-1-yl acetic acid using DCC/HOBt to afford the azide-based uracil monomer, compound **6** in 62% yield (Scheme 2).

Scheme 2. Reagents and conditions: (i) 3 equiv NaN_3, H_2O, 75°C, 24 h; (ii) 5.8 equiv NaOH, 0°C, Et_2O, 64% yield of **4**; (iii) 1 equiv NEt_3, 0.6 equiv ethyl 2-bromoacetate, DMF, 4 h, 83% yield of **5**; (iv) 1.1 equiv uracil-1-yl acetic acid, 1.1 equiv DCC, 1.1 equiv HOBt, DMF, 24 h, 62% yield of **6**.



Scheme 3. Reagents and conditions: (i) 2 equiv sodium ascorbate, 0.5 equiv Cu(II)SO_4, THF/*t*-BuOH/H_2O (1:1:1), 24 h, 98% yield of **7**; (ii) 2.5 equiv $LiBH_4$, THF/MeOH (12:1), reflux 1.5 h, 76% yield of **8**; (iii) 3 equiv DMT-Cl, pyridine, 24 h, 79% yield of **9**; (iv) 3 eq TBAF, THF, 12 h, 85% yield of **10**; (v) 2 equiv 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, 5.5 equiv DIPEA, 0.5 equiv DMAP, DCM, 8 h, 63% yield of **11**.



With respect to the synthesis of the phosphoramidite, the first task was to cyclize both monomers together. Under copper (I) conditions, 1,4-triazole formation occurred with copper sulfate and sodium ascorbate to afford a white solid **7** in 98% yield. This newly formed dimer **7** was selectively reduced with 2.5 equivalents of $LiBH_4$ in a methanol/THF mixture to afford the alcohol **8** in 76% yield. Alcohol **8** was treated with 4,4′-dimethoxytrityl-chloride (DMT-Cl) to afford the compound **9** in 79% yield. The subsequent removal of the TBS group using TBAF provided the alcohol **10** in 85% yield. This was then followed by synthesis of phosphoramidite **11** by reacting alcohol **10** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite to afford a clear oil in 63% yield (Scheme 3). With this phosphoramidite **11**, its incorporation within a DNA oligonucleotide was accomplished by employing solid-phase oligonucleotide synthesis on a 394 ABI synthesizer.

UV-melting denaturation studies indicate that moderate destabilization occurs with the UU-dimer on both the 5′ and 3′ position relative to the native strand (Table 1). A reduction in T_m of 4.5°C and 2.8°C is observed when native TT is replaced with the UU-dimer modification at the 5′ end and the 3′ end, respectively. However, this suggests a certain degree of compatibility as the T_m values for both modified oligonucleotides are higher when compared to corresponding 12-mer sequences lacking the heterocyclic pyrimidine dimer (**UU**) (Table 1). In comparison to other studies [28] and that of Kraicsovits and coworkers, a similar T_m drop was

Table 1

UV-melting denaturation studies.

Entry	Sequence	T_m (°C)	ΔT_m (°C)[a]
I	5′ TTTTTCTCTCTCTT 3′	40.9	0
II	5′ TTTTTCTCTCT**UU** 3′	38.1	−2.8
III	5′ TTTTTCTCTCTC 3′	37.1	−3.8
IV	5′ **UU**TTTCTCTCTCTT 3′	36.4	−4.5
V	5′ TTTCTCTCTCTT 3′	35.3	−5.6

UV melting temperatures for duplexes to complement strand DNA (5′-AAG AGA GAG AAA AA-3′). The total strand concentration ranged from 2.4 to 3.0 μM in 90 mM NaCl, 10 mM sodium phosphate, and 1 mM EDTA at pH 7.0.

[a]For the ΔT_m values, the T_m of Entry I is the reference (T_m = 40.9 °C).

observed when compared to a PNA-DNA chimera that contained a PNA element at the terminal end [29].

