JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

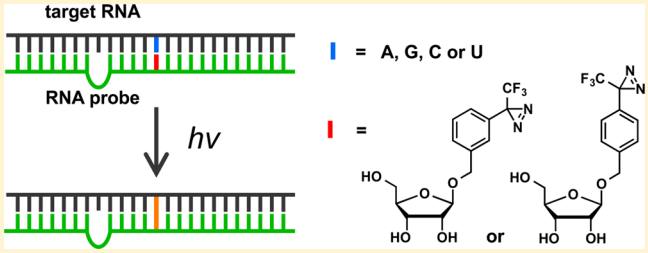
Diazirine-Containing RNA Photo-Cross-Linking Probes for Capturing microRNA Targets

Kosuke Nakamoto[†] and Yoshihito Ueno*[,†,‡]

[†]Course of Applied Life Science, Faculty of Applied Biological Sciences and [‡]United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Supporting Information

ABSTRACT: Here, we report the applicability of diazirine-containing RNA photo-cross-linking probes for the identification of microRNA (miRNA) targets. The RNA cross-linking probes were synthesized by substituting the RNA nucleobases with nucleoside analogues such as 1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose or 1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose that carry aryl trifluoromethyl diazirine moieties. The probes were successfully cross-linked with synthetic RNAs containing the four natural nucleosides on the opposite site of the nucleoside analogues. Furthermore, it was found that miRNAs containing these analogues were effective in regulating the expression of their target genes. Thus, RNAs containing the nucleoside analogues are promising candidates as photo-cross-linking probes to identify the target mRNAs of miRNAs.



INTRODUCTION

Micro ribonucleic acids (miRNAs) are small noncoding RNAs that modulate the function of specific mRNA targets by promoting their degradation and/or inhibiting their translational.[1] miRNAs are initially produced as long primary miRNAs (pri-miRNAs) that consist of several hundred to several thousand bases. pri-miRNAs are then processed in the nucleus into precursor miRNAs (pre-miRNAs) that have a stem-loop structure comprising 60−70 bases. pre-miRNAs are then transported from the nucleus to the cytoplasm where the loop moiety of the pre-miRNA is cleaved and eliminated by the dicer enzyme to create an miRNA duplex composed of 20−23 base pairs. One of the strands from the miRNA duplex (the guide strand) is incorporated into the RNA-induced silencing complex (RISC), leading to the formation of a mature, functional miRNA that regulates the functioning of multiple mRNA targets.[2−4] Currently, in the human genome, more than 1500 mature miRNA transcripts have been identified. Some of these are known to play important roles in crucial biological processes, including development, differentiation, apoptosis, and proliferation.[5−7]

miRNAs usually bind to their targets with incomplete complementarity, which allows the formation of short stretches of mismatched base pairs, resulting in bulges and interior looping. For this reason, an individual miRNA is able to bind to more than one target mRNA and consequently is able to inhibit the expression of multiple target genes. It has been considered that miRNAs potentially control the expression of about one-third of all human mRNAs.[8] Defining the functional mRNA targets for each individual miRNA is crucial in understanding the RNA-based mechanisms that regulate several biological processes.[9−11] Thus far, many miRNA targets have been computationally predicted.[10−15] However, only a limited number of these have been experimentally validated. Thus, the development of methods to accurately and conveniently identify the target mRNAs for all miRNAs is currently one of the most important subjects in this field.

Recently, Rana and co-workers reported a chemical method called miR-TRAP (miRNA target RNA affinity purification) to identify miRNA targets.[16] They incorporated a psoralen (pso) residue into the miRNA, which, upon ultraviolet (UV)-A irradiation, couples with uridine on the target mRNA, and they conjugated a biotin residue at the 3′ end of the miRNA. Thus, the pso-modified miRNA not only can cross-link to its target RNAs but can also be purified by biotin−streptavidin affinity column chromatography after UV-A irradiation. Thus, using this modified miRNA method, they successfully identified the functional mRNA targets for miRNA-135b and miRNA-29a. However, among the four natural nucleosides, the psoralen residue is able to react only with uridine, which limits the ability to capture all of its mRNA targets. Therefore, the development of photo-cross-linking miRNA probes that react efficiently with all four natural nucleosides is necessary for the comprehensive analysis of their mRNA targets.

The UV-A irradiation of aromatic diazirines leads to the generation of carbenes, which possess the ability to react with various chemical groups, including those containing the generally inactive C−H bond.[17,18] We recently reported the synthesis of small interfering RNAs (siRNAs) carrying an aryl

Received: December 10, 2013
Published: February 26, 2014

trifluoromethyldiazirine moiety (**1**) in the 3′-overhang region (Figure 1).[19] By using this photo-cross-linking siRNA probe,

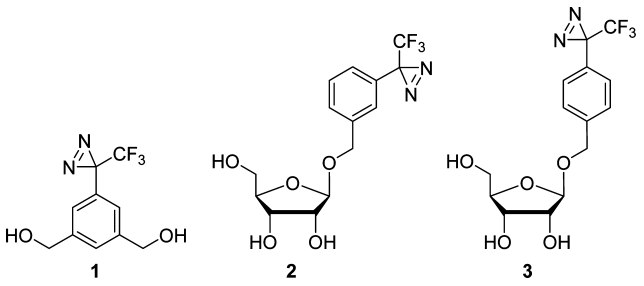


Figure 1. Structures of the diazirine derivatives.

we succeeded in capturing the argonaute (Ago) protein, a key component of RISC, which catalyzes the cleavage of target mRNAs. So far, a number of 2′-deoxyribonucleoside analogues with diazirinyl residues have been synthesized.[20−25] However, to the best of our knowledge, the synthesis of an oligoribonucleotide containing a ribonucleoside analogue with a diazirinyl residue has not been reported. From these experimental results and the literature, we designed photo-cross-linking miRNA probes consisting of nucleoside analogues, 1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-*β*-D-ribofuranose (**2**) or 1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]-benzyl-*β*-D-ribofuranose (**3**), in which the natural bases were substituted with diazirine residues via acetal linkages (Figure 1). We envisioned that the diazirine moieties on the benzyl residues would cross-link more efficiently with opposite bases in RNA duplexes than those on natural bases because those on benzyl residues are in closer proximity to the opposite bases in RNA duplexes than those on the natural bases.

In this article, we report the synthesis of photo-cross-linking miRNA probes containing diazirine-modified nucleoside analogues, **2** or **3**, and their abilities to capture RNA targets. In addition, we examined the gene-silencing activities of these diazirine-modified miRNA duplexes using a dual-luciferase reporter assay.

RESULTS AND DISCUSSION

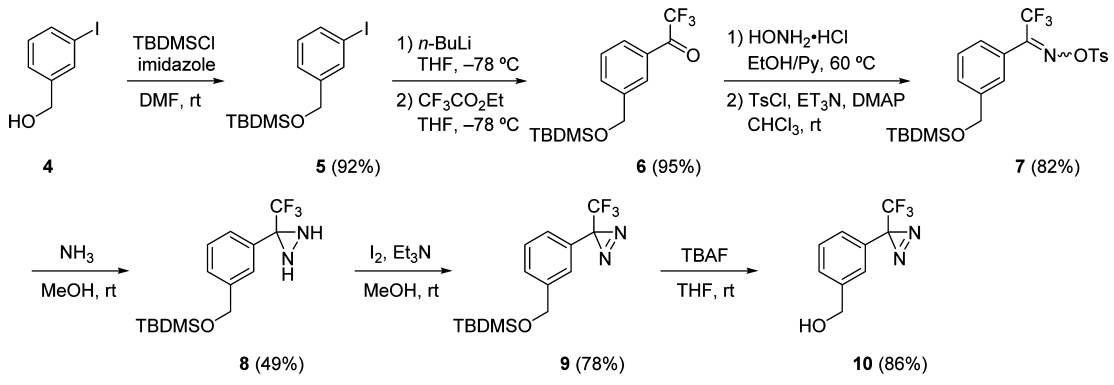
Synthesis. The synthesis of 3-iodo-1-[(*tert*-butyldimethylsilyl)oxymethyl]benzene (**5**) was achieved by reaction between 3-iodobenzyl alcohol (**4**) and *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole and was further trifluoroacetylated by lithium−halogen exchange at −78 °C using *n*-BuLi followed by the addition of ethyl trifluoroacetate (Scheme 1). The resulting trifluoroacetophenone (**6**) was converted to a stereoisomeric (E/Z) mixture of oximes by treatment with $HONH_2$·HCl. The crude mixture of oximes was tosylated to generate a mixture of tosyl-oximes (**7**). Upon addition of ammonia, this mixture was converted to diaziridine (**8**), which was oxidized to the corresponding diazirine by I_2 treatment. The deprotection of the silyl ether using tetra-*n*-butylammonium fluoride (TBAF) afforded the benzylic alcohol (**10**). In a similar manner, 3-[(4-hydroxymethyl)phenyl]-3-trifluoromethyldiazirine (**17**) was synthesized from 4-iodobenzyl alcohol in 79% yield (Scheme 2).

