JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Chimeric RNA Oligonucleotides Incorporating Triazole-Linked Trinucleotides: Synthesis and Function as mRNA in Cell-Free Translation Reactions

Tomoko Fujino,[†] Takeru Suzuki,[†] Koudai Okada,[†] Kanako Kogashi,[†] Ken-ichi Yasumoto,[‡] Kazuhiro Sogawa,[‡] and Hiroyuki Isobe*[,§,∥]

[†]Department of Chemistry, Tohoku University, Aoba-ku, Sendai 980-8578, Japan

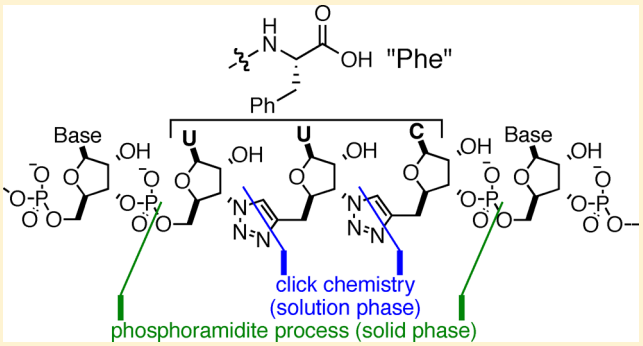
[‡]Department of Biomolecular Sciences, Tohoku University, Aoba-ku, Sendai 980-8578, Japan

[§]JST ERATO, Isobe Degenerate π-Integration Project and Advanced Institute for Materials Research, Tohoku University, Aoba-ku, Sendai 980-8577, Japan

[∥]Department of Chemistry, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Supporting Information

ABSTRACT: A method for the synthesis of chimeric oligonucleotides was developed to incorporate purine nucleobases and multiple triazole linkers in natural, phosphate-linked structures of RNA. A solution-phase synthesis method for triazole-linked RNA oligomers via copper-catalyzed azide−alkyne cycloaddition reaction was optimized and tolerated purine nucleobases and protecting groups for further transformations. Three ^{TL}RNA trinucleotides with 5′-protected hydroxy and 3′-phosphoramidite groups were prepared, and one congener with a representative sequence was subjected to automated, solid-phase phosphoramidite synthesis. The synthesis allowed the efficient preparation of 13-mer chimeric RNA oligonucleotides with two triazole linkers, ten phosphate linkers and purine/pyrimidine nucleobases. The chimeric oligonucleotide was found applicable to a cell-free translation system as mRNA and provided the genetic code for dipeptide production.



INTRODUCTION

Oligonucleotide analogues with artificial molecular structures are attracting much interest in life science and biotechnology.[1] Particularly, phosphate-replaced analogues of oligonucleotides are interesting as potential substrates for enzymatic reactions, which are enabled by their furanose-mimicking units.[2] Considering the obvious requirement of synthetic robustness for the preparation of functional analogues, we previously introduced click chemistry[3] in elongation reactions and designed triazole-linked DNA (^{TL}DNA) through the molecular design of deoxyribose-mimicking monomer units.[4,5] The multiple copper-catalyzed azide−alkyne cycloaddition (CuAAC) reactions, sequentially performed with 3′-azide and 5′-alkyne on solid supports, allowed us to synthesize oligothymine ^{TL}DNA **1** (Figure 1).[4,6]

A second analogue, an RNA congener, was then introduced to assemble ribofuranose-mimicking units in the form of oligomers via CuAAC. Although the synthesis of the triazole-linked RNA (^{TL}RNA) oligonucleotides (**2**) was synthetically improved from the ^{TL}DNA synthesis,[7] an inherent problem of low solubility with electroneutral linkers hindered the examination of the full potential of triazole-linked oligonucleotides.[8] We thus investigated chimeric RNA oligonucleotides to combine triazole linkages with phosphate linkages and

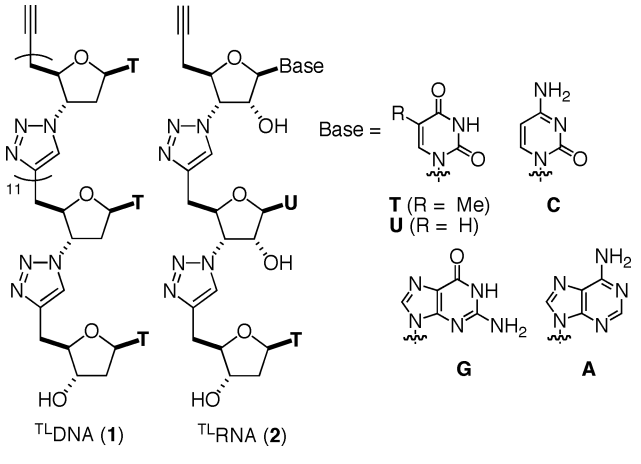


Figure 1. Triazole-linked DNA and RNA.

Received: July 6, 2016
Published: August 31, 2016

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

developed a method to introduce a single triazole linker in chimeric RNA oligonucleotides (**3**, Figure 2).[9] This method

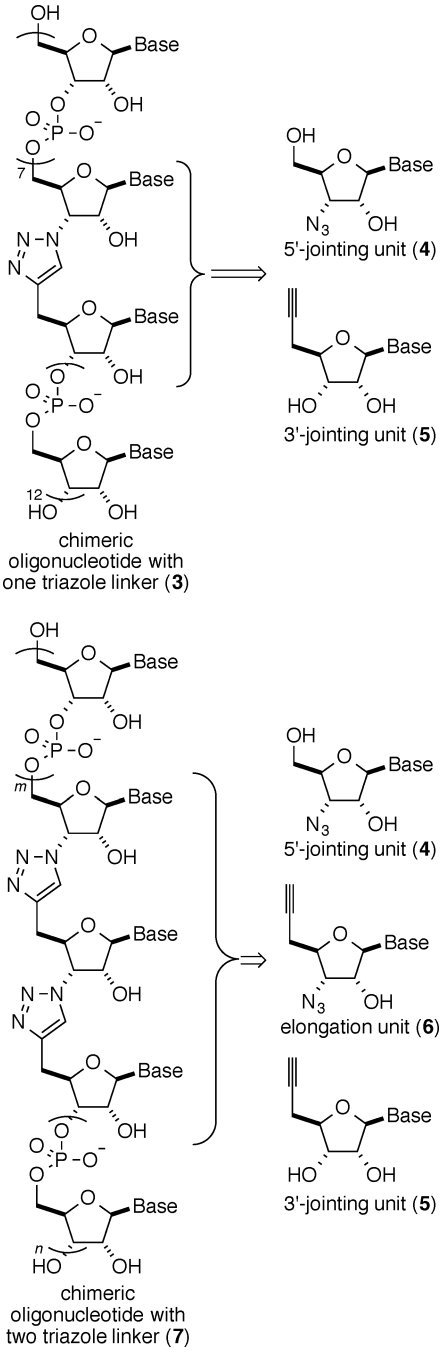


Figure 2. Structure and retrosynthesis of chimeric oligonucleotides with triazole linkers.

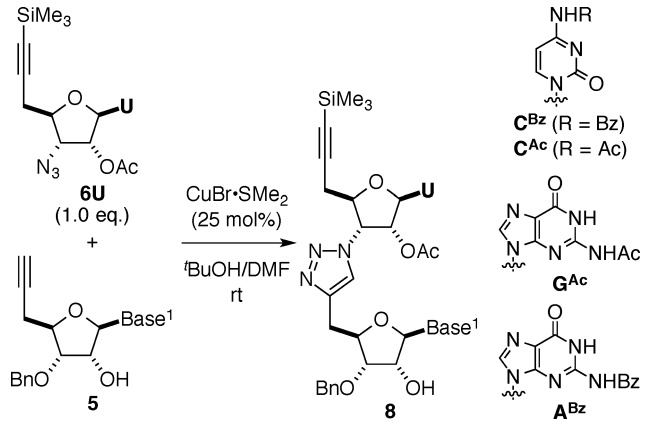
assembled a 5′-jointing unit (**4**, with 5′-OH and 3′-azide) and a 3′-jointing unit (**5**, with 5′-alkyne and 3′-OH) by solution-phase click synthesis and utilized a phosphoramidite protocol to synthesize chimeric RNA oligomers with a single triazole linker. To further expand the scope of the synthesis and the potential applications of the chimeric RNA, we herein explore a synthesis method to introduce multiple triazole linkers in chimeric RNA analogues. To introduce multiple triazole linkers, we adopted solution-phase synthesis to assemble ^{TL}RNA oligomers via a combination of **4**, **5** and an elongation unit **6** and applied oligomers with 5′-OH/3′-OH termini to the phosphoramidite protocol. Albeit a subtle change, the synthesis required careful reinvestigation of the solution-phase reactions, which may be informative for examinations of CuAAC reactions in the presence of biologically relevant substances. After succeeding in diversifying the nucleobases in the ^{TL}RNA trinucleotides, we synthesized chimeric 13-mer RNA oligonucleotides with two triazole linkers (**7**) and investigated their function as the mRNA substrate in translation reactions using a cell-free translation system.[10]

RESULTS AND DISCUSSION

The synthesis routes for monomer units (**4**, **5**, **6**) from D-xylose were previously established,[9] and we prepared pyrimidine ^{TL}RNA dinucleotides using a combination of the pyrimidine-bearing 5′- and 3′-jointing units (**4** and **5**) from the previous study for siRNA.[9] The next steps for synthetic developments involved: (1) inclusion of purine nucleobases and (2) inclusion of elongation units (**6**). We thus investigated the appropriate reaction conditions to combine all three monomer units and explored a synthetic route to utilize the phosphoramidite protocol for chimeric assembly.

The synthesis of ^{TL}RNA dinucleotides with purine nucleobases required substantial reoptimization of the reaction conditions. The reaction of 3′-jointing cytosine monomer **5C** and elongation uracil monomer **6U** proceeded under the previously optimized conditions to afford dinucleotide **8UC** in 97% yield (substrate concentration = 1.0 M; Table 1).