CONCLUSIONS

In conclusion, a facile and practical synthesis of a new uracil-uracil dimer linked together through a triazole functionality has been developed and is readily synthesized from inexpensive starting material precursors. Our alkyne-uracil monomer **3** was synthesized in 44% yield and the azide-uracil monomer **6** was generated in 62% yield. Following heterocyclic cyclization, the phosphoramidite **11** was generated in four steps in 32% yield. Incorporation of the heterocyclic dimer at both the 5′ and 3′ ends of DNA oligonucleotides suggests a certain degree of compatibility when analyzed by UV-monitored thermal denaturation. The effects of this modification within different positions of other various oligonucleotides will be investigated. Notwithstanding, these oligonucleotides may have potential use when targeted at messenger RNA in diseased cells, or for other downstream applications.

EXPERIMENTAL

Unless otherwise noted, all starting materials were obtained from commercial sources and were used without any additional purification. Anhydrous CH_2Cl_2, THF, and DMF were purchased from Sigma-Aldrich and degassed by stirring under a dry N_2 atmosphere. Purification by flash chromatography was carried out with Silicycle Siliaflash 60 (230–400 mesh) according to the procedure of Still et al. [30] ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at 400 or 500 MHz and ^{13}C NMR spectra were recorded at 100 or 125 MHz. High-Resolution MS (HRMS) was recorded on a Micromass AutoSpec Ultima Magnetic sector mass spectrometer.

***2-(tert*-Butyldimethylsilyloxy)ethanamine (1).** To a solution of ethanolamine (9.6 mL, 159 mmol) in 100 mL of CH_2Cl_2 was dissolved imidazole (10.8 g, 159 mmol) and this solution was cooled in an ice-water bath. To this solution was added *tert*-butyldimethylsilyl chloride (24 g, 159 mmol) and the reaction mixture was stirred overnight at room temperature. Sat. $NaHCO_3$ was added and the resulting mixture was partitioned. The organic fractions were concentrated *in vacuo* to afford the title compound as a clear light yellow oil (26.8 g, 96%) [31].

***N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)prop-2-yn-1-amine (2).** To a solution of **1** (10 g, 57 mmol) in 100 mL of CH_2Cl_2 was added diisopropylethylamine (3.7 g, 28.5 mmol), and this solution was cooled in an ice-water bath. To this solution was added 3-bromoprop-1-yne (3.4 g, 28.5 mmol) dropwise over 30 min. This reaction mixture was stirred at room temperature until TLC analysis indicated the complete consumption of starting material (3 h). Sat. $NaHCO_3$ was added and the reaction mixture was partitioned. The organic fraction was concentrated *in vacuo*, to afford an oil which was purified by silica gel chromatography eluting with a gradient of hexanes/EtOAc (7:3 to 3:7) to afford the title compound as a clear yellow oil (2.9 g, 71%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 6H), 0.90 (s, 9H), 1.67 (br s, 1H), 2.21 (s, 1H), 2.80 (t, 2H, J = 5.5 Hz), 3.46 (s, 2H), 3.75 (t, 2H, J = 5.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.36, 25.89, 29.66, 38.18, 50.52, 62.32, 71.21, 82.15; ESI-HRMS (ES^+) m/z calcd for $C_{11}H_{23}NOSi$: 213.1549, found 214.1626 $[M + H]^+$. Anal. Calcd. for $C_{11}H_{23}NOSi$: C, 61.91; H, 10.86. Found: C, 61.56; H, 10.49.