Glycosydation of benzylic alcohol **10** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**18**), which is commercially available, in the presence of TMSOTf at −30 °C in CH_2Cl_2 afforded an 89% yield of *β*-anomer **19** (Scheme 3). Subsequently, it was debenzoylated in the presence of a catalytic amount of $NaOCH_3$ in CH_3OH, and the primary hydroxy group of **20** was protected by a 4,4′-dimethoxytrityl (DMTr) group to produce 5-*O*-DMTr derivative **21**. Compound **21** was treated with TBDMSCl to afford 3-*O*-TBDMS and 2-*O*-TBDMS derivatives **22** and **23** in 30 and 32% yields, respectively. 2-*O*-TBDMS derivative **23** was phosphitylated by a standard procedure to give the corresponding phosphoramidite **24**. In a similar manner, phosphoramidite **30** with a 4-diazirinylbenzyl group was synthesized from 3-[(4-hydroxymethyl)phenyl]-3-trifluoromethyldiazirine (**17**) with a final yield of 23% (Scheme 4).

All of the RNAs were synthesized using a DNA/RNA synthesizer (Tables 1−3). Sequence of RNA **1** corresponds to that of let-7 miRNA. Fully protected RNAs (0.2 *μ*mol each) linked to solid supports were treated with concentrated NH_4OH/EtOH (3:1 v/v) at 55 °C for 4 h and then with Et_3N·3HF in DMSO at 65 °C for 90 min. The RNAs released after the treatment were purified using denaturing 20% polyacrylamide gel electrophoresis (PAGE) to afford deprotected RNAs **1**−**15** carrying the aryl diazirine in 5−12 OD_{260} absorbance units. These RNAs were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights were in agreement with their structures.

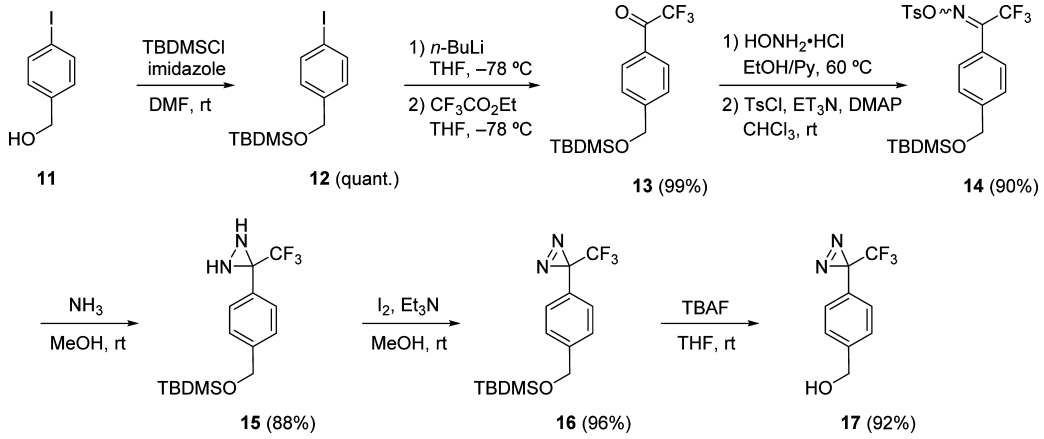
Thermal Stabilities of Duplexes. The thermal stabilities of the RNA duplexes containing analogue **2** or **3** were studied by evaluating their thermal denaturation in 0.01 M sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl (Tables 1 and 2). The UV absorbance of these RNA duplexes was measured at 260 nm. The T_m value of the complementary

Scheme 1

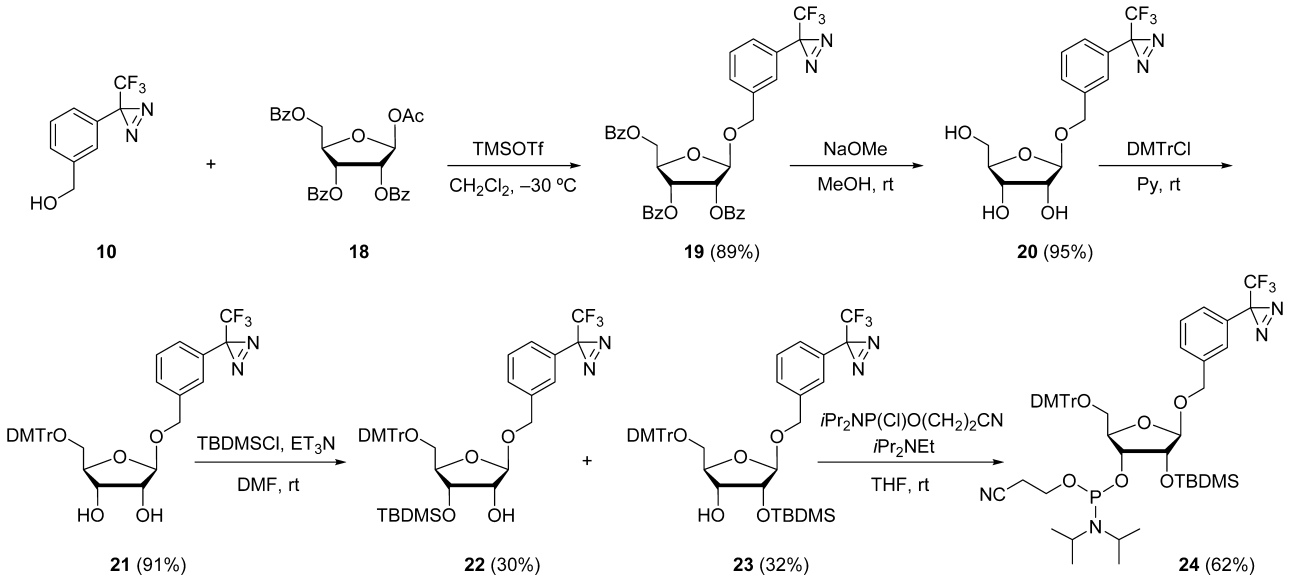


dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

Scheme 2



Scheme 3



duplex (duplex **1**) between RNA **1** and RNA **5** was 70.7 °C, whereas the T_m of the duplexes **2**–**4** containing analogue **2** were from 59.8 to 63.3 °C. The ΔT_m values [T_m (each duplex) − T_m (duplex **1**)] of duplexes **2**–**4** were from −7.4 to −10.9 °C (Table 1). It was found that analogue **2** thermally destabilizes the RNA duplexes. The ΔT_m value (−10.9 °C) of duplex **2** containing a **2**:rC base pair instead of the rG:rC base pair was smaller than those (−7.9 and −7.4 °C) of duplexes **3** and **4** containing a **2**:rA or **2**:rU base pair instead of the rU:rA base pair, respectively. Because analogue **2** does not pair with natural nucleosides, this difference in the ΔT_m values should be attributed to the stabilization effects of the duplexes by the base pairs that were lost as a result of the introduction of analogue **2**. The loss of the rG:rC base pair with three hydrogen bonds would more significantly reduce the thermal stability of the duplex than that of the rA:rU base pair with two hydrogen bonds.

However, the T_m value of duplex **5** containing a single mismatched base pair (duplex **5**) between RNA **1** and RNA **6** was 63.0 °C, whereas the T_m of duplexes **6**–**8** containing analogue **2** were from 56.8 to 60.4 °C. The ΔT_m values of duplexes **6**–**8** were from −2.6 to −6.2 °C. Destabilization of these duplexes by the introduction of a **2**:rG base pair instead of the rG:rG mismatched base pair (duplex **6**, ΔT_m = −2.6 °C) or a **2**:rA base pair next to the rG:rG mismatched base pair (duplex **8**, ΔT_m = −3.8 °C) were found to be smaller than those of other duplexes. miRNAs usually bind to their targets with incomplete complementarity. Thus, the introduction of analogue **2** near mismatched base pairs of miRNAs might not significantly reduce the thermal stabilities of the miRNA duplexes. This might be advantageous in terms of biological activities of miRNA duplexes, and perhaps the introduction of analogue **2** near a mismatched base pair might not significantly reduce the gene-silencing abilities of the miRNA duplexes. A similar tendency was observed with analogue **3**. However, the destabilization of the RNA duplexes by analogue **3** was smaller than that by analogue **2** (Table 2).

Photo-Cross-Linking. Next, we examined the photo-cross-linking abilities of the RNAs containing analogues **2** or **3**. The RNAs containing analogue **2** or **3** were annealed with the target RNAs, which were labeled with fluorescein at their 5′ end. The mixtures were irradiated with 365 nm UV-A for 30 min and then 302 nm UV-B for 10 min at 0 °C, and the cross-linked RNAs were separated by 20% polyacrylamide gel electrophoresis (PAGE) under denaturing conditions (Figure 2a,b).