Table 1. Solution-Phase Synthesis of Triazole-Linked Dinucleotides



5	concentration of 5/6U	8	yield (%)
5C (Base^1 = C^{Bz})	1.0 M	**8UC** (Base^1 = C^{Bz})	97
5G (Base^1 = G^{Ac})	200 mM	**8UG** (Base^1 = G^{Ac})	78
5G (Base^1 = G^{Ac})	50 mM	**8UG** (Base^1 = G^{Ac})	90

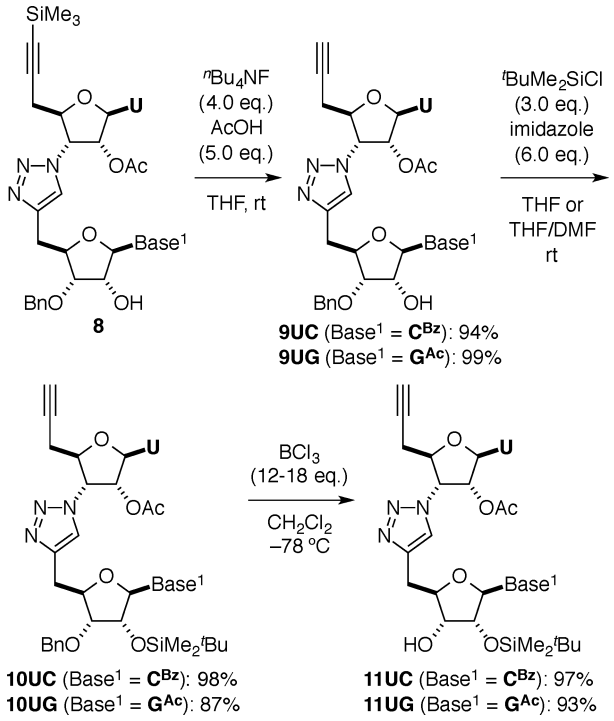
However, the reaction with 3′-jointing guanine monomer **5G** failed to proceed to completion under identical conditions. Assuming poor solubility of **5G** in the reaction medium as the origin of the low reactivity, we performed the reaction at lower substrate concentrations of 200 and 50 mM and obtained **8UG** in improved yields of 78% and 90%, respectively (Table 1).

The hydroxy groups of the ^{TL}RNA dinucleotides (**8UC** and **8UG**) were then modified to be compatible with the phosphoramidite protocol. Thus, after removal of the trimethylsilyl group of **8UC** at the acetylene moiety, we

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

protected the 2′-hydroxy group of the dinucleotide alkyne **9UC** with a TBDMS protective group to give dinucleotide **10UC** (Scheme 1). Removal of the 3′-benzyl group from **10UC** afforded ^{TL}RNA dinucleotide **11UC** in 89% yield (3 steps). The purine-containing ^{TL}RNA dinucleotide **11UG** was likewise transformed via a three-step route to afford **11UG** in 65% yield.

Scheme 1. Modification of ^{TL}RNA Dinucleotides

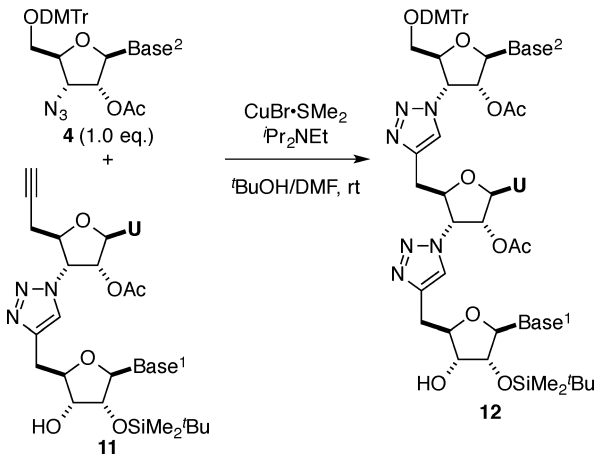


The ^{TL}RNA dinucleotides were then furnished with 5′-jointing units. Although a protocol for similar coupling reactions has been investigated in our previous studies,[9] we faced the issue of reproducibility of the synthesis. For instance, the yields of **12UUC** from a coupling reaction between **11UC** and **4U** varied from 51% to 99% (Table 2). Careful analysis of the crude materials showed that an undesired, detritylated trinucleotide was included in the byproducts, and we envisioned that removal of acid contaminants would improve the reproducibility. We thus added Hünig's base (*N*,*N*-diisopropylethylamine) to the reaction mixture and found that the desired ^{TL}RNA trinucleotide **12UUC** was obtained reproducibly in 97% yield. The revised coupling conditions were used to prepare ^{TL}RNA trinucleotide **12CUC** in 94% yield. The conditions for purine-containing congeners required further revision of the conditions: the reaction was sluggish and failed to complete even after 24 h. Assuming that the copper catalyst was captured by the purine nucleobase,[11] we doubled the amounts of the copper catalyst and Hünig's base to obtain **12AUG** in 77% yield.[12] The established protocols should allow us to access longer ^{TL}RNA oligonucleotides simply by increasing the number of elongation units.

The final solution-phase transformation before the solid-phase synthesis was the installation of the phosphoramidite moiety at the 3′-hydroxy group. Using 2-cyanoethyl *N*,*N*-diisopropyl chlorophosphoramidite, we converted **12UUC** to a ^{TL}RNA trinucleotide **13UUC** with a 3′-phosphoramidite moiety in 92% yield (Scheme 2). The other two ^{TL}RNA trinucleotides, **12CUC** and **12AUG**, were likewise converted to phosphoramidites **13CUC** and **13AUG** in 86% and 94% yields, respectively. With the synthetically robust procedures from abundantly available D-xylose, we synthesized a few hundred milligrams of each trinucleotide from a single set of synthetic operations.

With ^{TL}RNA trinucleotides possessing three nucleobases, we decided to examine their function as a codon for translation reactions. We thus designed a short chimeric RNA oligonucleotide comprising 13 nucleobases (Scheme 3). The 13-mer oligonucleotide possessed a nucleobase sequence (5′-G AUG UUC UAA GCU-3′; **19**) to describe the genetic code for a dipeptide, *N*-formyl-L-methionine-L-phenylalanine (fMet-Phe), and the ^{TL}RNA was installed as the second codon for Phe in the dipeptide. The 13-mer oligonucleotide was prepared by

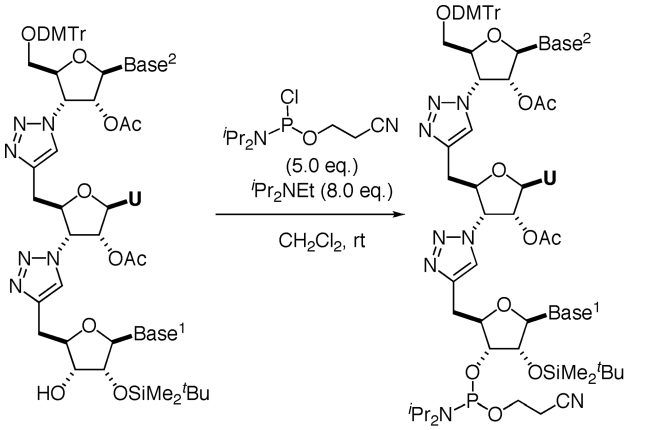
Table 2. Solution-Phase Synthesis of ^{TL}RNA Trinucleotides



4	11	CuBr·SMe$_2$ (mol %)	^{i}Pr$_2$NEt (mol %)	12	yield (%)
4U (Base^2 = U)	**11UC** (Base^1 = C^{Bz})	25	0	**12UUC** (Base^1 = C^{Bz}, Base^2 = U)	51–99
4U (Base^2 = U)	**11UC** (Base^1 = C^{Bz})	25	25	**12UUC** (Base^1 = C^{Bz}, Base^2 = U)	97
4C (Base^2 = C^{Bz})	**11UC** (Base^1 = C^{Bz})	25	25	**12CUC** (Base^1 = C^{Bz}, Base^2 = C^{Bz})	94
4A (Base^2 = A^{Bz})	**11UG** (Base^1 = G^{Ac})	50	50	**12AUG** (Base^1 = G^{Ac}, Base^2 = A^{Bz})	77

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967–8976

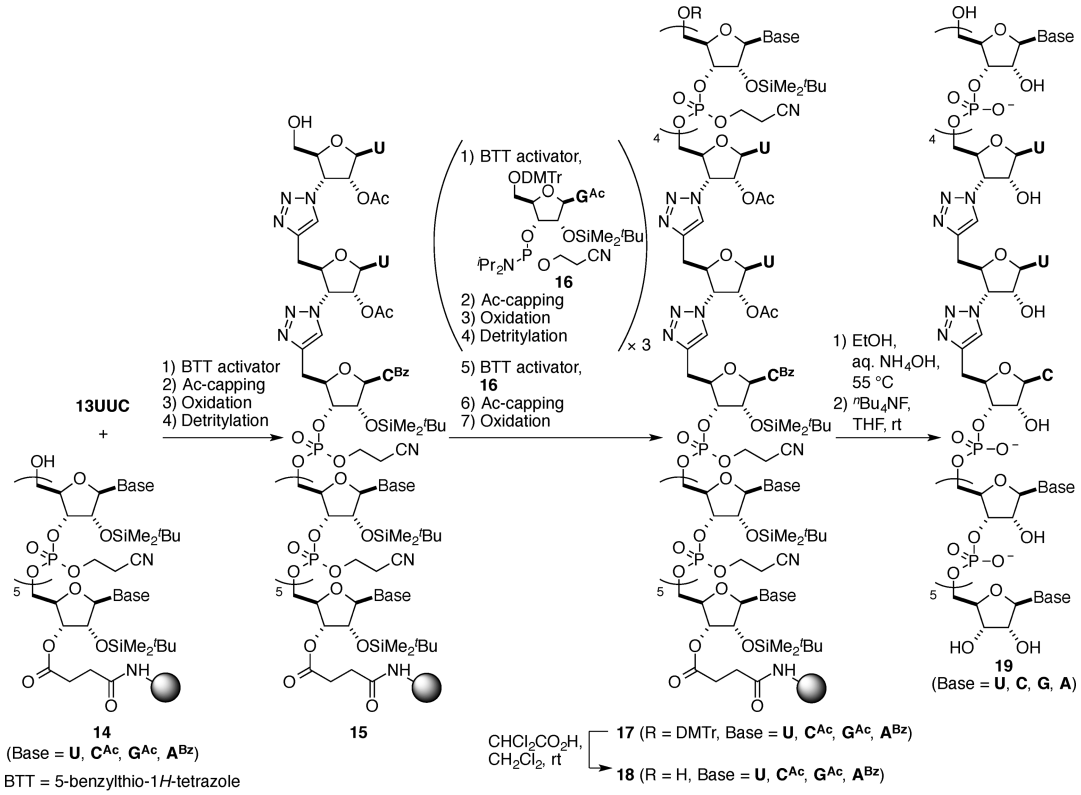
Scheme 2. Conversion of ^{TL}RNA Trinucleotides to Phosphoramidites



solid-phase synthesis on an automated synthesizer (M-2-MX, Nihon Techno Service).[9] After elongation of a 6-mer RNA oligonucleotide on CPG beads from the 3′-end, the ^{TL}RNA trinucleotide **13UUC** was incorporated, and subsequent elongation of four nucleotides afforded the target chimeric RNA oligonucleotide **19**. The yield was determined by a standard protocol to quantify the removed trityl cation from **17** using a UV−vis spectrometer, which showed a total yield of 60% for the 10 elongation reactions. The yield of a natural RNA oligonucleotide with an identical sequence (**19-*nat***) was 62%, which showed that the triazole linkers did not affect the coupling efficiency of the phosphoramidite elongation.