***N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-uracil-1-yl-*N*-(prop-2-yn-1-yl)acetamide (3).** To a solution of uracil-1-yl acetic acid (2.4 g, 14 mmol) in 240 mL of DMF was added diisopropylethylamine (1.8 g, 14 mmol) and this solution was cooled in an ice-water bath. The acid was activated with the addition of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate (5.3 g, 14 mmol) to the stirring solution over the course of 5 min, followed by the dropwise addition of **2** (2 g, 9.4 mmol) over 15 min. The reaction mixture was stirred for 24 h at ambient temperature and then extracted with EtOAc and the organic layer was washed with H_2O and brine (x3). The organic layer was collected and concentrated *in vacuo* to afford the crude product. This crude product was purified by flash chromatography with a gradient of hexanes/EtOAc (7:3) to 100% EtOAc to elute the title compound as a white solid (1.5 g, 44%). Compound **3** is a pair of rotamers; the signals to the major (ma.) and minor (mi.) rotamers are designated; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 2H, mi.), 0.08 (s, 4H, ma.), 0.88 (s, 3H, mi.), 0.89 (s, 6H, ma.), 2.25 (br s, 0.66H, ma.), 2.41 (br s, 0.33H, mi.), 3.58 (t, 0.6H, J = 5.3 Hz, mi.), 3.65 (t, 1.4H, J = 5.2 Hz, ma.), 3.76 (t, 0.6H, J = 5.3 Hz, mi.), 3.85 (t, 1.4H, J = 5.0 Hz, ma.), 4.27 (2s, 2H), 4.68 (m, 2H), 5.71–5.74 (m, 1H), 7.13 (d, 0.66H, J = 7.9 Hz, ma.), 7.17 (d, 0.33H, J = 7.9 Hz, mi.), 9.43 (br s, 0.66H, ma.), 9.48 (br s, 0.33H, mi.); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.61, −5.55, −5.41, −5.36, −3.72, −3.53, 17.94, 18.11, 18.28, 25.54, 25.69, 25.75, 25.81, 25.89, 25.96, 35.16, 38.67, 48.03, 48.06, 48.78, 49.55, 60.58, 61.67, 72.72, 73.59, 77.93, 78.13, 102.13, 102.24, 145.02, 145.09, 150.90, 150.93, 163.58, 166.30, 166.66; ESI-HRMS (ES^+) m/z calcd for $C_{17}H_{27}N_3O_4Si$: 365.1771, found 366.1845 $[M + H]^+$. Anal. Calcd. for $C_{17}H_{27}N_3O_4Si$: C, 55.86; H, 7.45; N, 11.50. Found: C, 55.67; H, 7.80; N, 11.89.

2-Azidoethanamine (4). To a solution of NaN_3 (23.8 g, 366 mmol) in 200 mL of water was added 2-bromoethylamine hydrobromide (25 g, 122 mmol) and this solution was heated to 75°C. The reaction mixture was stirred for 24 h and was then cooled in an ice-water bath. To the cooled solution was added NaOH (28.5 g, 712.5 mmol) and the reaction mixture was stirred until the NaOH was fully dissolved. To this aqueous solution was added Et_2O (x3). The ether fractions were collected, dried over Na_2SO_4, and evaporated *in vacuo* to afford the title compound as a clear oil (6.7 g, 64%). The ^{1}H proton and ^{13}C NMR shifts were confirmed with the report by Mayer and Maier [32].

Ethyl 2-(2-azidoethylamino)acetate (5). To a solution of **4** (5 g, 58.1 mmol) in 100 mL of DMF was added triethylamine (5.9 g, 58.1 mmol). Ethyl 2-bromoacetate (5.8 g, 34.9 mmol) was then added dropwise over 15 min to the stirring solution and the reaction was stirred for 4 h at room temperature. The solution was then extracted with EtOAc, washed with H_2O (three times) and the EtOAc fractions were collected and dried over Na_2SO_4. This solution was evaporated under reduced pressure to yield a crude orange oil. The oil was loaded directly onto a silica column for purification by gradient eluting with hexanes/EtOAc (5:5) to 100% EtOAc to afford the

title compound as a clear light yellow oil (5.0 g, 83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, 3H, J = 7.1 Hz), 1.98 (br s, 1H), 2.80 (t, 2H, J = 5.7 Hz), 3.39 (t, 2H, J = 5.9 Hz), 3.40 (s, 2H), 4.17 (q, 2H, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.09, 48.01, 50.50, 51.33, 60.74, 172.07; ESI-HRMS (ES^+) m/z calcd for $C_6H_{12}N_4O_2$: 172.0960, found 173.1034 $[M + H]^+$. Anal. Calcd. for $C_6H_{12}N_4O_2$: C, 41.85; H, 7.02; N, 32.54. Found: C, 41.65; H, 6.82; N, 32.12.