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

Scheme 4

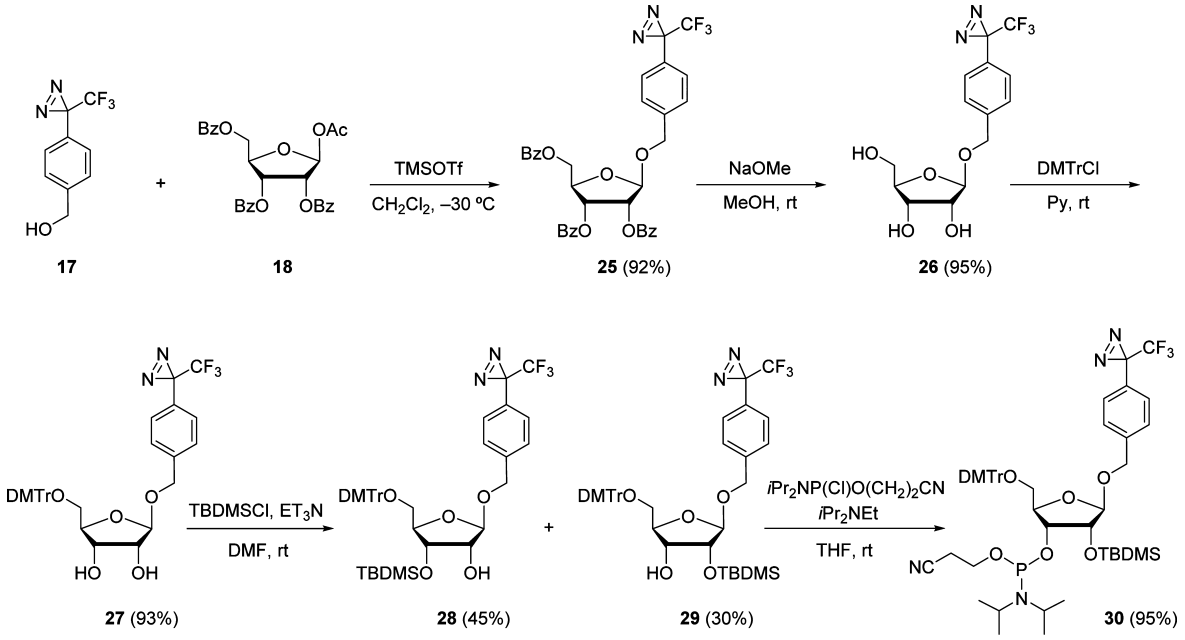


Table 1. Sequences of RNAs, T_m Values, and Photo-Cross-Linking Yields of RNA/RNA Duplexes Containing 2

abbreviation of duplex	abbreviation of RNA	sequence[a]	T_m (°C)	ΔT_m (°C)[b]	cross-linking yield (%)
duplex **1**	RNA **1**	5′-r(UGAGGUAGUAGGUUGUAUAGU)-3′	70.7		
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **2**	RNA **2**	5′-r(UGAGGUA**2**UAGGUUGUAUAGU)-3′	59.8	−10.9	5.9
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **3**	RNA **3**	5′-r(UGAGGUAG**2**AGGUUGUAUAGU)-3′	62.8	−7.9	18.0
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **4**	RNA **4**	5′-r(UGAGGUAGU**2**GGUUGUAUAGU)-3′	63.3	−7.4	13.1
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **5**	RNA **1**	5′-r(UGAGGUAGUAGGUUGUAUAGU)-3′	63.0		
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **6**	RNA **2**	5′-r(UGAGGUA**2**UAGGUUGUAUAGU)-3	60.4	−2.6	6.0
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **7**	RNA **3**	5′-r(UGAGGUAG**2**AGGUUGUAUAGU)-3′	59.2	−3.8	10.6
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **8**	RNA **4**	5′-r(UGAGGUAGU**2**GGUUGUAUAGU)-3′	56.8	−6.2	7.2
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			

[a]Underlined letters indicate mismatched bases. F denotes fluorescein. [b]ΔT_m = T_m (each duplex) − T_m (duplex **1** or duplex **5**).

The results for the RNA photo-cross-linking probes containing analogue **2** are shown in Figure 2a. For all probes containing analogue **2**, bands with slower mobilities than that of single-stranded RNA **5** or **6** were observed on the gels. The band with slower mobility observed in the reaction between RNA **3** and RNA **5** was excised, extracted from the gel, and analyzed by electrospray ionization time-of-flight mass spectrometry (ESI-TOF/MS). The observed molecular weight was in agreement with the cross-linked structure. The net photo-cross-linking yields were 6−18% for the reactions between RNAs **2**−**4** and target RNA **5** and 6−11% for reactions with target RNA **6**. The yields for the duplexes with RNA **5** composed of complementary sequences were slightly higher than those with RNA **6** with mismatched base pairs. The yield for the duplex involving the **2**:rA base pair (18%) was the highest in the tested sequences.

Similar results were observed for the RNA probes **7**−**9** containing analogue **3** (Figure 2b). The band with slower mobility than that of single-stranded RNA **6** observed in the reaction between RNA **6** and RNA **7** was also analyzed by ESI-TOF/MS. The observed molecular weight supported the cross-linked structure. The photo-cross-linking yields were 13−32% for the reactions between RNAs **7**−**9** and target RNA **5** and 9−29% for the reactions with target RNA **6**. The photo-cross-linking yields with RNA probes **7**−**9** containing analogue **3** were higher than those with the corresponding RNA probes containing analogue **2**. The yield for the duplex with the **3**:rC base pair (32%) was the highest in the tested sequences. From these results, it was found that RNA probes **2**−**4** containing analogue **2** or **3** can photo-cross-link to the target RNAs with all four natural nucleosides complementary to that of analogue **2** or **3**.

Dual-Luciferase Assay. Lastly, we investigated the ability of miRNAs modified with analogue **3** to suppress gene expression using a dual-luciferase reporter assay. The psiCHECK-2 vector encoding *Renilla* and firefly luciferase

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

Table 2. Sequences of RNAs, T_m Values, and Photo-Cross-Linking Yields of RNA/RNA Duplexes Containing 3

abbreviation of duplex	abbreviation of RNA	sequence[a]	T_m (°C)	ΔT_m (°C)[b]	cross-linking yield (%)
duplex **1**	RNA **1**	5′-r(UGAGGUAGUAGGUUGUAUAGU)-3′	70.7		
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **9**	RNA **7**	5′-r(UGAGGUA**3**UAGGUUGUAUAGU)-3′	61.0	−9.7	32.1
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **10**	RNA **8**	5′-r(UGAGGUAG**3**AGGUUGUAUAGU)-3′	64.1	−6.6	24.9
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **11**	RNA **9**	5′-r(UGAGGUAGU**3**GGUUGUAUAGU)-3′	64.4	−6.3	13.0
	RNA **5**	3′-r(ACUCCAUCAUCCAACAUAUCA)-5′-F			
duplex **5**	RNA **1**	5′-r(UGAGGUAGUAGGUUGUAUAGU)-3′	63.0		
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **12**	RNA **7**	5′-r(UGAGGUA**3**UAGGUUGUAUAGU)-3	61.3	−1.7	28.8
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **13**	RNA **8**	5′-r(UGAGGUAG**3**AGGUUGUAUAGU)-3′	59.6	−3.4	15.2
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			
duplex **14**	RNA **9**	5′-r(UGAGGUAGU**3**GGUUGUAUAGU)-3′	57.6	−5.4	9.0
	RNA **6**	3′-r(ACUCCAUGAUCCAACAUAUCA)-5′-F			

[a]Underlined letters indicate mismatched bases. F denotes fluorescein. [b]ΔT_m = T_m (each duplex) − T_m (duplex **1** or duplex **5**).

Table 3. Sequences of miRNAs and Their Ability to Suppress Gene Expression

abbreviation of duplex	abbreviation of RNA	sequence[a]	5p vector (%) (upper, 1 nM; lower, 10 nM)[b]	3p vector (%) (upper, 1 nM; lower, 10 nM)[c]
duplex **15**	RNA **10**	miR-199a-5p 5′-r(CCCAGUGUUCAGACUACCUGUUC)-3′	19.1 ± 3.5	10.4 ± 1.5
	RNA **11**	3′-r(AUUGGUUACACGUCUGAUG_ACA)-5′ miR-199a-3p	14.8 ± 2.2	6.5 ± 1.0
duplex **16**	RNA **12**	5′-r(CCCAGUG**3**UCAGACUACCUGUUC)-3′	54.6 ± 5.3	26.0 ± 1.8
	RNA **11**	3′-r(AUUGGUUACACGUCUGAUG_ACA)-5′	23.7 ± 3.0	11.8 ± 1.0
duplex **17**	RNA **13**	5′-r(CCCAGUGU**3**CAGACUACCUGUUC)-3′	69.0 ± 6.3	19.3 ± 2.9
	RNA **11**	3′-r(AUUGGUUACACGUCUGAUG_ACA)-5′	63.1 ± 5.2	11.1 ± 1.4
duplex **18**	RNA **10**	5′-r(CCCAGUGUUCAGACUACCUGUUC)-3′	43.2 ± 4.5	53.9 ± 7.4
	RNA **14**	3′-r(AUUGGUUACACGUC**3**GAUG_ACA)-5′	33.7 ± 4.2	21.7 ± 3.5
duplex **19**	RNA **10**	5′-r(CCCAGUGUUCAGACUACCUGUUC)-3′	92.2 ± 2.7	62.2 ± 2.2
	RNA **15**	3′-r(AUUGGUUACACGU**3**UGAUG_ACA)-5′	45.7 ± 4.2	26.7 ± 3.4

[a]Underlined letters indicate mismatched bases. [b]Luciferase activity (%) of the psi-199a-5p vector. [c]Luciferase activity (%) of the psi-199a-3p vector.