Finally, the 13-mer chimeric RNA oligonucleotide **19** was subjected to translation reactions with the cell-free translation system using an *in vitro* protein synthesis kit (PURExpress, New England Biolabs).[10] The translation system was reconstructed with purified components for the synthesis of peptides encoded on oligonucleotides (mRNA and/or DNA). The 13-mer RNA oligonucleotide **19** was introduced in the translation reaction, and radioactive L-methionine, [^{35}S]Met, was added to label the translation products with radioactive fMet encoded in the initiation codon of AUG. The translation products were separated by HPLC equipped with an ODS column, and the fractions were analyzed by a scintillation detector to produce a radiochromatogram, as shown in Figure 3a. We also synthesized a nonradioactive dipeptide, fMet-Phe, and used it as a reference for the HPLC analysis (Figure 3b). As shown in Figure 3a and 3b, the target dipeptide was detected at ca. 22 min under the present HPLC conditions. The translation products were also analyzed by LC−MS, and the extracted-ion chromatogram for fMet-Phe, with an m/z of 323 ([M − H]$^-$), identified the target dipeptide, fMet-Phe, at an identical elution time (22 min; Figure 3c). The results showed that the chimeric RNA oligonucleotide was recognized as mRNA by the

Scheme 3. Synthesis of Chimeric Oligonucleotide[a]



chimeric oligonucleotides	**19**	5′-Gp ApUpGp **UtUtC**p UpApAp GpCpU-3′	60% (13-mer; 10 elongations)
cf. natural oligonucleotides	**19-*nat***	5′-Gp ApUpGp UpUpCp UpApAp GpCpU-3′	62% (13-mer; 12 elongations)

[a]The triazole linkage is represented as *t*.

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

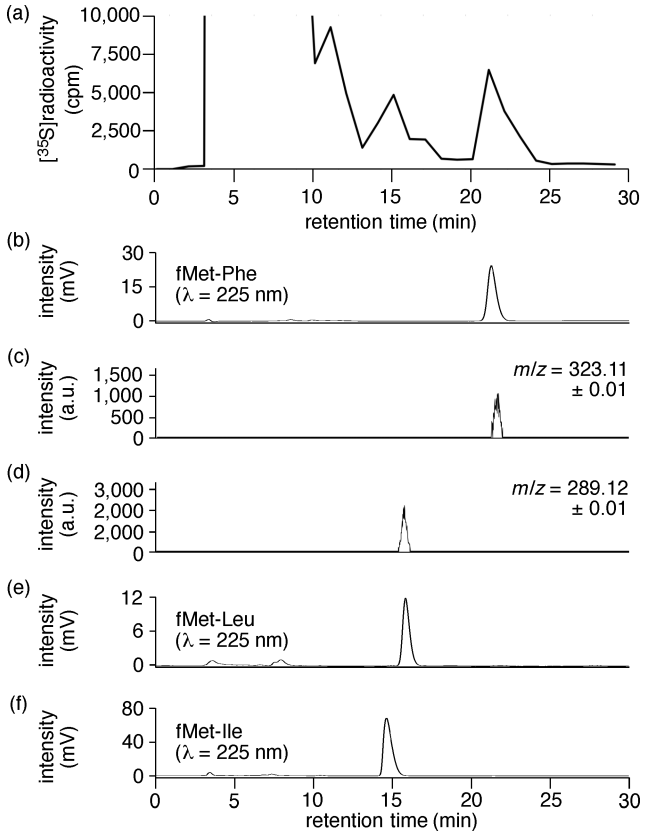


Figure 3. Chromatograms for the product analysis of the translation reaction. The analytical conditions for the HPLC were identical for all chromatograms. Column = ODS, eluent = 33% v/v methanol/water containing 0.1% v/v trifluoroacetic acid, flow rate = 1 mL/min. (a) Radiochromatograms of the translation products from the cell-free translation reaction using 13-mer chimeric RNA oligonucleotide **19**. The peptide products were labeled with [^{35}S]Met. The peak around 10 min in the radiochromatogram originated from contamination in [^{35}S]Met (PerkinElmer, NEG009A). (b) Reference chromatogram of the synthetic dipeptide, fMet-Phe, monitored by absorption at 225 nm. (c) Chromatogram of the translation products when monitoring the dipeptide, fMet-Phe, by MS at m/z = 323.11 ± 0.01 ([M − H]$^-$). (d) Chromatogram of the translation products when monitoring the dipeptide, fMet-Leu/fMet-Ile, by MS at m/z = 289.12 ± 0.01 ([M − H]$^-$). (e) Reference chromatogram of the synthetic dipeptide, fMet-Leu, monitored by absorption at 225 nm. (f) Reference chromatogram of the synthetic dipeptide, fMet-Ile, monitored by absorption at 225 nm.

reconstructed translation system and that the triazole-linked codon of UUC was translated into Phe in the peptide product.

In addition to an intense, broad peak originating from the tailing peak of the starting materials (fMet, < 12 min), the radiochromatogram showed a second major peak at ca. 15 min (Figure 3a). As shown in the MS chromatogram in Figure 3d, the peak contained a compound with an m/z of 289 that corresponded to dipeptides fMet-Leu/fMet-Ile. These dipeptides were synthesized separately and were detected at 15 (fMet-Ile) and 16 (fMet-Leu) min (Figure 3e and 3f). Although the present analysis precluded further, decisive assignments of the 15 min peak in the radiochromatogram, the results showed that both or one of the dipeptides were produced in the translation reaction. Misreading of a single nucleotide at the triazole-linked codon (UUC) could produce the corresponding dipeptides. Thus, Ile can be translated from AUC, and Leu can be translated from CUC, UUA or UUG. Quantification of the radioactivities of the target and misread products in the radiochromatograms allowed us to estimate the production ratio as target:misread = 57:43 (see below). The results indicated that chimeric RNA oligonucleotides with triazole-linkages may tolerate misreading of the encoding sequence in the translation reaction.[13]

Performing a control translation reaction with natural RNA **19-*nat***, we compared the target-to-misread ratio and the production efficiency. A quantitative data from quadruplicate reactions are shown in Figure 4. The target-to-misread ratio was

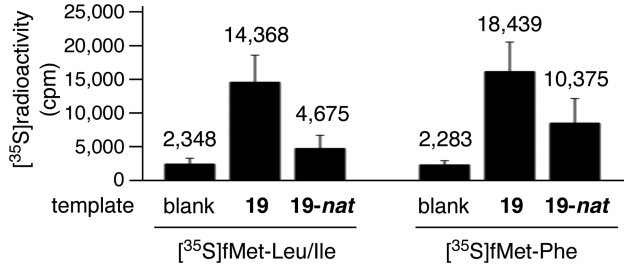


Figure 4. Quantitative data for the dipeptide products. The data were obtained from quadruplicate translation reactions. See Figure S45 for the representative chromatogram.

57:43 and 77:23 for **19** and **19-*nat***, respectively,[14] which showed a higher frequency of the misread production with ^{TL}RNA. Despite the misread production, the target dipeptide was produced with ^{TL}RNA more effectively, and the amount of the fMet-Phe translated from **19** was 2.0-fold higher than that from **19-*nat***.

In summary, we have developed synthesis methods for ^{TL}RNA oligonucleotides that can be incorporated in chimeric RNA oligonucleotides through phosphoramidite protocols. Three monomer units, i.e., 5′-jointing unit **4**, elongation unit **6** and 3′-jointing unit **5**, with pyrimidine and purine nucleobases were assembled by solution-phase click reactions under reoptimized, reproducible conditions. This method is versatile and can be extended to synthesize phosphoramidite-compatible ^{TL}RNA oligonucleotides with longer sequences. The ^{TL}RNA trinucleotide was successfully incorporated in the 13-mer chimeric RNA oligonucleotide **19** using the automated synthesizer. The chimeric RNA oligonucleotide was applicable as an mRNA substrate in the cell-free translation reaction. During the translation reaction, single nucleotide misreading was noted, and further investigation may identify its unique utility as a biological tool, for instance, as an error-prone mRNA in translation reactions.

EXPERIMENTAL SECTION

General Methods. Compounds from solution-phase synthesis were purified by flash column chromatography with 60N (spherical and neutral gel, 40−50 μm) and/or by MPLC with a Biotage SNAP Ultra column (50 g). Compounds from solid-phase synthesis (oligonucleotides and dipeptides) were purified by HPLC with a COSMOSIL 5C$_{18}$-MS-II column (20 × 250 mm). Translation products, dipeptides and oligonucleotides were analyzed by HPLC with a COSMOSIL 5C$_{18}$-MS-II column (4.6 × 250 mm). IR spectra were recorded on an FT-IR instrument and were reported as wavenumbers (ν) in cm^{-1}. NMR spectra were recorded at 400 MHz (^{1}H), 100 or 150 MHz (^{13}C) and 160 MHz (^{31}P) and were reported as chemical shift values with respect to chloroform (δ 7.26: ^{1}H NMR; δ 77.16: ^{13}C NMR), DMSO (δ 2.50: ^{1}H NMR; δ 39.52: ^{13}C NMR) or

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

85% v/v aqueous H_3PO_4 (δ 0.0: ^{31}P NMR, external standard). MS spectra were obtained by ESI-TOF MS.

Anhydrous THF (stabilizer free), DMF and toluene were purified by a solvent purification system equipped with columns of activated alumina and supported copper catalyst. Water was purified by a Milli-Q ultrapure water system. Other solvents were purified by distillation from calcium hydride and were dried over 4 Å molecular sieves. Phosphoramidite **16** and 500 Å controlled pore glass (CPG) support for the solid-phase oligonucleotide synthesis were purchased from Glen Research. The *in vitro* protein synthesis kit (PURExpress) was purchased from New England Biolabs.