Ethyl 2-(*N*-(2-azidoethyl)-uracil-1-yl-acetamido)acetate (6). To a solution of uracil-1-yl acetic acid (1.1 g, 6.4 mmol) in 100 mL of anhydrous DMF under N_2 was added dicyclohexylcarbodiimide (1.3 g, 6.4 mmol) and 1*N*-hydroxybenzotriazole (1.0, 6.4 mmol). This solution was stirred for 15 min on an ice-water bath, followed by 1 h of stirring at ambient temperature. To this solution was added compound **5** (1 g, 5.8 mmol) dropwise over 10 min, and the reaction mixture was stirred for 24 h after which the DCU precipitate was collected by filtration. The filtrate was dried down *in vacuo*, dissolved in EtOAc, and washed with water and brine (x3). The EtOAc solutions were concentrated *in vacuo*. The resulting crude product was dissolved in a minimal amount of CH_2Cl_2 and purified by flash chromatography with a gradient of hexanes/EtOAc (5:5) to 100% EtOAc to elute the title compound as a white solid (1.2 g, 62%). Compound **6** is comprised of a pair of rotamers, each displaying signals of equal intensity; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (t, 1.5H, J = 7.1 Hz), 1.33 (t, 1.5H, J = 7.1 Hz), 3.55 (s, 2H), 3.59 (t, 1H, J = 5.6 Hz), 3.66 (t, 1H, J = 5.4 Hz), 4.12 (s, 1H), 4.21 (q, 1H, J = 7.1 Hz), 4.26 (s, 1H), 4.28 (q, 1H, J = 7.1 Hz), 4.49 (s, 1H), 4.72 (s, 1H), 5.74 (d, 0.5H, J = 6.0 Hz), 5.75 (d, 0.5H, J = 5.6 Hz), 7.20 (d, 0.5H, J = 2.0 Hz), 7.21 (d, 0.5H, J = 2.0 Hz), 9.08 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 47.75, 47.82, 48.05, 48.31, 48.86, 49.84, 49.92, 50.95, 61.67, 62.31, 102.30, 102.38, 145.02, 145.14, 150.90, 150.92, 163.44, 167.16, 167.46, 168.64, 169.03; ESI-HRMS (ES^+) m/z calcd for $C_{12}H_{16}N_6O_5$: 324.1182, found 325.1262 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{16}N_6O_5$: C, 44.44; H, 4.97; N, 25.91. Found: C, 44.40; H, 5.10; N, 25.41.

Ethyl 2-(*N*-(2-(4-((*N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-uracil-1-yl-acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)-uracil-1-yl-acetamido)acetate (7). To a solution of compounds **3** (677 mg, 1.9 mmol) and **6** (600 mg, 1.9 mmol) dissolved in 18 mL of THF/*t*-BuOH/H_2O (1:1:1) was added sodium ascorbate (733 mg, 3.7 mmol) and Cu(II)SO_4 (231 mg, 925 μmol). This reaction mixture was stirred for 24 h at room temperature and then extracted with CH_2Cl_2. The organic layer was washed with H_2O (three times) to wash off the excess copper. The precipitate in the organic layer was collected by filtration and washed with cold H_2O to afford the crude product. This crude product was redissolved in minimal CH_2Cl_2/MeOH for 1 h and purified by flash column chromatography eluting with 5% (10% NH_4OH in MeOH) in CH_2Cl_2 to afford the title compound as a white solid (1.3 g, 98%). Compound **7** is a mixture of rotamers with varying signal intensities. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.03 (s, 2H), 0.05 (m, 4H), 0.85, 0.86 (2s, 9H), 1.18 (t, 1.5H, J = 7.2 Hz), 1.23 (t, 1.5H, J = 7.0 Hz), 3.48–3.50 (m, 1H), 3.63–3.91 (m, 4H), 4.01–4.03 (m, 1H), 4.08 (q, 1.2H, J = 7.3 Hz), 4.16 (q, 0.8H, J = 7.3 Hz), 4.24, 4.29 (2s, 1H), 4.44–4.77 (m, 9H), 5.56 (d, 2H, J = 7.8 Hz), 7.31–7.47 (m, 2H), 7.89, 8.05, 8.13, 8.24 (4s, 1H), 11.29 (br m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ −5.45, −5.39, 13.99, 14.04, 17.89, 18.04, 25.82, 25.90, 41.05, 47.31, 47.58, 47.91, 48.00, 60.18, 60.65, 60.83, 61.25, 100.57, 100.79, 123.70, 124.04, 124.12, 124.61, 143.18, 143.34, 143.41, 143.56, 146.12, 146.19, 146.29, 146.50, 146.54, 150.92, 150.98, 151.04, 151.07, 151.11, 163.76, 163.80, 163.81, 163.85, 163.88, 166.74, 166.82, 166.88, 166.90, 167.43, 167.97, 168.03, 168.85, 169.15; ESI-HRMS (ES^+) m/z calcd for $C_{29}H_{43}N_9O_9Si$: 689.2953, found 690.3030 $[M + H]^+$. Anal. Calcd. for $C_{29}H_{43}N_9O_9Si$: C, 50.50; H, 6.28. Found: C, 50.47; H, 6.24.