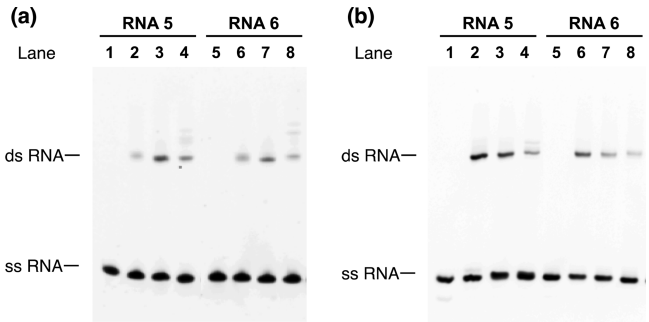


Figure 2. Polyacrylamide gel electrophoresis (PAGE) of the photo-cross-linked products. The mixtures of the target RNAs and the RNA probes were irradiated with 365 nm UV-A for 30 min and then 302 nm UV-B for 10 min at 0 °C, and the products were analyzed by 20% PAGE under denaturing conditions. (a) Photo-cross-linking to target RNAs **5** or **6** by the RNA probes containing analogue **2**. Lanes 1 and 5, target RNA; lanes 2 and 6, target RNA + RNA **2**; lanes 3 and 7, target RNA + RNA **3**; and lanes 4 and 8, target RNA + RNA **4**. (b) Photo-cross-linking to target RNAs **5** or **6** by the RNA probes containing analogue **3**. Lanes 1 and 5, target RNA; lanes 2 and 6, target RNA + RNA **7**; lanes 3 and 7, target RNA + RNA **8**; and lanes 4 and 8, target RNA + RNA **9**.

genes was used as the reporter. miR-199a has been known to exhibit aberrant expression patterns in human cancer and infection.[26−28] Thus, we selected miR-199a as a target candidate for the novel photo-cross-linking probe. To examine the ability of each strand of a miRNA duplex to suppress gene expression, psi-199a-5p and psi-199a-3p vectors were constructed by inserting sequences complementary to the miR-199a-5p and miR-199a-3p strands in the *Luc* 3′-UTR region of the psiCHECK-2 vectors according to a reported method.[29] The miRNA duplexes and the psi-199a-5p or psi-199a-3p vector were cotransfected into HeLa cells with the TransFast transfection reagent. After 24 h, luciferase activities were measured. The signals of *Renilla* luciferase were normalized to those of firefly luciferase (Table 3).

An unmodified miR-199a duplex, duplex **15**, effectively suppressed the expression of *Renilla* luciferase in both the psi-199a-5p and psi-199a-3p vectors in a dose-dependent manner. *Renilla* luciferase activity of the psi-199a-5p vector decreased from 19 to 15% and that of the psi-199a-3p vector decreased from 10 to 7%. The region encompassing the first−seventh positions at the 5′ terminus of each strand of the miRNA duplex is known as the seed region, which is important for binding to target mRNAs. Therefore, we introduced analogue **3** next to the seed region. The gene-silencing activities of the modified miRNAs were dependent on the positions of analogue

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

3 and the kind of vectors used. When duplexes **16** and **17**, modified with analogue **3** at the eighth or ninth position from the 5′ termini of miR-199a-5p strands, were transfected, the *Renilla* luciferase activity of the psi-199a-5p vector (24 and 63% at 10 nM for duplexes **16** and **17**, respectively) was higher than that of the psi-199a-3p vector (12 and 11% at 10 nM for duplexes **16** and **17**, respectively). Thus, it was found that the modifications on the miR-199a-5p strands exhibited a more significant influence on the expression of the *Renilla* luciferase of the psi-199a-5p vector than that of the psi-199a-3p vector. However, duplex **16**, with analogue **3** on the miR-199a-5p strand, still exhibited sufficient gene-silencing activity at 10 nM for the psi-199a-5p vector, although the gene-silencing activity of duplex **17** for the psi-199a-5p vector was markedly decreased.

In contrast, when duplexes **18** and **19**, modified with analogue **3** at the eighth or ninth position from the 5′ termini of miR-199a-3p strands, were transfected, it was intriguing to note that the *Renilla* luciferase activity of the psi-199a-3p vector (22 and 27% at 10 nM for duplexes **18** and **19**, respectively) was lower than that of the psi-199a-5p vector (34 and 46% at 10 nM for duplexes **18** and **19**, respectively). It has been reported that strand selectivity of an miRNA duplex depends on the thermodynamic stability of the duplex.[30,31] Furthermore, it has been reported that loading and unwinding of miRNAs in RISCs are dependent on the positions of the mismatched base pairs and bulge bases in the miRNAs. A mismatched base pair at a central region promotes loading of the miRNA into RISC, whereas the same at a seed region enhances unwinding of the miRNA.[32] Thus, the result may reflect the change in the thermodynamic features of the miRNAs caused by the introduction of analogue **3** into each strand. These results indicate that the modified miRNAs with their guide strands carrying analogue **3** still exhibit sufficient gene-silencing activity, although the gene-silencing activity of the modified miRNAs are slightly lower compared with the unmodified miRNA.

■ CONCLUSIONS

We have successfully synthesized RNA probes substituted with photoactivatable residues, 1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose (**2**) or 1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose (**3**), instead of the natural nucleosides in their strands. It was revealed that the RNAs containing analogue **2** or **3** can photo-cross-link to the target RNAs with four natural nucleosides complementary to analogue **2** or **3**. Furthermore, it turned out that in some cases the miRNAs modified with analogue **3** have sufficient gene-silencing activity even if the guide strands of the miRNAs are modified with analogue **3**. Therefore, the RNA containing analogue **3** is a promising candidate for use as a photo-cross-linking probe to identify the target mRNAs for each miRNA.

■ EXPERIMENTAL SECTION

General Remarks. $CDCl_3$ (CIL) or DMSO-d_6 (CIL) was used as the solvent for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) downfield from $(CH_3)_4Si$ (δ 0.00 for ^1H NMR in $CDCl_3$), CF_3CO_2H (δ 0.00 for ^{19}F NMR), or solvent (for ^{13}C NMR and ^1H NMR in DMSO-d_6) as an internal reference with coupling constants (J) in Hz. The abbreviations s, d, and q signify singlet, doublet, and quartet, respectively.

3-Iodo-1-[(*tert*-butyldimethylsilyl)oxymetyl]benzene (5). A mixture of 3-iodobenzylalchol (**4**) (1.00 g, 4.27 mmol), TBDMSCl (0.708 g, 4.70 mmol), and imidazole (0.639 g, 9.39 mmol) in DMF (10 mL) was stirred at room temperature for 2 h. EtOH (1 mL) was added to the mixture, and the mixture was stirred for 10 min. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 1% EtOAc in hexane) to give **5** (1.38 g, 3.96 mmol, 92%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 9H), 4.68 (s, 2H), 7.06 (t, 1H, J = 7.6), 7.28 (d, 1H, J = 7.8), 7.57 (d, 1H, J = 7.8), 7.67 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.3, 18.4, 25.9, 64.1, 94.2, 126.0, 135.0, 135.9, 143.9. Anal. Calcd for $C_{13}H_{21}IOSi·1/10H_2O$: C, 44.60; H, 6.10. Found: C, 44.42; H, 5.94.

1-[(*tert*-Butyldimethylsilyl)oxymethyl]-3-trifluoroacetylbenzene (6). To a solution of **5** (1.44 g, 4.13 mmol) in THF (30 mL) at −78 °C was added dropwise over 30 min *n*-BuLi (1.64 M in hexane, 5.3 mL, 8.67 mmol). The solution was stirred for 15 min, and then ethyl trifluoroacetate (1.0 mL, 8.67 mmol) was added over 15 min. The resulting mixture was stirred at −78 °C for 1 h, quenched at −78 °C using saturated $NaHCO_3$ (15 mL), and then extracted three times with hexane. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 33% EtOAc in hexane) to give **6** (1.25 g, 3.93 mmol, 95%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.12 (s, 6H), 0.96 (s, 9H), 4.81 (s, 2H), 7.52 (t, 1H, J = 7.8), 7.67 (d, 1H, J = 8.2), 7.96 (d, 1H, J = 7.8), 8.05 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.4, 18.3, 25.8, 64.0, 116.7 (q, $^1J_{C-F}$ = 292.0), 127.4, 128.6, 129.0, 129.9, 133.0, 142.9, 180.6 (q, $^2J_{C-F}$ = 34.7). ^{19}F NMR (376 MHz, $CDCl_3$) δ 5.1. Anal. Calcd for $C_{15}H_{21}F_3O_2Si·3/20H_2O$: C, 56.11; H, 6.69. Found: C, 55.90; H, 6.39.