Monomer Units (4, 5, 6). Monomer units were synthesized by procedures previously reported in the literature.[7,9,15] The characterization data of the newly prepared compounds are as follows.

5G. Yield 31% (853 mg, 3 steps from glycosylation); IR (neat) 2940, 1679, 1607, 1557, 1438, 1111 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.59 (ddd, J = 2.6, 6.0, 18.8 Hz, 1H), 2.70 (ddd, J = 2.6, 7.2, 18.8 Hz, 1H), 2.97 (dd, J = 2.6, 2.6 Hz, 1H), 4.02 (dd, J = 2.4, 4.8 Hz, 1H), 4.19 (ddd, J = 2.4, 6.0, 7.2 Hz, 1H), 4.68 (d, J = 11.6 Hz, 1H), 4.76 (d, J = 11.6 Hz, 1H), 4.85 (ddd, J = 4.8, 5.6, 6.8 Hz, 1H), 5.72 (d, J = 5.6 Hz, 1H), 5.85 (d, J = 6.8 Hz, 1H), 7.29−7.43 (m, 5H), 8.24 (s, 1H), 11.6−12.0 (br s, 1H), 11.6−12.0 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.0, 23.8, 71.2, 72.2, 73.2, 79.7, 80.5, 80.7, 86.5, 120.2, 127.5, 127.6, 128.2, 137.8, 138.3, 148.1, 149.0, 154.8, 173.5; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{21}H_{21}N_5O_5Na$ 446.1440, found 446.1457.

6U. Yield 97% (6.72 mg, 1 step from glycosylation); IR (neat) 3197, 2177, 2116, 1693, 1222, 843 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.19 (s, 9H), 2.19 (s, 3H), 2.69 (dd, J = 4.1, 17.6 Hz, 1H), 2.81 (dd, J = 4.5, 17.6 Hz, 1H), 4.06 (ddd, J = 4.1, 4.5, 5.2 Hz, 1H), 4.28 (dd, J = 5.2, 5.7 Hz, 1H), 5.48 (dd, J = 5.4, 5.7 Hz, 1H), 5.76 (dd, J = 2.2, 8.1 Hz, 1H), 6.00 (d, J = 5.4 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 8.11−8.46 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.0, 20.5, 24.4, 62.1, 75.0, 79.9, 87.3, 89.7, 100.9, 103.3, 139.9, 150.0, 162.4, 169.9; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{16}H_{21}N_5O_5SiNa$ 414.1210, found 414.1229.

Click Assembly of Dinucleotides. Dinucleotide **8UC** was synthesized from **5C** and **6U** using a procedure reported in the literature,[4] and dinucleotide **8UG** was synthesized using reoptimized conditions as follows. A solution of **5G** (20.0 mg, 47.2 μmol), **6U** (18.5 mg, 47.2 μmol) and copper(I) bromide dimethyl sulfide complex (2.42 mg, 11.8 μmol) in *t*-butanol/DMF (1:2 v/v, 0.94 mL) was stirred at ambient temperature for 21 h. After the addition of a saturated aqueous solution of ammonium chloride (0.94 mL), the mixture was extracted with dichloromethane (4 × 10 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 7% v/v methanol/dichloromethane) to give the dinucleotide (**8UG**; 35.2 mg, 43.2 μmol, 90%) as an amorphous solid.

8UC. Yield 97% (104 mg); IR (neat) 3421, 2923, 1697, 1485, 1300, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.17 (s, 9H), 1.80 (s, 3H), 2.74 (dd, J = 4.0, 17.8 Hz, 1H), 2.91 (dd, J = 4.0, 17.8 Hz, 1H), 3.08 (dd, J = 7.4, 15.4 Hz, 1H), 3.24 (dd, J = 4.0, 15.4 Hz, 1H), 3.45−3.65 (br s, 1H), 4.04 (dd, J = 5.8, 6.2 Hz, 1H), 4.39 (dd, J = 6.0, 7.0 Hz, 1H), 4.44 (ddd, J = 4.0, 7.0, 7.4 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 5.00 (ddd, J = 4.0, 4.0, 5.8 Hz, 1H), 5.36 (dd, J = 3.6, 6.0 Hz, 1H), 5.58 (dd, J = 5.2, 6.2 Hz, 1H), 5.71 (d, J = 3.6 Hz, 1H), 5.77 (d, J = 8.0 Hz, 1H), 6.17 (d, J = 5.2 Hz, 1H), 7.30−7.40 (m, 5H), 7.43 (s, 1H), 7.46−7.76 (m, 4H), 7.72 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 8.02 (s, 1H), 8.49 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.0, 20.2, 24.4, 29.3, 61.5, 72.8, 73.7, 74.5, 77.3, 78.9, 79.8, 81.1, 88.4, 89.5, 94.2, 97.6, 100.9, 103.3, 124.3, 128.0, 128.2, 128.3, 128.7, 129.0, 133.0, 133.3, 137.3, 140.2, 143.5, 145.2, 150.2, 162.7, 162.9, 163.3, 169.6; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{41}H_{44}N_8O_{10}SiNa$ 859.2847, found 859.2865.

8UG. Yield 90% (35.2 mg); IR (neat) 2940, 1679, 1607, 1557, 1251, 1112 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.17 (s, 9H), 1.72 (s, 3H), 2.37 (s, 3H), 2.68 (dd, J = 3.6, 18.2 Hz, 1H), 2.85 (dd, J = 3.6, 18.2 Hz, 1H), 3.12 (dd, J = 3.6, 15.4 Hz, 1H), 3.50 (dd, J = 3.6, 15.4 Hz, 1H), 4.33 (dd, J = 6.0, 6.4 Hz, 1H), 4.47 (ddd, J = 3.6, 3.6, 8.0 Hz, 1H), 4.68 (d, J = 11.6 Hz, 1H), 4.85 (d, J = 11.6 Hz, 1H), 4.89 (dd, J = 3.6, 3.6, 6.0 Hz, 1H), 5.05 (dd, J = 6.0, 8.0 Hz, 1H), 5.25−5.35 (br s, 1H), 5.34 (dd, J = 6.0, 6.4 Hz, 1H), 5.48 (dd, J = 5.2, 6.0 Hz, 1H), 5.77 (d, J = 8.0 Hz, 1H), 5.78 (d, J = 6.0 Hz, 1H), 6.19 (d, J = 5.2 Hz, 1H), 7.33−7.37 (m, 5H), 7.44 (s, 1H), 7.52 (s, 1H), 7.73 (d, J = 8.0 Hz, 1H), 8.70−8.95 (br s, 1H), 10.7−10.9 (br s, 1H), 13.0 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.0, 20.1, 24.4, 24.7, 30.0, 61.7, 72.8, 73.1, 74.4, 79.2, 80.7, 82.5, 87.9, 89.6, 90.8, 100.9, 103.4, 121.3, 123.9, 128.0, 128.0, 128.6, 137.9, 139.1, 140.2, 144.2, 147.7, 148.0, 150.3, 155.4, 163.2, 169.5, 173.7; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{37}H_{42}N_{10}O_{10}SiNa$ 837.2752, found 837.2751.

A Typical Procedure for the Desilylation of 5′-Silylalkyne of Dinucleotides. A 1.0 M aqueous solution of acetic acid (5.05 mL, 5.05 mmol) and tetra-*n*-butylammonium fluoride (1.0 M in THF, 4.04 mL, 4.04 mmol) were added to a solution of dinucleotide **8UC** (847 mg, 1.01 mmol) in THF (50 mL) at 0 °C. The mixture was allowed to warm to ambient temperature and stirred for 6 h. After the addition of a saturated aqueous solution of ammonium chloride (50 mL), the mixture was extracted with ethyl acetate (4 × 100 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 5% v/v methanol/dichloromethane) to give the desilylated dinucleotide (**9UC**; 723 mg, 0.944 mmol, 94%) as an amorphous solid.

9UC. Yield 94% (723 mg); IR (neat) 3283, 1694, 1485, 1261, 1101 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.83 (s, 3H), 2.13 (dd, J = 2.4, 2.8 Hz, 1H), 2.74 (ddd, J = 2.6, 4.4, 17.8 Hz, 1H), 2.89 (ddd, J = 2.6, 4.4, 17.8 Hz, 1H), 3.09 (dd, J = 7.4, 15.6 Hz, 1H), 3.21 (dd, J = 4.4, 15.6 Hz, 1H), 3.46−3.65 (br s, 1H), 4.05 (dd, J = 6.4, 6.6 Hz, 1H), 4.38 (ddd, J = 2.6, 4.4, 7.0 Hz, 1H), 4.41 (ddd, J = 4.4, 6.6, 7.4 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 5.05 (ddd, J = 3.4, 4.4, 7.4 Hz, 1H), 5.39 (dd, J = 7.0, 7.4 Hz, 1H), 5.65 (dd, J = 4.0, 6.4 Hz, 1H), 5.68 (d, J = 3.4 Hz, 1H), 5.77 (d, J = 8.0 Hz, 1H), 5.96 (d, J = 4.0 Hz, 1H), 7.30−7.40 (m, 5H), 7.41 (s, 1H), 7.50−7.65 (m, 5H), 7.81 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 8.11 (s, 1H), 8.70−8.80 (br s, 1H); ^{13}C NMR (100 MHz, 10% v/v CD_3OD/$CDCl_3$) δ 20.0, 22.4, 29.0, 61.4, 72.4, 72.6, 73.2, 74.2, 78.2, 78.3, 79.4, 80.9, 90.2, 93.9, 97.7, 102.9, 124.3, 127.9, 128.1, 128.2, 128.6, 128.8, 132.9, 133.2, 137.1, 141.1, 143.4, 144.7, 150.2, 156.2, 163.4, 163.8, 167.4, 169.5; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{38}H_{37}N_8O_{10}$ 765.2633, found 765.2635.