***N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-uracil-1-yl-*N*-((1-(2-(uracil-1-yl-*N*-(2-hydroxyethyl)acetamido)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8).** To a solution of compound **7** (300 mg, 435 μmol) suspended in 12 mL of dry THF, drops of MeOH were added until the compound was dissolved. To this solution was then added $LiBH_4$ (544 μL, 1.09 mmol) and the reaction mixture was refluxed until TLC analysis indicated the complete consumption of starting material (1.5 h). This reaction mixture was quenched with MeOH and dried down *in vacuo* to afford the crude product. The crude product was then redissolved in minimal CH_2Cl_2/MeOH and purified by flash column chromatography eluting with a gradient of 10% NH_4OH in MeOH (5 to 15%) in CH_2Cl_2 to afford the title compound as a white crystalline solid (213 mg, 76%). Compound **8** is a mixture of rotamers with varying signal intensities; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.03 (s, 2H), 0.05 (s, 4H), 0.85, 0.87 (2s, 9H), 3.16–3.27 (m, 2H), 3.43–3.54 (m, 4H), 3.63–3.74 (m, 3H), 3.80–3.87 (m, 2H), 4.36–4.38 (m, 1H), 4.46 (t, 1.2H, J = 6.3 Hz), 4.51 (t, 0.8H, J = 6.3 Hz), 4.56–4.81 (m, 6H), 4.94–5.00 (m, 1H), 5.54–5.58 (m, 2H), 7.35–7.47 (m, 2H), 7.87, 8.04, 8.13, 8.23 (4s, 1H), 11.30 (br s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ −5.44, −5.37, 17.91, 18.05, 25.83, 25.92, 30.74, 41.04, 46.24, 46.55, 46.71, 46.92, 48.29, 48.32, 48.51, 49.07, 49.26, 58.49, 58.81, 58.85, 60.21, 60.85, 100.57, 100.63, 123.76, 124.11, 124.55, 143.23, 143.39, 143.43, 143.58, 146.48, 146.52, 146.58, 151.04, 151.08, 151.11, 163.84, 163.87, 166.69, 166.74, 166.81, 167.43, 167.54; ESI-HRMS (ES^+) m/z calcd for $C_{27}H_{41}N_9O_8Si$: 647.2847, found 648.2918 $[M + H]^+$. Anal. Calcd. for $C_{27}H_{41}N_9O_8Si$: C, 50.06; H, 6.38; N, 19.46. Found: C, 50.23; H, 6.84; N, 19.36.