3-[3-[(*tert*-Butyldimethylsilyl)oxymethyl]phenyl]-3-trifluoromethyldiaziridine (8). To a solution of **6** (1.07 g, 3.36 mmol) in pyridine (10 mL) and EtOH (10 mL) was added $HONH_2·HCl$ (0.35 g, 5.04 mmol). The resulting mixture was stirred at 60 °C overnight, cooled to room temperature, and concentrated. The residual oil was dissolved in $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residual oil was dissolved in CH_2Cl_2 (10 mL), and then Et_3N (1.72 mL, 12.43 mmol), a catalytic amount of DMAP, and *p*-toluenesulfonyl chloride (1.28 g, 6.72 mmol) were added. The final mixture was allowed to react at room temperature overnight. The volatiles were evaporated, and the residue was dissolved in $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 7% EtOAc in hexane) to give oxime **7** (1.34 g, 4.02 mmol, 82%, two stereoisomers). ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.94 (s, 9H), 2.46−2.49 (m, 3H), 4.72−4.76 (m, 2H), 7.35−7.91 (m, 8H).

Oxime **7** (1.13 g, 2.32 mmol) was dissolved in a 7 M methanolic ammonia solution (17.0 mL) in a stainless steel portable reactor at −78 °C. The portable reactor was sealed, and the resulting solution was stirred at room temperature for 2 days. After cooling the mixture, the tube was opened, and the excess NH_3 was allowed to escape slowly. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 5% EtOAc in hexane) to give **8** (0.38 g, 1.14 mmol, 49%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.94 (s, 9H), 2.22 (d, 1H, J = 8.7), 2.79 (d, 1H, J = 8.7), 4.77 (s, 2H), 7.36−7.42 (m, 2H), 7.49 (d, 1H, J = 6.4), 7.59 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.3, 18.4, 25.9, 58.1 (q, $^2J_{C-F}$ = 36.1), 64.4, 123.5 (q, $^1J_{C-F}$ = 277.6), 125.6, 126.6, 127.7, 128.6, 131.6, 142.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ 0.9. Anal. Calcd for $C_{15}H_{23}F_3N_2OSi$: C, 54.19; H, 6.97; N, 8.43. Found: C, 54.06; H. 6.80; N, 8.41.

3-[3-[(*tert*-Butyldimethylsilyl)oxymethyl]phenyl]-3-trifluoromethyl-3*H*-diazirine (9). To a solution of diaziridine **8** (0.42 g, 1.25 mmol) in MeOH (5 mL) were added Et_3N (0.43 mL, 3.13 mmol) and I_2 (0.35 g, 1.38 mmol). The mixture was stirred overnight at room temperature. The mixture was partitioned between EtOAc and aqueous $Na_2S_2O_3$. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 17% EtOAc in hexane) to give **9** (0.60 g, 2.78 mmol, 78%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.95 (s, 9H), 4.73 (s, 2H), 7.04 (s, 1H), 7.20 (s, 1H), 7.35 (d, 2H, J = 4.6). ^{13}C

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

NMR (151 MHz, $CDCl_3$) δ −5.3, 18.3, 25.9, 28.5 (q, $^2J_{C-F}$ = 40.5), 64.3, 122.2 (q, $^1J_{C-F}$ = 274.7), 123.9, 124.9, 127.0, 128.7, 129.1, 142.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.2. Anal. Calcd for $C_{15}H_{21}F_3N_2OSi\cdot 3/10H_2O$: C, 53.65; H, 6.48; N, 8.34. Found: C, 53.52; H, 6.20; N, 8.32.

3-[(3-Hydroxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (10). To a solution of diazirine **9** (1.07 g, 3.24 mmol) in THF (10 mL) was added TBAF (1 M in THF, 3.6 mL), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 17% EtOAc in hexane) to give **10** (0.60g, 2.78 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57−1.77 (m, 1H), 4.70−4.72 (m, 2H), 7.18−7.43 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.4 (q, $^2J_{C-F}$ = 40.5), 64.6, 122.1 (q, $^1J_{C-F}$ = 274.7), 124.7, 125.7, 128.0, 129.1, 129.4, 141.7. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.2. Anal. Calcd for $C_9H_7F_3N_2O\cdot 1/5H_2O$: C, 49.19; H, 3.39; N, 12.75. Found: C, 49.21; H, 3.23; N, 12.78.

4-Iodo-1-[(*tert*-butyldimethylsilyl)oxymetyl]benzene (12). A mixture of 4-iodobenzylalchol (**11**) (2.00 g, 8.58 mmol), TBDMSCl (1.42 g, 9.44 mmol), and imidazole (1.29 g, 18.88 mmol) in DMF (20 mL) was stirred at room temperature for 2 h. EtOH (1 mL) was added to the mixture, and the mixture was stirred for 10 min. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 3% EtOAc in hexane) to give **12** (2.98 g, 8.56 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.93 (s, 9H), 4.68 (s, 2H), 7.07 (d, 2H, J = 8.2), 7.65 (d, 2H, J = 8.2). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.3, 18.4, 25.9, 64.4, 92.0, 128.0, 137.2, 141.2. Anal. Calcd for $C_{13}H_{21}IOSi$: C, 44.83; H, 6.08. Found: C, 44.68; H, 5.99.

1-[(*tert*-Butyldimethylsilyl)oxymethyl]-4-trifluoroacetylbenzene (13). To a solution of **12** (2.98 g, 8.56 mmol) in THF (30 mL) at −78 °C was added dropwise over 30 min *n*-BuLi (1.64 M in hexane, 11.0 mL, 17.98 mmol). The solution was stirred for 15 min, and then ethyl trifluoroacetate (2.2 mL, 17.98 mmol) was added over 15 min. The resulting mixture was stirred at −78 °C for 1 h, quenched at −78 °C using saturated $NaHCO_3$ (15 mL), and then extracted three times with hexane. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 3% EtOAc in hexane) to give **13** (2.69 g, 8.46 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.12 (s, 6H), 0.96 (s, 9H), 4.83 (s, 2H), 7.50 (d, 2H, J = 8.2), 8.05 (d, 2H, J = 8.2). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.4, 18.4, 25.9, 64.3, 116.7 (q, $^1J_{C-F}$ = 292.0), 126.2, 128.6, 130.2, 150.1, 180.2 (q, $^2J_{C-F}$ = 34.7). ^{19}F NMR (376 MHz, $CDCl_3$) δ 5.1. Anal. Calcd for $C_{15}H_{21}F_3O_2Si$: C, 56.58; H, 6.65. Found: C, 56.62; H, 6.55.

3-[4-[(*tert*-Butyldimethylsilyl)oxymethyl]phenyl]-3-trifluoromethyldiaziridine (15). To a solution of **13** (2.18 g, 6.85 mmol) in pyridine (10 mL) and EtOH (10 mL) was added $HONH_2\cdot HCl$ (0.71 g, 10.28 mmol). The resulting mixture was stirred at 60 °C overnight, cooled to room temperature, and concentrated. The residual oil was dissolved in $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residual oil was dissolved in CH_2Cl_2 (10 mL), and then Et_3N (3.5 mL, 25.35 mmol), a catalytic amount of DMAP, and *p*-toluenesulfonyl chloride (2.61 g, 13.70 mmol) were added. The final mixture was allowed to react at room temperature overnight. The volatiles were evaporated, and the residue was dissolved in $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 6% EtOAc in hexane) to give oxime **14** (3.01 g, 6.17 mmol, 90%, two stereoisomers). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 9H), 2.47 (m, 3H), 4.77 (m, 2H), 7.36−7.91 (m, 8H).

Oxime **14** (0.80 g, 1.64 mmol) was dissolved in conc. NH_3(aq) (10 mL) and THF (8 mL) in a stainless steel portable reactor at −78 °C. The portable reactor was sealed, and the resulting solution was stirred at room temperature for 2 days. After cooling the mixture, the tube was opened, and the excess NH_3 was allowed to escape slowly. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 5% EtOAc in hexane) to give **15** (0.48 g, 1.44 mmol 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 9H), 2.20 (d, 1H, J = 8.7), 2.78 (d, 1H, J = 8.2), 4,76 (s, 2H), 7.38 (d, 2H, J = 8.5), 7.58 (d, 2H, J = 8.5). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.3, 18.4, 25.9, 57.9 (q, $^2J_{C-F}$ = 34.7), 123.6 (q, $^2J_{C-F}$ = 279.0), 126.2, 128.0, 130.2, 143.8. ^{19}F NMR (376 MHz, $CDCl_3$) δ 0.7. Anal. Calcd for $C_{15}H_{23}F_3N_2OSi$: C, 54.19; H, 6.97; N, 8.43. Found: C, 54.07; H. 6.77; N, 8.21.