9UG. Yield 99% (21.4 mg); ^1H NMR (400 MHz, 10% v/v CD_3OD/$CDCl_3$) δ 1.68 (s, 3H), 2.10 (dd, J = 2.8, 2.8 Hz, 1H), 2.19 (s, 3H), 2.58 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 2.73 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 4.18 (dd, J = 4.8, 5.2 Hz, 1H), 4.32 (ddd, J = 5.2, 5.2, 6.0 Hz, 1H), 4.55 (d, J = 8.8 Hz, 1H), 4.57 (d, J = 11.6 Hz, 1H), 4.64 (d, J = 11.6 Hz, 1H), 4.84 (ddd, J = 4.8, 4.8, 8.8 Hz, 1H), 5.34 (dd, J = 6.8, 7.2 Hz, 1H), 5.50 (dd, J = 4.0, 6.4 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 5.75 (d, J = 4.0 Hz, 1H), 5.90 (d, J = 4.0 Hz, 1H), 7.20−7.32 (m, 5H), 7.41 (s, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.57 (s, 1H), 8.49 (s, 1H), three protons overlapped with solvent signals; ^{13}C NMR (100 MHz, 10% v/v CH_3OD/$CDCl_3$) δ 19.8, 22.5, 23.7, 29.3, 29.6, 61.6, 72.4, 72.7, 73.1, 74.3, 78.2, 78.4, 80.0, 81.3, 89.8, 102.9, 121.2, 124.4, 128.0, 128.2, 128.6, 137.2, 138.2, 141.0, 143.4, 147.7, 148.3, 150.2, 155.9, 163.9, 169.5, 173.2; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{34}H_{34}N_{10}O_{10}Na$ 765.2357, found 765.2360.

A Typical Procedure for the Silyl Protection of the 2′-Hydroxy Group of Dinucleotides. A solution of **9UC** (77.3 mg, 101 μmol), *t*-butyldimethylsilyl chloride (45.7 mg, 303 μmol) and imidazole (41.3 mg, 606 μmol) in THF (0.79 mL) was stirred at ambient temperature for 6 h. After the addition of a saturated aqueous solution of ammonium chloride (0.79 mL), the mixture was extracted with dichloromethane (4 × 5 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 3% v/v methanol/dichloromethane) to give the silyl-protected dinucleotide (**10UC**; 86.7 mg, 98.6 μmol, 98%) as an amorphous solid.

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

10UC. Yield 98% (86.7 mg); IR (neat) 3395, 2923, 1696, 1464, 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.15 (s, 3H), 0.20 (s, 3H), 0.92 (s, 9H), 1.82 (s, 3H), 2.16 (dd, J = 2.8, 2.8 Hz, 1H), 2.72 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 2.88 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 3.09 (dd, J = 8.6, 15.6 Hz, 1H), 3.29 (dd, J = 3.6, 15.6 Hz, 1H), 3.69 (dd, J = 4.2, 7.8 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.50 (ddd, J = 3.6, 7.8, 8.6 Hz, 1H), 4.67 (dd, J = 1.8, 4.2 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 5.01 (ddd, J = 4.4, 4.4, 7.0 Hz, 1H), 5.38 (dd, J = 6.8, 7.0 Hz, 1H), 5.61 (dd, J = 4.6, 6.8 Hz, 1H), 5.64 (d, J = 1.8 Hz, 1H), 5.78 (d, J = 8.0 Hz, 1H), 6.01 (d, J = 4.6 Hz, 1H), 7.25−7.40 (m, 5H), 7.45 (s, 1H), 7.47−7.68 (m, 5H), 7.72 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 8.12 (s, 1H), 8.80−8.95 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, −4.5, 18.0, 20.1, 22.5, 25.7, 29.2, 61.1, 72.3, 72.5, 73.2, 74.4, 78.3, 78.7, 80.1, 80.5, 89.4, 94.0, 96.6, 102.8, 124.6, 127.7, 127.9, 128.4, 128.8, 132.9, 133.0, 137.4, 140.5, 140.5, 143.5, 144.9, 150.3, 154.6, 162.7, 163.4, 166.9, 169.4; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{44}H_{50}N_8O_{10}SiNa$ 901.3317, found 901.3293.

10UG. Yield 87% (7.3 mg); IR (neat) 2926, 1686, 1611, 1560, 1251 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.23 (s, 3H), −0.03 (s, 3H), 0.85 (s, 9H), 1.74 (s, 3H), 2.13 (dd, J = 2.4, 2.4 Hz, 1H), 2.38 (s, 3H), 2.66 (ddd, J = 2.4, 4.0, 17.2 Hz, 1H), 2.80 (ddd, J = 2.4, 4.0, 17.2 Hz, 1H), 3.07 (dd, J = 3.8, 15.2 Hz, 1H), 3.59 (dd, J = 9.6, 15.2 Hz, 1H), 4.08 (dd, J = 2.8, 4.0 Hz, 1H), 4.57 (ddd, J = 2.8, 3.8, 9.6 Hz, 1H), 4.63 (d, J = 11.6 Hz, 1H), 4.72 (dd, J = 4.0, 5.8 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 4.90 (ddd, J = 4.0, 4.0, 6.8 Hz, 1H), 5.41 (dd, J = 6.8, 6.8 Hz, 1H), 5.53 (dd, J = 4.4, 6.8 Hz, 1H), 5.78 (d, J = 8.0 Hz, 1H), 5.79 (d, J = 5.8 Hz, 1H), 5.99 (d, J = 4.4 Hz, 1H), 7.33−7.39 (m, 5H), 7.41 (s, 1H), 7.50 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 8.25 (s, 1H), 10.0 (s, 1H), 11.9 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −4.8, 18.0, 20.1, 22.8, 24.4, 25.7, 30.5, 61.6, 72.5, 72.7, 74.5, 74.5, 78.7, 80.9, 82.1, 89.7, 89.9, 90.0, 103.0, 122.4, 124.3, 124.4, 127.8, 128.1, 128.6, 137.9, 138.5, 141.1, 144.1, 147.8, 148.2, 150.1, 155.8, 169.4, 172.9; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{40}H_{48}N_{10}O_{10}SiNa$ 879.3222, found 879.3228.

A Typical Procedure for the Debenzylation of the 3′-Hydroxy Group of Dinucleotides. Boron trichloride (1.0 M in dichloromethane, 980 μL, 980 μmol) was added to a solution of silyl-protected dinucleotide **10UC** (70.4 mg, 80.2 μmol) in dichloromethane (4.0 mL) at −78 °C, and the mixture was stirred at −78 °C for 2 d. After the addition of methanol (4.0 mL) at −78 °C, the mixture was allowed to warm to 0 °C and was mixed with a saturated aqueous solution of sodium bicarbonate (4.0 mL). The mixture was extracted with dichloromethane (5 × 4.0 mL), dried over sodium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 95% v/v ethyl acetate/dichloromethane and 10% v/v methanol/dichloromethane) to give the debenzylated dinucleotide (**11UC**; 61.1 mg, 77.5 μmol, 97%) as an amorphous solid.

11UC. Yield 97% (61.1 mg); IR (neat) 3277, 1697, 1485, 1260, 1099, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.13 (s, 3H), 0.19 (s, 3H), 0.91 (s, 9H), 1.80 (s, 3H), 2.16 (dd, J = 2.8, 2.8 Hz, 1H), 2.74 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 2.76 (d, J = 7.8 Hz, 1H), 2.90 (ddd, J = 2.8, 4.4, 17.6 Hz, 1H), 3.18 (dd, J = 7.6, 15.2 Hz, 1H), 3.33 (dd, J = 4.6, 15.2 Hz, 1H), 3.98 (ddd, J = 5.4, 7.8, 7.8 Hz, 1H), 4.24 (ddd, J = 4.6, 7.6, 7.8 Hz, 1H), 4.47 (dd, J = 2.4, 5.4 Hz, 1H), 5.07 (ddd, J = 4.4, 4.4, 7.0 Hz, 1H), 5.46 (dd, J = 7.0, 7.0 Hz, 1H), 5.64 (dd, J = 4.4, 7.0 Hz, 1H), 5.70 (d, J = 2.4 Hz, 1H), 5.75 (d, J = 8.0 Hz, 1H), 5.97 (d, J = 4.4 Hz, 1H), 7.48−7.52 (m, 3H), 7.58 (dd, J = 8.0, 9.2 Hz, 2H), 7.63 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 7.2 Hz, 2H), 8.70−9.05 (br s, 1H), 9.05−9.20 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.4, 18.2, 20.3, 22.7, 25.9, 29.1, 61.4, 72.6, 73.3, 74.5, 75.4, 78.6, 78.7, 82.7, 90.2, 93.5, 96.9, 103.1, 124.5, 127.9, 129.1, 133.1, 133.4, 140.9, 143.6, 145.2, 150.1, 154.8, 162.8, 163.1, 166.7, 169.6; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{37}H_{44}N_8O_{10}SiNa$ 811.2847, found 811.2857.

11UG. Yield 93% (76.1 mg); IR (neat) 2968, 1682, 1610, 1560, 1252, 1048 cm^{-1}; ^1H NMR (400 MHz, 6% v/v $CD_3OD/CDCl_3$) δ −0.15 (s, 3H), −0.05 (s, 3H), 0.79 (s, 9H), 1.78 (s, 3H), 2.12 (s, 1H), 2.28 (s, 3H), 2.61−2.63 (m, 1H), 2.75−2.78 (m, 1H), 3.17 (dd, J = 7.6, 14.8 Hz, 1H), 3.30 (dd, J = 6.8, 14.8 Hz, 1H), 4.26 (ddd, J = 6.8, 6.8, 7.6 Hz, 1H), 4.32 (dd, J = 4.0, 6.8 Hz, 1H), 4.91−4.93 (m, 2H), 5.46 (dd, J = 4.8, 6.8 Hz, 1H), 5.57 (dd, J = 3.6, 6.8 Hz, 1H), 5.73 (d, J = 8.4 Hz, 1H), 5.75 (d, J = 4.8 Hz, 1H), 5.92 (d, J = 3.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.73 (s, 1H), 8.27−8.35 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.9, −4.9, 18.1, 20.2, 22.9, 24.4, 25.7, 29.6, 61.6, 72.6, 73.4, 74.5, 75.5, 78.7, 78.7, 83.6, 89.8, 90.1, 103.1, 122.2, 124.6, 138.4, 141.1, 143.8, 148.0, 148.1, 150.2, 155.8, 163.4, 169.7, 173.1; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{33}H_{42}N_{10}O_{10}SiNa$ 789.2752, found 789.2752.

A Typical Procedure for the Click Elongation for Trinucleotides. A solution of alkyne **8UC** (20.9 mg, 26.5 μmol), azide **9U** (18.2 mg, 26.5 μmol), copper(I) bromide dimethyl sulfide complex (1.36 mg, 6.62 μmol) and *N*,*N*-diisopropylethylamine (1.09 μL, 6.62 μmol) in *t*-butanol/DMF (1:2 v/v, 0.13 mL) was stirred at ambient temperature for 24 h. After the addition of a saturated aqueous solution of ammonium chloride (0.13 mL), the mixture was extracted with dichloromethane (5 × 4 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 3−8% v/v methanol/dichloromethane) to give the trinucleotide (**12UUC**; 37.9 mg, 25.7 μmol, 97%) as an amorphous solid.