***N*-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-2-(uracil-1-yl)-*N*-(2-(4-((2-(uracil-1-yl)-*N*-(2-((2,3,3-trimethylbutan-2-yl)oxy)ethyl)acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)acetamide (9).** To a solution of compound **8** (1.06 g, 1.64 mmol) dissolved in 10 mL of dry pyridine under N_2, was added an excess of 4,4′-dimethoxytrityl chloride (1.66 g, 4.91 mmol) until TLC analysis revealed the consumption of starting material. This reaction mixture was stirred overnight at room temperature. while under N_2, after which the entire mixture was extracted with CH_2Cl_2 and washed with H_2O (two times). The organic fractions were collected, dried over Na_2SO_4 and subsequently condensed *in vacuo* to afford the crude product. The crude was dissolved in minimal CH_2Cl_2/MeOH and purified by flash column chromatography eluting with a gradient of MeOH (5 to 15%) in CH_2Cl_2 to afford the title compound as a white solid (1.22 g, 79%). Compound **9** is a mixture of rotamers with varying signal intensities; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 2H), 0.06 (s, 4H), 0.86, 0.88 (2s, 9H), 2.87–2.94 (m, 1.5H), 3.03–3.08 (m, 0.5H), 3.20 (t, 1.5H, J = 4.7 Hz), 3.26 (t, 0.5H, J = 4.5 Hz), 3.29–3.31 (m, 0.2H), 3.36–3.43 (m, 0.3H), 3.46–3.55 (m, 2.5H), 3.60–3.67 (m, 2H), 3.72–3.75 (m, 0.75H), 3.76, 3.77 (2s, 7H), 3.80 (t, 1.25H, J = 4.9 Hz), 4.51 (t, 1.5H, J = 5.3 Hz), 4.55 (t, 0.5H, J = 5.3 Hz),

4.59 (s, 1H), 4.63–4.64 (m, 1H), 4.67 (s, 3H), 4.73 (d, 1H, J = 7.4 Hz), 5.51–5.55 (m, 1H), 5.62 (d, 0.1H, J = 1.6 Hz), 5.64 (d, 0.1H, J = 1.6 Hz), 5.64–5.68 (m, 0.8H), 6.57 (d, 0.1H, J = 7.8 Hz), 6.66 (d, 0.7H, J = 7.8 Hz), 6.80–6.84 (m, 4.3H), 7.06–7.09 (m, 0.8H), 7.17–7.36 (m, 7.2H), 7.71, 7.81, 7.88, 7.94 (4s, 1H), 10.11 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.44, −5.16, 18.13, 18.29, 25.61, 25.83, 25.90, 41.33, 44.08, 47.64, 47.74, 48.27, 48.50, 48.62, 48.87, 48.93, 49.09, 55.23, 60.41, 60.66, 60.79, 60.98, 87.24, 87.39, 102.02, 102.21, 102.25, 113.15, 113.25, 124.37, 125.24, 127.18, 127.24, 127.86, 127.98, 128.15, 129.91, 130.08, 135.03, 135.09, 135.70, 143.49, 143.76, 144.01, 144.05, 144.65, 145.00, 145.10, 145.15, 145.21, 145.54, 151.26, 151.32, 151.38, 151.46, 158.42, 158.67, 158.71, 163.91, 164.00, 164.08, 166.48, 167.25, 167.67. ESI-HRMS (ES^+) m/z calcd for $C_{48}H_{59}N_9O_{10}Si$ 949.4154, found 972.4035 $[M + Na]^+$. Anal. Calcd. for $C_{49}H_{59}N_9O_{10}Si$: C, 60.68; H, 6.26; N, 13.27. Found: C, 60.50; H, 6.28; N, 12.90.