3-[4-[(*tert*-Butyldimethylsilyl)oxymethyl]phenyl]-3-trifluoromethyl-3*H*-diazirine (16). To a solution of diaziridine **15** (1.48 g, 4.46 mmol) in MeOH (15 mL) was added Et_3N (1.5 mL, 11.15 mmol) and I_2 (1.25 g, 4.91 mmol). The mixture was stirred overnight at room temperature. The mixture was partitioned between EtOAc and aqueous $Na_2S_2O_3$. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 2% EtOAc in hexane) to give **16** (1.41 g, 4.27 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.94 (s, 9H), 4.74 (s, 2H), 7.17 (d, 2H, J = 8.2), 7.35 (d, 2H, J = 8.2). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.3, 18.4, 28.4 (q, $^2J_{C-F}$ = 40.5), 64.2, 122.2 (q, $^1J_{C-F}$ = 274.7), 126.2, 126.4, 127.6, 143.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.1. Anal. Calcd for $C_{15}H_{21}F_3N_2OSi\cdot 3/10H_2O$: C, 53.94; H, 6.46; N, 8.39. Found: C, 53.81; H, 6.24; N, 8.43.

3-[(4-Hydroxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (17). To a solution of diazirine **16** (0.78 g, 2.36 mmol) in THF (10 mL) was added TBAF (1 M in THF, 2.6 mL), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 25% EtOAc in hexane) to give **17** (0.47 g, 2.18 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70−1.73 (m, 1H), 4.73 (d, 2H, J = 5.6), 7.20 (d, 2H, J = 7.8), 7.40 (d, 2H, J = 8.2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.3 (q, $^2J_{C-F}$ = 40.5), 64.2, 122.1 (q, $^1J_{C-F}$ = 274.7), 126.7, 127.1, 128.3, 142.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.1. Anal. Calcd for $C_9H_7F_3N_2O\cdot 3/20H_2O$: C, 49.39; H, 3.36; N, 12.80. Found: C, 49.26; H, 3.31; N, 12.66.

2,3,5-Tri-*O*-benzoyl-1-*O*-[3-(3-trifluoromethyl-3*H*-diazirine-3-yl)]benzyl-β-D-ribofuranose (19). To a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**18**) (0.32 g, 0.63 mmol) in CH_2Cl_2 (3 mL) at −30 °C was added dropwise TMSOTf (0.14 mL, 0.76 mmol) followed by a solution of diazirine **10** (0.17 g, 0.79 mmol) in CH_2Cl_2 (2 mL). The resulting mixture was stirred at −30 °C for 2 h, quenched at −30 °C using aqueous $NaHCO_3$ (saturated) (10 mL), then partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 13% EtOAc in hexane) to give **19** (0.37 g, 0.56 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52−4.58 (m, 2H), 4.74−4.80 (m, 3H), 5.31 (s, 1H), 5.77 (d, 1H, J = 4.6), 5.92 (t, 1H, J = 5.7), 7.05−8.02 (m, 16H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 14.2, 21.1, 28.3 (q, $^2J_{C-F}$ = 40.5), 60.4, 64.4, 69.0, 72.1, 75.5, 76.8, 79.3, 104.7, 122.0 (q, $^1J_{C-F}$ = 274.7), 125.4, 126.0, 128.3, 128.4, 128.5, 128.8, 128.9, 129.0, 129.1, 129.3, 129.5, 129.7, 129.7, 129.8, 133.1, 133.4, 133.5, 137.9, 165.2, 165.4, 166.2, 171.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.3. Anal. Calcd for $C_{35}H_{27}F_3N_2O_8$: C, 63.64; H, 4.12; N, 4.24. Found: C, 63.41; H, 3.95; N, 4.24.

1-*O*-[3-(3-Trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose (20). To a solution of **19** (0.37 g, 0.56 mmol) in MeOH (5 mL) was added a catalytic amount of a 28% MeOH solution of sodium methoxide. The mixture was stirred at room temperature for 2 h and quenched using aqueous NH_4Cl (saturated) (1 mL). The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 9% MeOH in $CHCl_3$) to give **20** (0.18 g, 0.52 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 1H), 2.52 (d, 1H, J = 6.4), 2.71 (d, 1H, J = 3.7), 3.81 (s, 2H), 4.01−4.15 (m, 2H), 4.40−4.41 (m, 1H), 4.54 (d, 1H, J = 12.4), 4.75 (d, 1H, J = 12.4), 5.04 (s, 1H), 7.12−7.18 (m, 2H), 7.38−7.39 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.3 (q, $^2J_{C-F}$ = 40.5), 63.1, 67.4, 71.1, 74.6, 84.0, 106.5, 122.1 (q, $^1J_{C-F}$ = 274.7), 125.4, 125.7, 127.8, 129.6, 129.7, 140.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.3. Anal. Calcd for

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

$C_{14}H_{15}F_3N_2O_5$: C, 48.28; H, 4.34; N, 8.04. Found: C, 48.17; H, 4.26; N, 8.01.

1-*O*-[3-(3-Trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4-dimethoxy)trityl-β-D-ribofuranose (21). A mixture of **20** (0.36 g, 1.03 mmol) and DMTrCl (0.42 g, 1.24 mmol) in pyridine (4 mL) was stirred at room temperature. After 3 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 33% EtOAc in hexane) to give **21** (0.61 g, 0.94 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27−2.54 (m, 2H), 3.29−3.35 (m, 2H), 3.77−3.80 (m, 7H), 4.12−4.14 (m, 1H), 4.33 (s, 1H), 4.43 (d, 1H, *J* = 11.9), 4.70 (d, 1H, *J* = 11.9), 5.03 (s, 1H), 6.78−7.46 (m, 17H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 14.2, 21.0, 28.4 (q, $^2J_{C-F}$ = 40.5), 55.2, 60.4, 64.8, 68.7, 72.7, 75.3, 82.2, 86.1, 106.3, 113.1, 122.0 (q, $^1J_{C-F}$ = 274.7), 125.5, 125.8, 126.8, 127.8, 128.1, 128.9, 129.0, 129.2, 130.0, 135.9, 138.5, 144.7, 158.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.3. Anal. Calcd for $C_{35}H_{33}F_3N_2O_7$: C, 64.61; H, 5.11; N, 4.31. Found: C, 64.47; H, 5.10; N, 4.24.

3-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-β-D-ribofuranose (22) and 2-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-β-D-ribofuranose (23). A mixture of **21** (1.30 g, 2.00 mmol), Et_3N (0.92 mL, 6.75 mmol), and TBDMSCl (0.47 g, 3.15 mmol) in DMF (15 mL) was stirred at room temperature. After 12 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 17% EtOAc in hexane) to give **22** (0.46 g, 0.60 mmol, 30%) and **23** (0.48 g, 0.63 mmol, 32%). Physical data for **22**: ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.13 (s, 3H), 0.00 (s, 3H), 0.81 (s, 9H), 2.75 (d, 1H, *J* = 1.8), 3.08 (dd, 2H, *J* = 5.0 and 10.1), 3.38 (dd, 2H, *J* = 2.8 and 10.1), 3.76−3.80 (m, 6H), 3.97−3.98 (m, 1H), 4.12−4.13 (m, 1H), 4.35−4.38 (m, 1H), 4.51 (d, 1H, *J* = 11.9), 4.78 (d, 1H, *J* = 11.9), 5.09 (s, 1H), 6.76−7.50 (m, 17H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −4.9, 17.9, 25.6, 28.4 (q, $^2J_{C-F}$ = 40.5), 55.2, 63.8, 68.8, 72.3, 75.4, 82.9, 85.9, 106.4, 113.0, 122.1 (q, $^1J_{C-F}$ = 274.7), 125.6, 125.8, 126.7, 127.6, 127.7, 128.3, 128.9, 129.2, 129.2, 129.5, 130.0, 136.1, 136.1, 138.7, 144.7, 158.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.2. Anal. Calcd for $C_{41}H_{47}F_3N_2O_7Si$: C, 64.38; H, 6.19; N, 3.66. Found: C, 64.16; H, 6.27; N, 3.57. Physical data for **23**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10−0.12 (m, 6H), 0.90−0.92 (m, 9H), 2.46 (d, 1H, *J* = 7.4), 3.06 (dd, 1H, *J* = 4.6 and 10.1), 3.26 (dd, 1H, *J* = 3.2 and 10.1), 3.76 (s, 6H), 4.11−4.13 (m, 2H), 4.19−4.20 (m, 1H), 4.47 (d, 1H, *J* = 12.4), 4.76 (d, 1H, *J* = 12.4), 4.94 (d, 1H, *J* = 1.8), 6.78−7.47 (m, 17H). ^{13}C NMR (151 MHz, DMSO-d_6) δ −5.0, −4.7, 25.7, 28.4 (q, $^2J_{C-F}$ = 40.5), 55.1, 64.6, 68.8, 72.2, 76.5, 83.8, 85.9, 106.6, 113.0, 122.1 (q, $^1J_{C-F}$ = 274.7), 125.4, 125.8, 126.7, 127.7, 128.2, 128.9, 129.0, 129.2, 130.1, 130.1, 136.1, 136.1, 138.7, 144.9, 158.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.3. Anal. Calcd for $C_{41}H_{47}F_3N_2O_7Si·1/5H_2O$: C, 63.63; H, 6.25; N, 3.62. Found: C, 63.38; H, 6.03; N, 3.53.