12UUC. Yield 97% (37.9 mg); IR (neat) 3286, 1695, 1381, 1251, 1103, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.12 (s, 3H), 0.17 (s, 3H), 0.92 (s, 9H), 1.84 (s, 3H), 1.89 (s, 3H), 2.72 (d, J = 6.8 Hz, 1H), 3.17 (dd, J = 5.6, 16.0 Hz, 1H), 3.25 (dd, J = 5.2, 16.0 Hz, 1H), 3.26 (dd, J = 5.2, 16.0 Hz, 1H), 3.34 (dd, J = 4.4, 16.0 Hz, 1H), 3.46 (dd, J = 2.4, 11.2 Hz, 1H), 3.66 (dd, J = 2.0, 11.2 Hz, 1H), 3.78 (s, 3H), 3.79 (s, 3H), 4.01 (ddd, J = 4.0, 6.8, 7.8 Hz, 1H), 4.22 (ddd, J = 4.4, 5.2, 5.6 Hz, 1H), 4.54 (dd, J = 2.0, 4.8 Hz, 1H), 4.90 (ddd, J = 2.0, 2.4, 4.8 Hz, 1H), 5.11 (ddd, J = 5.2, 5.6, 7.8 Hz, 1H), 5.41 (dd, J = 2.0, 2.4 Hz, 1H), 5.44 (dd, J = 3.6, 4.0 Hz, 1H), 5.48 (dd, J = 5.6, 5.6 Hz, 1H), 5.62 (d, J = 3.6 Hz, 1H), 5.64 (d, J = 2.4 Hz, 1H), 5.68 (dd, J = 5.6, 5.6 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 6.27 (d, J = 5.6 Hz, 1H), 6.81 (d, J = 3.6 Hz, 2H), 6.83 (d, J = 4.0 Hz, 2H), 7.21−7.25 (m, 5H), 7.27−7.30 (m, 4H), 7.33 (s, 1H), 7.35 (s, 1H), 7.48−7.62 (m, 6H), 7.71 (d, J = 6.0 Hz, 1H), 7.74 (d, J = 6.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.68−8.73 (br s, 1H), 8.73−8.78 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.5, 18.1, 20.2, 20.3, 25.8, 28.5, 28.9, 55.3, 59.8, 61.7, 62.4, 73.2, 74.0, 74.5, 75.4, 79.4, 80.8, 82.6, 87.5, 87.8, 92.4, 93.5, 97.0, 102.9, 103.1, 113.4, 124.2, 124.7, 127.3, 128.0, 128.2, 128.3, 129.0, 130.2, 130.3, 133.0, 133.3, 134.9, 135.0, 140.2, 142.4, 143.4, 144.0, 145.4, 150.1, 150.6, 154.7, 158.8, 162.9, 163.3, 163.5, 169.6, 169.7; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{69}H_{75}N_{13}O_{18}SiNa$ 1424.5020, found 1424.5049.

12CUC. Yield 94% (49.8 mg); IR (neat) 2931, 1697, 1482, 1251, 1108 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.14 (s, 3H), 0.88 (s, 9H), 1.75 (s, 3H), 1.77 (s, 3H), 2.87−2.95 (br s, 1H), 3.12 (dd, J = 7.2, 15.2 Hz, 1H), 3.09−3.15 (m, 1H), 3.21−3.29 (m, 2H), 3.37 (d, J = 10.4 Hz, 1H), 3.64 (d, J = 10.4 Hz, 1H), 3.77 (s, 3H), 3.78 (s, 3H), 3.90−3.98 (m, 1H), 4.19 (ddd, J = 4.8, 7.0, 11.8 Hz, 1H), 4.45 (d, J = 2.8 Hz, 1H), 5.11−5.17 (m, 2H), 5.40 (dd, J = 6.8, 6.8 Hz, 1H), 5.62−5.70 (m, 4H), 5.84 (d, J = 3.6 Hz, 1H), 5.98−6.04 (m, 1H), 6.12 (s, 1H), 6.78 (d, J = 6.8 Hz, 2H), 6.80 (d, J = 6.8 Hz, 2H), 7.17−7.25 (m, 7H), 7.31 (s, 1H), 7.33 (s, 1H), 7.36−7.47 (m, 7H), 7.52−7.58 (m, 4H), 7.65 (s, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.95 (d, J = 7.6 Hz, 2H), 8.50 (d, J = 7.2 Hz, 1H), 9.10−9.40 (br s, 1H), 9.40−9.45 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.4, 18.2, 20.2, 20.4, 25.9, 28.9, 29.0, 55.4, 58.5, 60.8, 61.8, 73.1, 73.9, 73.9, 75.4, 79.7, 80.4, 82.6, 87.5, 89.8, 89.8, 91.4, 93.8, 103.1, 103.1, 113.5, 124.9, 124.9, 127.3, 127.9, 127.9, 128.0, 128.1, 128.2, 129.0, 129.0, 130.1, 133.1, 133.1, 133.3, 133.3, 135.1, 135.2, 141.9, 142.0, 142.0, 142.1, 143.4, 144.0, 150.0, 158.8, 158.8, 162.8, 162.8, 163.1, 163.1, 169.5, 169.6; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{76}H_{80}N_{14}O_{18}SiNa$ 1527.5442, found 1527.5464.

12AUG. Yield 77% (113 mg); ^1H NMR (400 MHz, $CDCl_3$) δ −0.19 (s, 3H), −0.10 (s, 3H), 0.76 (s, 9H), 1.19 (s, 3H), 1.15−1.20 (m, 1H), 1.70 (s, 3H), 2.21 (s, 3H), 2.97−3.22 (m, 5H), 3.37−3.50 (m, 1H), 3.70 (s, 3H), 3.70 (s, 3H), 4.11−4.22 (m, 1H), 4.17−4.26 (m, 1H), 4.72−4.82 (m, 1H), 4.92−5.06 (m, 1H), 5.08−5.26 (m, 1H),

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

5.22−5.40 (m, 1H), 5.46−5.70 (m, 6H), 5.66−5.78 (m, 1H), 5.72−6.00 (m, 1H), 6.62−6.84 (m, 5H), 7.00−7.52 (m, 16H), 7.49 (s, 1H), 7.64 (s, 1H), 8.04−8.20 (br s, 1H), 8.20−8.40 (br s, 1H), 8.58−8.76 (br s, 1H), 8.74−8.98 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −5.1, −5.0, 18.1, 20.1, 20.4, 24.4, 25.6, 29.8, 32.1, 55.3, 59.5, 61.5, 62.2, 73.2, 73.2, 73.9, 74.0, 80.1, 80.8, 83.6, 87.3, 89.4, 90.2, 103.1, 103.1, 113.5, 125.4, 125.7, 127.2, 127.8, 127.9, 128.2, 128.5, 128.9, 129.3, 130.2, 131.0, 131.1, 132.3, 132.5, 135.1, 135.1, 141.8, 142.1, 143.0, 143.1, 144.1, 148.0, 148.2, 150.0, 155.6, 158.8, 163.0, 169.5, 169.5, 173.3, 175.9, 179.1; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{73}H_{78}N_{18}O_{17}SiNa$ 1529.5459, found 1529.5432.

A Typical Procedure for the Transformation of a Trinucleotide to Phosphoramidite. A solution of trinucleotide **12UUC** (182 mg, 0.130 mmol), *N*,*N*-diisopropylethylamine (180 μL, 1.03 mmol) and 2-cyanoethyl diisopropylchlorophosphoramidite (144 μL, 0.647 mmol) in dichloromethane (1.4 mL) was stirred at ambient temperature for 4 h. After the addition of a saturated aqueous solution of sodium bicarbonate (1.4 mL), the mixture was extracted with dichloromethane (4 × 2 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography using silica gel treated with 0.1% v/v triethylamine (eluent: 5% v/v methanol/dichloromethane) to give a mixture of 2:1 diastereomers of trinucleotide phosphoramidite (**13UUC**; 193 mg, 0.120 mmol, 93%) as an amorphous solid.

13UUC. Yield 93% (193 mg); IR (neat) 2966, 1695, 1483, 1381, 1251, 1030, 833 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (major diastereomer) 0.13 (s, 3H), 0.13 (s, 3H), 0.89 (s, 9H), 1.12−1.30 (m, 12H), 1.83 (s, 3H), 1.85 (s, 3H), 2.54−2.72 (m, 2H), 2.73−2.80 (m, 2H), 3.08−3.84 (m, 8H), 3.77 (s, 3H), 3.77 (s, 3H), 3.83−3.96 (m, 1H), 4.02−4.38 (m, 3H), 4.86−5.00 (m, 1H), 5.04−5.18 (m, 1H), 5.42 (d, J = 8.0 Hz, 1H), 5.43−5.78 (m, 7H), 6.65−6.88 (m, 4H), 7.10−8.08 (m, 20H), 8.70−10.4 (m, 3H); δ (minor diastereomer) 0.10 (s, 3H), 0.13 (s, 3H), 0.88 (s, 9H), 1.12−1.30 (m, 12H), 1.81 (s, 3H), 1.87 (s, 3H), 2.54−2.72 (m, 2H), 2.70−2.82 (m, 2H), 3.08−3.84 (m, 8H), 3.77 (s, 3H), 3.77 (s, 3H), 3.83−3.96 (m, 1H), 4.02−4.38 (m, 3H), 4.27−4.34 (m, 1H), 4.40−4.64 (m, 3H), 4.69−4.75 (m, 1H), 5.43−5.78 (m, 2H), 6.14 (s, 1H), 6.23 (d, J = 5.2 Hz, 1H), 6.24 (d, J = 5.2 Hz, 1H), 6.65−6.88 (m, 4H), 7.10−8.08 (m, 20H), 8.70−10.4 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.9, −4.7, −4.6, −4.5, 18.1, 18.5, 20.2, 20.2, 20.2, 20.3, 20.4, 20.5, 20.5, 20.6, 23.0, 23.1, 24.7, 24.8, 24.8, 24.8, 24.9, 24.9, 25.9, 25.9, 43.2, 43.3, 43.4, 43.4, 45.4, 45.4, 45.4, 45.4, 55.3, 55.3, 57.8, 58.3, 58.3, 59.8, 59.8, 60.1, 60.1, 61.4, 62.3, 62.4, 73.9, 74.3, 74.4, 74.9, 75.0, 75.2, 75.8, 79.3, 79.4, 80.8, 81.5, 81.6, 87.6, 87.7, 92.4, 92.4, 93.8, 94.5, 96.6, 96.7, 96.9, 96.9, 103.0, 103.0, 103.2, 103.2, 113.4, 113.5, 116.5, 117.1, 117.1, 117.4, 117.8, 117.9, 118.2, 118.5, 124.2, 124.2, 124.5, 124.7, 127.0, 127.3, 127.4, 128.0, 128.0, 128.1, 128.9, 130.1, 130.2, 130.3, 133.0, 133.1, 133.2, 134.8, 134.9, 140.1, 142.4, 143.7, 144.0, 144.2, 145.3, 145.4, 145.7, 145.7, 150.0, 150.5, 154.8, 154.8, 158.6, 158.8, 158.8, 162.6, 162.8, 163.2, 163.3, 166.8, 166.9, 168.6, 169.5, 169.6, 169.7, 173.2; ^{31}P NMR (160 MHz, $CDCl_3$) δ 135.0, 135.7; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{78}H_{92}N_{15}O_{19}PSiNa$ 1624.6098, found 1624.6073.