***N*-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-2-(uracil-1-yl)-*N*-((1-(2-(2-(uracil-1-yl)-*N*-(2-hydroxyethyl)acetamido)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (10).** To a solution of compound **9** (1.12 g, 1.18 mmol) dissolved in 26 mL of dry THF was added TBAF (926 mg, 3.54 mmol). This reaction mixture was stirred for 12 h at room temperature and then extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine (×2), at which point the combined organic fractions were dried over Na_2SO_4, and evaporated under reduced pressure. The crude was dissolved in minimal CH_2Cl_2/MeOH and purified by flash column chromatography, first eluting with 100% CH_2Cl_2 followed by a gradient of MeOH (5 to 15%) in CH_2Cl_2 to afford the title compound as a yellow solid (840 mg, 85%). Compound **10** is a mixture of rotamers with varying signal intensities; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (br s, 1H), 2.98 (br s, 1H), 3.16–3.20 (br m, 2H), 3.27–3.31 (m, 3H), 3.45–3.57 (m, 4H), 3.68–3.81 (m, 8H), 4.45–4.80 (m, 8H), 5.50–5.53 (m, 0.8H), 5.56–5.60 (m, 1.2H), 6.63 (d, 0.15H, J = 8.2 Hz), 6.66 (d, 0.35H, J = 7.8 Hz), 6.78–6.83 (m, 4H), 7.17–7.31 (m, 8.5H), 7.69, 7.88, 7.90, 8.09 (4s, 1H), 10.20–10.42 (br m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.13, 27.75, 29.14, 29.64, 42.18, 47.37, 48.29, 49.13, 50.35, 55.24, 59.15, 60.78, 68.46, 87.23, 87.32, 101.65, 101.91, 107.85, 113.16, 113.26, 124.97, 127.16, 127.99, 128.17, 129.92, 130.09, 135.15, 135.73, 143.56, 144.11, 144.28, 145.47, 146.36, 151.41, 151.47, 158.40, 158.64, 164.30, 164.39, 164.56, 167.63, 167.68; ESI-HRMS (ES^+) m/z calcd for $C_{42}H_{45}N_9O_{10}$: 835.3289, found 858.3217 $[M + Na]^+$. Anal. Calcd. for $C_{42}H_{45}N_9O_{10}$: C, 60.35; H, 5.43. Found: C, 60.72; H, 5.81.

2-(*N*-(2-(4-((*N*-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)-2-(uracil-1-yl)acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)-2-(uracil-1-yl)acetamido)ethyl (2-cyanoethyl) diisopropylphosphoramidite (11). To a solution of compound **10** (230 mg, 275 μmol) dissolved in 6 mL of dry CH_2Cl_2 under N_2, was added diisopropylethylamine (196 mg, 1.51 mmol) along with 4-dimethylaminopyridine (16.8 mg, 138 μmol). To this solution was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (195 mg, 825 μmol), until TLC analysis revealed the maximum consumption of starting material. This mixture was then stirred for 6 h at room temperature under N_2 and dried *in vacuo* to afford the crude product. The crude was dissolved in minimal 2% triethylamine in hexanes/acetone and purified by flash column chromatography eluting with a gradient of 2% triethylamine in acetone/hexanes (1:1 to 4:1) to afford the title compound as a white solid (180 mg, 63%). Compound **11** is a mixture of rotamers with varying signal intensities; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14–1.19 (m, 12H), 2.62–2.66 (m, 3.25H), 2.92 (t, 1H, J = 3.9 Hz), 3.07 (t, 0.75H, J = 4.3 Hz), 3.21 (t, 1.25H, J = 4.7 Hz), 3.26 (t, 0.5H, J = 4.3 Hz), 3.29–3.37 (m, 0.25H), 3.54–3.61 (m, 5.5H), 3.70–3.77 (m, 2H), 3.78 (s, 6.25H), 3.81–3.90 (m, 2.25H), 4.52 (t, 1H, J = 5.3 Hz), 4.55 (t, 0.5H, J = 5.5 Hz), 4.61–4.65 (m, 2H), 4.70 (s, 2.5H), 4.74–4.77 (m, 1H), 5.52–5.55 (m, 1H), 5.66–5.70 (m, 1H), 6.56 (d, 0.33H, J = 7.8 Hz), 6.63 (d, 0.66H, J = 7.8 Hz), 6.81–6.84 (m, 4H), 7.17–7.31 (m, 12H), 7.70, 7.76, 7.89, 7.95 (4s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.10, 14.84, 20.45, 20.52, 22.66, 23.34, 24.58, 24.62, 24.65, 24.69, 24.77, 29.24, 29.34, 29.66, 31.72, 31.89, 33.80, 34.43, 41.51, 43.02, 43.14, 45.82, 47.67, 47.80, 48.29, 48.60, 48.71, 48.93, 53.76, 55.26, 58.08, 58.30, 60.53, 60.70, 60.80, 69.48, 87.29, 87.42, 102.08, 102.20, 102.25, 113.27, 117.90, 118.12, 125.13, 127.24, 128.01, 128.20, 130.12, 135.05, 135.09, 143.39, 143.82, 144.05, 145.07, 145.46, 151.24, 151.34, 151.45, 158.71, 158.75, 163.63, 163.87, 163.91, 166.67, 167.41, 167.74; ESI-HRMS (ES^+) m/z calcd for $C_{51}H_{62}N_{11}O_{11}P$: 1035.4368, found 1058.4298 $[M + Na]^+$.