2-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[3-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-3-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropyamino)]phosphanyl-β-D-ribofuranose (24). A mixture of **23** (0.50 g, 0.65 mmol), *N*,*N*-diisopropylethylamine (0.55 mL, 3.25 mmol), and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.29 mL, 1.30 mmol) in THF (3.3 mL) was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (a neutralized SiO_2, 50% EtOAc in hexane) to give **24** (0.39 g, 0.40 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06−0.10 (m, 6H), 0.88−0.90 (m, 9H), 0.96−1.13 (m, 12H), 2.32 (t, 1H, *J* = 6.9), 2.56−2.61 (m, 1H), 3.09 (dd, *J* = 3.2 and 10.1), 3.37−3.39 (m, 1H), 3.47−3.67 (m, 4H), 3.74−3.75 (m, 6H), 4.09−4.15 (m, 1H), 4.20−4.22 (m, 1H), 4.24−4.30 (m, 1H), 4.49 (dd, *J* = 7.8 and 12.4), 4.81 (d, *J* = 12.4), 4.93 (d, *J* = 8.7), 6.74−7.51 (m, 17H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.5, 150.0. HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{50}H_{65}F_3N_4O_8PSi$, 965.4261; found, 965.4243.

2,3,5-Tri-*O*-benzoyl-1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose (25). To a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**18**) (0.46 g, 0.91 mmol) in CH_2Cl_2 (5 mL) at −30 °C was added dropwise TMSOTf (0.14 mL, 0.76 mmol) followed by a solution of diazirine **17** (0.18 g, 0.83 mmol) in CH_2Cl_2 (3 mL). The resulting mixture was stirred at −30 °C for 2 h, quenched at −30 °C using aqueous $NaHCO_3$ (saturated) (10 mL), then partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 13% EtOAc in hexane) to give **25** (0.50 g, 0.76 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49−4.59 (m, 2H), 4.76−4.81 (m, 3H), 5.31 (s, 1H), 5.77 (d, 1H, *J* = 4.6), 5.93 (t, 1H, *J* = 6.0), 7.11−8.02 (m, 16H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.3 (q, $^2J_{C-F}$ = 40.5), 64.2, 68.7, 72.0, 75.5, 79.3, 104.6, 122.1 (q, $^1J_{C-F}$ = 274.7), 126.5, 128.0, 128.3, 128.4, 128.5, 128.8, 129.1, 129.5, 129.7, 129.7, 129.8, 133.1, 133.4, 133.5, 138.6, 165.2, 165.4, 166.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.2. Anal. Calcd for $C_{28}H_{22}F_3N_2O_6$: C, 63.64; H, 4.12; N, 4.24. Found: C, 63.66; H, 4.12; N, 4.15.

1-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-β-D-ribofuranose (26). To a solution of **25** (1.22 g, 1.85 mmol) in MeOH (5 mL) was added a catalytic amount of a 28% MeOH solution of sodium methoxide. The mixture was stirred at room temperature for 2 h and quenched using aqueous NH_4Cl (saturated) (1 mL). The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 9% MeOH in $CHCl_3$) to give **26** (0.61 g, 1.75 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 1H), 2.44 (d, 1H, *J* = 6.0), 2.65 (s, 1H), 3.68 (d, 1H, *J* = 11.5), 3.82 (dd, 1H, *J* = 3.2 and 12.0), 4.09−4.12 (m, 2H), 4.41 (d, 1H, *J* = 5.5), 4.55 (d, 1H, *J* = 12.4), 4.66 (d, 1H, *J* = 12.4), 5.04 (s, 1H), 7.19 (d, 2H, *J* = 7.8), 7.35 (d, 2H, *J* = 8.2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.2 (q, $^2J_{C-F}$ = 40.5), 63.9, 68.9, 71.9, 75.2, 83.6, 106.6, 122.0 (q, $^1J_{C-F}$ = 274.7), 126.6, 128.0, 128.8, 138.7. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.7. Anal. Calcd for $C_{14}H_{15}F_3N_2O_5$: C, 48.28; H, 4.34; N, 8.04. Found: C, 48.35; H, 4.33; N, 7.95.

1-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-β-D-ribofuranose (27). A mixture of **26** (1.01 g, 2.90 mmol) and DMTrCl (1.18 g, 3.48 mmol) in pyridine (15 mL) was stirred at room temperature. After 3 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 33% EtOAc in hexane) to give **27** (1.77 g, 2.72 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (d, 1H, *J* = 6.0), 2.52 (d, 1H, *J* = 3.2), 3.27−3.32 (m, 2H), 3.77 (s, 6H), 4.11−4.15 (m, 2H), 4.32−4.36 (m, 1H), 4.42 (d, 1H, *J* = 12.4), 4.70 (d, 1H, *J* = 11.9), 5.03 (s, 1H), 6.77−7.46 (m, 17H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.3 (q, $^2J_{C-F}$ = 40.5), 55.2, 64.7, 68.6, 72.8, 75.4, 82.3, 86.2, 106.4, 113.1, 122.5 (q, $^1J_{C-F}$ = 274.7), 126.5, 126.8, 127.8, 128.1, 128.2, 128.4, 130.0, 135.9, 139.3, 144.7, 158.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.3. Anal. Calcd for $C_{35}H_{33}F_3N_2O_7·1/10H_2O$: C, 64.43; H, 5.13; N, 4.29. Found: C, 64.25; H, 5.14; N, 4.32.

3-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-β-D-ribofuranose (28) and 2-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-β-D-ribofuranose (29). A mixture of **27** (2.01 g, 3.09 mmol), Et_3N (1.3 mL, 9.27 mmol), and TBDMSCl (0.93 g, 6.18 mmol) in DMF (20 mL) was stirred at room temperature. After 12 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 17% EtOAc in hexane) to give **28** (1.07 g, 1.40 mmol, 45%) and **29** (0.71 g, 0.93 mmol, 30%). Physical data for **28**: ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03 (s, 3H), 0.01 (s, 3H), 0.91 (s, 9H), 2.85 (d, 1H, *J* = 2.3), 3.17 (dd, 1H, *J* = 5.0 and 10.1), 3.49 (dd, 1H, *J* = 3.2 and 10.1), 3.86−3.90 (m, 6H), 4.06−4.08 (m, 1H), 4.19−4.23 (m, 1H), 4.48 (q, 1H, *J* = 1.8 and 4.6), 4.60 (d, 1H, *J* = 11.9), 4.89 (d, 1H, *J* = 11.9), 5.19 (s, 1H), 6.86−7.59 (m, 17H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −4.9, 17.9 25.6, 28.1 (q, $^2J_{C-F}$ = 40.5), 55.2, 63.7, 68.8, 72.2,

dx.doi.org/10.1021/jo402738t | J. Org. Chem. 2014, 79, 2463−2472

75.4, 82.3, 85.9, 106.6, 113.0, 122.1 (q, $^2J_{C-F}$ = 274.7), 125.6, 126.5, 127.7, 128.3, 128.4, 128.9, 130.0, 136.1, 139.4, 144.7, 158.4. Anal. Calcd for $C_{41}H_{47}F_3N_2O_7Si$: C, 64.38; H, 6.19; N, 3.66. Found: C, 64.09; H, 6.22; N, 3.50. Physical data for **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09−0.11 (m, 6H), 0.91 (d, 9H, J = 4.6), 2.45 (d, 1H, J = 7.8), 3.15 (dd, 1H, J = 5.0 and 10.1), 3.35 (dd, 1H, J = 3.2 and 10.1), 3.76 (d, 6H, J = 1.4), 4.10−4.20 (m, 3H), 4.46 (d, 1H, J = 12.4), 4.78 (d, 1H, J = 11.9), 4.95 (d, 1H, J = 1.4), 6.76−7.49 (m, 17H). ^{13}C NMR (151 MHz, $CDCl_3$) δ −5.0, −4.7, 18.1, 25.7, 28.3 (q, $^2J_{C-F}$ = 40.5), 55.1, 64.6, 68.8, 72.2, 76.5, 83.8, 85.9, 106.7, 113.0, 122.1 (q, $^1J_{C-F}$ = 274.7), 126.5, 126.7, 127.7, 128.1, 128.2, 128.3, 130.1, 136.0, 136.1, 139.4, 144.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ 11.1. Anal. Calcd for $C_{41}H_{47}F_3N_2O_7Si$: C, 64.38; H, 6.19; N, 3.66. Found: C, 64.39; H, 6.22; N, 3.61.