13CUC. Yield 90% (20.3 mg); IR (neat) 2963, 1691, 1480, 1245 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (major diastereomer) 0.11 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 1.10−1.28 (m, 12H), 1.81 (s, 3H), 1.83 (s, 3H), 2.54−2.68 (m, 2H), 3.04−3.97 (m, 12H), 3.78 (s, 3H), 3.78 (s, 3H), 3.84−3.96 (m, 1H), 4.02−4.14 (m, 1H), 5.06−5.14 (m, 2H), 5.36−5.44 (m, 1H), 5.48−5.56 (m, 1H), 5.56−5.68 (m, 3H), 5.72−5.86 (m, 1H), 5.80−5.88 (m, 1H), 6.74−6.84 (m, 4H), 7.10−7.96 (m, 26H), 8.77−9.30 (br s, 3H); δ (minor diastereomer) 0.09 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 1.10−1.28 (m, 12H), 1.81 (s, 3H), 1.83 (s, 3H), 2.54−2.68 (m, 2H), 2.70−2.78 (m, 2H), 3.04−3.97 (m, 9H), 3.78 (s, 3H), 3.78 (s, 3H), 4.02−4.34 (m, 3H), 4.38−4.56 (m, 3H), 4.66−4.72 (m, 1H), 5.06−5.14 (m, 1H), 5.56−5.68 (m, 1H), 6.02−6.26 (m, 3H), 6.74−6.84 (m, 4H), 7.10−7.96 (m, 26H), 8.36−8.44 (br s, 1H), 8.38−8.46 (br s, 1H), 8.77- 9.30 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.8, −4.7, −4.5, −4.4, 18.1, 18.1, 20.2, 20.2, 20.2, 20.3, 20.3, 20.5, 20.5, 20.6, 23.0, 23.1, 24.7, 24.8, 24.8, 24.8, 24.9, 24.9, 25.9, 25.9, 43.2, 43.3, 43.4, 43.5, 45.4, 45.4, 45.4, 45.4, 55.4, 55.4, 57.7, 57.9, 58.3, 58.3, 58.5, 58.5, 58.6, 60.9, 61.7, 65.7, 65.8, 73.9, 74.4, 74.9, 74.9, 75.2, 75.3, 75.4, 80.0, 80.3, 81.4, 81.5, 87.5, 89.6, 91.7, 91.7, 94.0, 94.5, 96.9, 97.0, 97.3, 97.5, 103.0, 103.0, 113.3, 113.5, 117.1, 117.8, 118.2, 124.5, 124.6, 124.9, 124.9, 125.4, 127.3, 127.9, 127.9, 127.9, 127.9, 128.1, 128.1, 128.2, 128.2, 128.3, 129.0, 129.0, 129.0, 129.0, 129.1, 130.1, 130.1, 130.1, 130.1, 131.1, 132.3, 133.1, 133.2, 13.2, 135.1, 135.2, 142.1, 142.1, 142.3, 142.3, 143.7, 143.9, 143.9, 144.2, 144.8, 145.3, 145.3, 145.6, 145.6, 150.0, 150.0, 154.8, 154.9, 158.8, 158.8, 158.8, 158.8, 162.6, 162.7, 163.0, 163.0, 166.7, 166.7, 167.8, 168.9, 169.4, 169.5, 169.6; ^{31}P NMR ($CDCl_3$, 160 MHz) δ 149.6, 150.3; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{85}H_{97}N_{16}O_{19}PSiNa$ 1727.6520, found 1727.6554.

13AUG. Yield 94% (163 mg); IR (neat) 2963, 1670, 1252, 1031 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (major diastereomer) −0.29 (s, 3H), −0.09 (s, 3H), 0.74 (s, 9H), 1.16−1.28 (m, 12H), 1.70 (s, 3H), 1.72 (s, 3H), 2.21 (s, 3H), 2.06−2.92 (m, 5H), 2.61 (t, J = 6.0 Hz, 1H), 3.77 (s, 3H), 3.77 (s, 3H), 3.04−6.00 (m, 19H), 6.72−6.84 (m, 4H), 7.14−7.76 (m, 18H), 7.90 (s, 1H), 8.16−8.36 (m, 3H), 8.72 (s, 1H), 9.34−9.46 (br s, 1H); δ (minor diastereomer) −0.21 (s, 3H), −0.05 (s, 3H), 0.80 (s, 9H), 1.16−1.28 (m, 12H), 1.70 (s, 3H), 1.72 (s, 3H), 2.13 (s, 3H), 2.06−2.92 (m, 6H), 3.77 (s, 3H), 3.77 (s, 3H), 3.04−6.00 (m, 19H), 6.72−6.84 (m, 4H), 6.98 (s, 1H), 7.14−7.76 (m, 17H), 7.91 (s, 1H), 8.16−8.36 (m, 3H), 8.78 (s, 1H), 9.34−9.46 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −5.3, −5.1, −4.9, −4.7, 18.0, 18.0, 19.9, 20.1, 20.1, 20.2, 20.3, 20.3, 20.6, 20.6, 24.1, 24.3, 24.7, 24.7, 24.8, 24.8, 24.8, 24.8, 24.9, 24.9, 25.6, 25.7, 29.7, 29.7, 29.8, 29.8, 43.1, 43.2, 43.5, 43.5, 55.3, 55.3, 57.0, 57.0, 58.5, 58.5, 59.2, 59.5, 60.4, 61.5, 61.8, 61.9, 62.1, 74.0, 74.2, 79.5, 80.6, 80.9, 82.6, 82.7, 82.7, 82.8, 82.8, 82.9, 87.3, 87.4, 87.9, 88.0, 88.1, 89.2, 89.4, 90.3, 90.8, 90.9, 102.9, 102.9, 113.5, 113.5, 118.3, 118.5, 121.5, 122.3, 123.8, 124.7, 124.7, 125.3, 125.4, 125.7, 127.2, 127.2, 127.8, 127.8, 128.1, 128.1, 128.1, 128.1, 128.2, 128.2, 128.5, 128.5, 129.2, 129.2, 130.1, 130.1, 130.1, 130.1, 132.5, 135.0, 135.1, 135.2, 137.0, 138.4, 141.7, 142.1, 142.4, 143.2, 144.0, 144.1, 144.3, 147.7, 147.8, 148.0, 148.4, 150.0, 155.6, 155.8, 158.7, 158.7, 163.0, 163.0, 163.1, 163.1, 169.4, 169.4, 169.4, 169.5, 172.5, 172.5, 172.6, 172.6; ^{31}P NMR (160 MHz, $CDCl_3$) δ 149.6, 150.3; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{82}H_{95}N_{20}O_{18}PSiNa$ 1729.6538, found 1729.6532.

Solid-Phase Synthesis of Chimeric RNA Oligonucleotide (19). Chimeric oligonucleotide **19** was synthesized on an automated synthesizer by using CPG supports loaded with rU (1.02 μmol on 32.0-mg CPG) as the starting material. The 5′-hydroxy terminus of rU was coupled with *N*-acetylcytidine phosphoramidite (**16C**) in the presence of 5-benzylthio-1*H*-tetrazole (BTT) activator (450 mM in acetonitrile, 440 μL, 198 μmol). After a 240 s coupling reaction, the residual 5′-hydroxy end of rU was capped with acetic anhydride (1.06 M in THF, 500 μL, 530 μmol). After oxidation of the phosphorus linker with iodine (20 mM in THF, 700 μL, 14.0 μmol), the 5′-dimethoxytrityl (DMTr) group on the extended nucleoside (C) was removed with trichloroacetic acid (300 mM in dichloromethane, 1.3 mL, 390 μmol). The DMTr cation was roughly quantified by an LED detector equipped with the synthesizer to ensure a coupling efficiency >99%. This process was repeated five times using the corresponding phosphoramidite **16** with a designated nucleobase to assemble 5′-UAAGCU-3′ on CPG. The trinucleotide **13UUC** was then loaded to assemble 9-mer **15** on CPG under the identical conditions for the phosphoramidite of natural ribonucleosides except for a longer coupling time of 1440 s. Elongation with phosphoramidite of natural ribonucleoside **16** was repeated four times on **15** to afford the 13-mer chimeric oligonucleotide **17** on CPG. After the final elongation with G at the 5′-end, the amount of **17** on CPG was carefully determined by quantification of the DMTr cation using a UV−vis spectrometer in the following manner. The CPG loaded with **17** was treated with a solution of 4% v/v dichloroacetic acid in 1,2-dichloroethane (2.0 mL) for 30 s to release the DMTr cation. After separation of CPG, the solvent was removed in vacuo to afford a yellow residue. The residue was dissolved in 0.1 M tosyl chloride solution in acetonitrile (5.0 mL), and 1/5 v/v of the solution was diluted with acetonitrile to 10 mL. The absorbance at 498 nm was 0.878, and using the absorption coefficient of the DMTr cation (ε_{498} = 72 000 mol^{-1}·cm^{-1}[16]), the

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

amount of DMTr at the 5′-end was calculated as 0.612 μmol. Based on this value, the total yield of **18** from the 40-step transformation was 60%. Finally, **18** on CPG was treated with a 28% solution of ammonium hydroxide/ethanol (3:1 v/v) at 55 °C for 5 h to afford **19** via cleavage from the solid support. The crude materials were then mixed with tetra-*n*-butylammonium fluoride (1.0 M) in THF (1.0 mL) at ambient temperature for 24 h to remove the 2′-TBDMS group. After removal of volatile materials from the mixture, the residual material was dissolved in water and desalted on a sep-pak column. The filtered material was purified by HPLC with an eluent of 0−34% acetonitrile in 100 mM triethylammonium acetate buffer (pH 6.8) at a flow rate of 10 mL/min. The fractions containing the target oligonucleotide were collected and freeze-dried to give the chimeric oligonucleotide **19**. The purity of oligonucleotide **19** was checked by HPLC analysis at 260 nm (98% purity; Figure S44). **19**: HRMS (ESI-TOF) *m*/*z* $[M + 5H]^{5-}$ calcd for $C_{127}H_{147}N_{52}O_{83}P_{10}$ 807.5257, found 807.5258. The mass spectra are shown in Figures S42 and S43.