Table 2

The oligonucleotides characterized by MALDI-TOF mass spectrometry.

Sequence	Calcd mass (g/mol)	Observed mass (g/mol)	Observed molecular ion
5′ TTTTTCTCTCTC**UU** 3′	4123	4122	$[M - H]^-$
5′ TTTTTCTCTCTC 3′	3529	3530	$[M + H]^-$
5′ **UU**TTTCTCTCTCTT 3′	4123	4164	$[M - H + K]^-$
5′ TTTCTCTCTCTT 3′	3529	3530	$[M + H]^-$

Observed masses obtained from MALDI-TOF Mass Spectrometry. Polyacrylamide gel-purified samples were desalted using Millipore Zip-Tip C18-column micropipette tips to a final concentration of 5 μM. 5 μL of matrix solution (3-hydroxypicolinic acid at 25 mg/mL, dissolved in 50/50 acetonitrile/water with 5 mg/mL ammonium citrate) was added to each sample vial.

Oligonucleotide synthesis. All standard β-cyanoethyl DNA phosphoramidites, solid supports and reagents were purchased from Glen Research. All oligonucleotides were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer using a 0.2 μM cycle with a 25 s coupling time for unmodified phosphoramidites. All phosphoramidites were dissolved in anhydrous acetonitrile to a 0.1M concentration immediately prior to synthesis. Synthesis on solid phase was accomplished using 0.2 μM solid supports. Cleavage of the unmodified oligonucleotides from their solid supports was performed through exposure to 1.5 mL of 30% NH_4OH for 2 h at room temperature, followed by incubation in 30% NH_4OH at 55°C overnight. All oligonucleotides were purified on a 20% denaturing polyacrylamide gel by utilizing the ''crush and soak'' method, followed by ethanol precipitation and desalting through Millipore 3000 MW cellulose centrifugal filters. The oligonucleotides were characterized by MALDI-TOF mass spectrometry (Waters Tofspec-2E) (Table 2).

Synthesis of 5′ TTTTTCTCTCTCUU 3′ and 5′ UUTTTCTCTCTCTT 3′. Phosphoramidite **11** was attached to the 5′ end of the growing oligonucleotide being synthesized on the ABI 394 DNA/RNA synthesizer, with an increased coupling time of 600 s. To perform the 3′ modification, compound **11** was attached to a Universal III solid support (Glen Research) via the ABI 394 synthesizer with a 600 s coupling time. Cleavage from the Universal III solid support was performed through exposure to 1.5 mL of 2M NH_3 in MeOH for 30 min at room temperature, followed by incubation in 30% NH_4OH at 55°C for 8 h.

Synthesized sequences. Oligonucleotides synthesized using standard dT/dA supports: (i) 5′-AAG AGA GAG AAA AA–3′; (ii) 5′–TTT TTC TCT CTC TT–3′; and (iii) 5′–**UU**T TTC TCT CTC TT–3′. The **UU**–3′ modified sequence synthesized on the Universal III solid support: 5′–TTT TTC TCT CTC **UU**–3′. The sequence 5′-AAG AGA GAG AAA AA-3′ was purchased and purified from Integrated DNA Technologies.

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