Dipeptides (fMet-Phe, fMet-Leu, fMet-Ile). Dipeptides were synthesized using the procedures reported in the literature.[17] The characterization data of the compounds are as follows.

fMet-Phe. Yield 67% (504 mg); IR (neat) 3322, 1728, 1649, 1610, 1536, 1392 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.77 (ddd, *J* = 8.0, 8.0, 14.0 Hz, 1H), 1.88 (ddd, *J* = 8.0, 8.0, 14.0 Hz, 1H), 2.05 (s, 3H), 2.43 (dd, *J* = 8.0, 8.0 Hz, 2H), 2.95 (dd, *J* = 8.0, 14.0 Hz, 1H), 3.10 (dd, *J* = 4.8, 14.0 Hz, 1H), 4.30−4.36 (m, 1H), 4.43 (ddd, *J* = 4.8, 8.0, 8.8 Hz, 1H), 7.20−7.30 (m, 6H), 8.02 (s, 1H), 8.08−8.20 (br s, 1H), 8.31 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.0, 29.5, 32.7, 36.8, 50.5, 53.8, 126.8, 128.5, 129.4, 137.5, 161.2, 170.6, 172.7; HRMS (ESI-TOF) *m*/*z* $[M - H]^-$ calcd for $C_{15}H_{19}N_2O_4S$ 323.1066, found 323.1078.

fMet-Leu.[18] Yield 33% (53.8 mg); IR (neat) 3340, 1699, 1665, 1593, 1514 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.80 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 3H), 1.43−1.51 (m, 2H), 1.55−1.63 (m, 1H), 1.70−1.80 (m, 1H), 1.80−1.89 (m, 1H), 1.99 (s, 3H), 2.41 (dd, *J* = 8.0, 8.0 Hz, 2H), 4.16 (ddd, *J* = 5.6, 8.0, 9.6 Hz, 1H), 4.41 (ddd, *J* = 5.2, 8.0, 8.0 Hz, 1H), 7.97 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.40 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.6, 21.2, 22.9, 24.2, 29.2, 32.4, 39.7, 50.1, 50.2, 160.8, 170.7, 173.9; HRMS (ESI-TOF) *m*/*z* $[M - H]^-$ calcd for $C_{12}H_{21}N_2O_4S$ 289.1222, found 289.1212.

fMet-Ile. Yield 42% (13.9 mg); fMet-Ile: IR (neat) 3295, 1716, 1650, 1538, 1213 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.84 (t, *J* = 7.2 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H), 1.12−1.23 (m, 1H), 1.35−1.44 (m, 1H), 1.73−1.82 (m, 2H), 1.84−1.92 (m, 1H), 2.03 (s, 3H), 2.43 (t, *J* = 8.0 Hz, 2H), 4.15 (dd, *J* = 6.0, 8.0 Hz, 1H), 4.52 (ddd, *J* = 5.6, 8.0, 8.0 Hz, 1H), 8.01 (s, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 8.28 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.3, 14.6, 15.5, 24.7, 29.2, 32.4, 36.1, 50.2, 56.4, 160.9, 170.8, 172.8; HRMS (ESI-TOF) *m*/*z* $[M - H]^-$ calcd for $C_{12}H_{21}N_2O_4S$ 289.1222, found 289.1216.

Cell-Free Translation Reaction Using Chimeric Oligonucleotide 19. The cell-free translation reaction with chimeric oligonucleotide **19** was performed using PURExpress Solution A and Solution B. A mixture of **19** (270 μM in diethylpyrocarbonate-treated (DEPC) water, 3.7 μL, 1.00 nmol), Solution A (7.5 μL), Solution B (10 μL) and [^{35}S]Met (370 KBq/μL in 50 mM pH 7.4 tricine and 10 mM 2-mercaptoethanol, 3.8 μL) was incubated at 37 °C for 24 h. After the addition of DEPC water (25 μL) at 0 °C, the mixture was applied to a 100 K nanosep device and centrifuged at 1500*g* at 4 °C for 30 min. The flow-through was mixed with prewashed nickel-nitrilotriacetic acid (Ni-NTA)-immobilized agarose (5.0 μL) and was gently shaken at 4 °C for 1 h. The mixture was collected by filtration, and 24% v/v of the aliquot was analyzed on an HPLC system equipped with an ODS column to separate the translation products. Each fraction, collected per minute (1 mL), was mixed with a scintillation cocktail (Liquiscint; 1.0 mL), and the [^{35}S] radioactivity of the mixture was quantified using a [^{35}S] scintillation detector. The radiochromatograms are shown in Figure 3a. For the LC−MS analysis of the translation products, the translation reaction was performed in decuple scale of the above-mentioned procedure without the addition of [^{35}S]Met. After the workup procedure by centrifugal separation and treatment with prewashed Ni-NTA agarose (50 μL), the resulting materials were analyzed by an LC−MS system equipped with an ODS column. The extracted-ion chromatograms are shown in Figure 3c and 3d.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01618.

^1H and ^{13}C NMR spectra of compounds (**5G**, **6U**, **8**-**13**, fMet-Phe, fMet-Leu, fMet-Ile), ^{31}P NMR spectra of compounds (**13**), HRMS spectra and HPLC chart of the oligonucleotide (**19**) and a radiochromatogram of translation products (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: isobe@m.tohoku.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partly supported by KAKENHI (26810083, 24241036). We thank Research and Analytical Center for Giant Molecules (Tohoku Univ.) for mass spectrometry analysis.

■ REFERENCES

(1) (a) Sahin, U.; Karikó, K.; Türeci, Ö. *Nat. Rev. Drug Discovery* **2014**, *13*, 759−780. (b) Quabius, E. S.; Krupp, G. *New Biotechnol.* **2015**, *32*, 229−235. (c) Dorsett, Y.; Tuschl, T. *Nat. Rev. Drug Discovery* **2004**, *3*, 318−329. (d) Deleavey, G. F.; Damha, M. J. *Chem. Biol.* **2012**, *19*, 937−954. (e) Bramsen, J. B.; Kjems, J. *Front. Genet.* **2012**, *3*, 1−22.

(2) (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−584. (b) Isobe, H.; Fujino, T. *Chem. Rec.* **2014**, *14*, 41−51. (c) El-Sagheer, A. H.; Brown, T. *Acc. Chem. Res.* **2012**, *45*, 1258−1267.

(3) (a) Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302−1315. (b) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952−3015. (c) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128−1137.

(4) (a) Isobe, H.; Fujino, T.; Yamazaki, N.; Guillot-Nieckowski, M.; Nakamura, E. *Org. Lett.* **2008**, *10*, 3729−3732. (b) Fujino, T.; Yamazaki, N.; Isobe, H. *Tetrahedron Lett.* **2009**, *50*, 4101−4103.

(5) Fujino, T.; Tsunaka, N.; Guillot-Nieckowski, M.; Nakanishi, W.; Iwamoto, T.; Nakamura, E.; Isobe, H. *Tetrahedron Lett.* **2010**, *51*, 2036−2038.

(6) Fujino, T.; Yasumoto, K.; Yamazaki, N.; Hasome, A.; Sogawa, K.; Isobe, H. *Chem. - Asian J.* **2011**, *6*, 2956−2960.

(7) Fujino, T.; Endo, K.; Yamazaki, N.; Isobe, H. *Chem. Lett.* **2012**, *41*, 403−405.

(8) *Peptide Nucleic Acids: Protocols and Applications*, 2nd ed.; Nielsen, P. E., Ed.; Horizon Bioscience: Norfolk, 2004, and references cited therein.

(9) Fujino, T.; Kogashi, K.; Okada, K.; Mattarella, M.; Suzuki, T.; Yasumoto, K.; Sogawa, K.; Isobe, H. *Chem. - Asian J.* **2015**, *10*, 2683−2688.

(10) Shimizu, Y.; Inoue, A.; Tomari, Y.; Suzuki, T.; Yokogawa, T.; Nishikawa, K.; Ueda, T. *Nat. Biotechnol.* **2001**, *19*, 751−755.

(11) Choquesillo-Lazarte, D.; Brandi-Blanco, M. P.; García-Santos, I.; González-Pérez, J. M.; Castiñeiras, A.; Niclós-Gutiérrez, J. *Coord. Chem. Rev.* **2008**, *252*, 1241−1256.

(12) Addition of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine was also examined for this reaction, but the reaction remained sluggish.

(13) (a) Chen, X.; El-Sagheer, A. H.; Brown, T. *Chem. Commun.* **2014**, *50*, 7597−7600. (b) Hansen, D. J.; Manuguerra, I.; Kjelstrup, M. B.; Gothelf, K. V. *Angew. Chem., Int. Ed.* **2014**, *53*, 14415−14418.

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976

(14) The ratio was obtained after subtracting the ratioactivity value of the blank experiment as the background.
(15) Fujino, T.; Okada, K.; Isobe, H. *Tetrahedron Lett.* **2014**, *55*, 2659−2661.
(16) Fisher, E. F.; Caruthers, M. H. *Nucleic Acids Res.* **1983**, *11*, 1589−1599.
(17) (a) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012−5017. (b) *Fmoc Solid Phase Peptide Synthesis*; Chan, W. C.; White, P. D., Eds.; Oxford University Press: New York, 2000, and references cited therein.
(18) Spectral data of fMet-Leu were not reported in a preceding paper reporting its synthesis. See: Spisani, S.; Turchetti, M.; Varani, K.; Falzarano, S.; Cavicchioni, G. *Eur. J. Pharmacol.* **2003**, *469*, 13−19.

DOI: 10.1021/acs.joc.6b01618
J. Org. Chem. 2016, 81, 8967−8976