2-*O*-(*tert*-Butyldimethyl)silyl-1-*O*-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl-5-*O*-(4,4′-dimethoxy)trityl-3-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropyamino)]phosphanyl-β-D-ribofuranose (30). A mixture of **29** (0.75 g, 0.98 mmol), *N*,*N*-diisopropylethylamine (0.85 mL, 4.90 mmol), and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.43 mL, 1.96 mmol) in THF (4.9 mL) was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (neutralized SiO_2, 50% EtOAc in hexane) to give **30** (0.90 g, 0.93 mmol, 95%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.06−0.0.11 (m, 6H), 0.87−1.17 (m, 21H), 2.30 (t, J = 6.9), 2.56−2.60 (m, 1H), 3.06−3.10 (m, 1H), 3.39 (d, 1H, J = 10.3), 3.49−3.63 (m, 4H), 4.14−4.15 (m, 1H), 4.19−4.20 (m, 1H), 4.25−4.32 (m, 1H), 4.50 (dd, 1H, J = 16.6 and 18.5), 4.83 (dd, 1H, J = 7.6 and 12.4), 4.94 (d, 1H, J = 10.3), 6.74−7.50 (m, 17H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1, 149.8. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{50}H_{65}F_3N_4O_8PSi$, 965.4261; found, 965.4264.

RNA Synthesis. Synthesis was carried out with a DNA/RNA synthesizer by the phosphoramidite method. Deprotection of bases and phosphates was performed in concentrated NH_4OH/EtOH (3:1 v/v) at 55 °C for 4 h. 2′-*O*-TBDMS groups were removed by Et_3N·3HF (125 μL) in DMSO (100 μL) at 65 °C for 90 min. The reaction was quenched with 0.1 M TEAA buffer (pH 7.0) and desalted on a Sep-Pak C18 cartridge. Deprotected RNAs were purified by 20% PAGE containing 7 M urea to give highly purified RNA **1** (5), RNA **2** (7), RNA **3** (5), RNA **4** (4), RNA **5** (8), RNA **6** (8), RNA **7** (12), RNA **8** (8), RNA **9** (10), RNA **10** (7), RNA **11** (6), RNA **12** (7), RNA **13** (7), RNA **14** (7), and RNA **15** (8). The yields are indicated in parentheses as OD units at 260 nm starting from 0.2 μmol scale.

MALDI-TOF/MS Analysis of RNAs. Spectra were obtained with a time-of-flight mass spectrometer equipped with a nitrogen laser (337 nm, 3 ns pulse). A solution of 3-hydroxypicolinic acid (3-HPA) and diammonium hydrogen citrate in H_2O was used as the matrix. Data for synthetic RNAs: RNA **1** m/z = 6791.5 ($[M - H]^-$, calcd 6794.1; $C_{202}H_{244}N_{81}O_{148}P_{20}$); RNA **2** m/z = 6854.6 ($[M - H]^-$, calcd 6859.1; $C_{206}H_{246}F_3N_{78}O_{148}P_{20}$); RNA **3** m/z = 6897.7 ($[M - H]^-$, calcd 6898.2; $C_{207}H_{247}F_3N_{81}O_{147}P_{20}$); RNA **4** m/z = 6875.9 ($[M - H]^-$, calcd 6875.1; $C_{206}H_{246}F_3N_{78}O_{149}P_{20}$); RNA **5** m/z = 7079.1 ($[M - H]^-$, calcd 7080.4; $C_{224}H_{271}N_{75}O_{151}P_{21}$); RNA **6** m/z = 7118.0 ($[M - H]^-$, calcd 7120.5; $C_{225}H_{271}N_{77}O_{151}P_{21}$); RNA **7** m/z = 6790.0 ($[M - H]^-$, calcd 6859.1; $C_{206}H_{246}F_3N_{78}O_{148}P_{20}$); RNA **8** m/z = 6897.7 ($[M - H]^-$, calcd 6898.2; $C_{207}H_{247}F_3N_{81}O_{147}P_{20}$); RNA **9** m/z = 6875.9 ($[M - H]^-$, calcd 6875.1; $C_{206}H_{246}F_3N_{78}O_{149}P_{20}$); RNA **10** m/z = 7219.3 ($[M - H]^-$, calcd 7219.3; $C_{215}H_{269}N_{78}O_{162}P_{22}$); RNA **11** m/z = 7000.6 ($[M - H]^-$, calcd 7002.2; $C_{209}H_{257}N_{81}O_{153}P_{21}$); RNA **12** m/z = 7324.5 ($[M - H]^-$, calculated 7323.4; $C_{220}H_{272}F_3N_{78}O_{161}P_{22}$); RNA **13** m/z = 7322.2 ($[M - H]^-$, calcd 7323.4; $C_{220}H_{272}F_3N_{78}O_{161}P_{22}$); RNA **14** m/z = 7106.2 ($[M - H]^-$, calcd 7106.3; $C_{214}H_{260}F_3N_{81}O_{152}P_{21}$); and RNA **15** m/z = 7104.8 ($[M - H]^-$, calcd 7107.3; $C_{214}H_{259}F_3N_{80}O_{153}P_{21}$).

Thermal Denaturation Study. The solution containing the duplex in a buffer comprising 10 mM sodium phosphate (pH 7.0) and 0.1 M NaCl was heated at 95 °C for 3 min, cooled gradually to an appropriate temperature, and then used for the thermal denaturation study. The thermal-induced transition of each mixture was monitored at 260 nm on UV−vis spectrophotometer fitted with a temperature controller in quartz cuvettes with a path length of 1.0 cm and a 3.0 μM duplex concentration in a buffer of 10 mM sodium phosphate (pH 7.0) and 0.1 M NaCl. The sample temperature was increased by 0.5 °C/min.

Photo-Cross-Linking. The RNA probes containing the analogue **2** or **3** were annealed with the target RNAs, which were labeled with fluorescein at their 5′ end, in a buffer of 10 mM Tris-HCl (pH 7.2) and 0.1 M NaCl. The mixtures were irradiated with 365 nm UV-A for 30 min and then 302 nm UV-B for 10 min at 0 °C (∼1 cm under 6 W UV-B or UV-A bulb), and the photo-cross-linked products were analyzed by 20% polyacrylamide gel electrophoresis (PAGE) under denaturing conditions.

ESI-TOF/MS Analysis of Photo-Cross-Linked Products. The band containing the photo-cross-linked product was excised from the gel, crushed, and then soaked at room temperature for 12 h in 10 mM TEAA buffer (pH 7.0). The resulting solution was desalted on a SepPak C18 cartridge to afford the cross-linked RNA. Spectra were obtained with a mass spectrometry system UPLC-MS. Data of the photo-cross-linked products: RNA **3** + RNA **5** m/z = 13 952.5 ($[M - H]^-$, calcd 13 951.6; $C_{431}H_{519}F_3N_{154}O_{298}P_{41}$); RNA **6** + RNA 7 m/z = 13 953.0 ($[M - H]^-$, calcd 13 952.6; $C_{431}H_{518}F_3N_{153}O_{299}P_{41}$).

Dual-Luciferase Assay. HeLa cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 in air in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Twenty-four hours before transfection, HeLa cells (4×10^4/mL) were transferred to 96-well plates (100 μL per well). They were transfected using TransFast according to the manufacturer's instructions for the transfection of adherent cell lines. Cells in each well were transfected with a solution (35 μL) of 20 ng of psiCHECK-2 vector, the indicated amounts of miRNAs, and 0.3 μg of TransFast in Opti-MEM I reduced-serum medium and incubated at 37 °C. Transfection without miRNA was used as a control. After 1 h, MEM (100 μL) containing 10% FBS and antibiotics was added to each well, and the cells were further incubated at 37 °C. After 24 h, cell extracts were prepared in passive lysis buffer. Activity of firefly and *Renilla* luciferases in cell lysates was determined with a dual-luciferase assay system according to the manufacturer's protocol. The results were confirmed by at least three independent transfection experiments with two cultures each and are expressed as the average from four experiments as the mean ± SD.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of compounds **5**−**10**, **12**−**17**, **19**−**24**, and **25**−**30**; ^{13}C NMR spectra of compounds **5**−**10**, **12**−**17**, **19**−**23**, and **25**−**29**; ^{19}F NMR spectra of compounds **6**−**10**, **13**−**17**, **19**−**23**, and **25**−**29**; ^{31}P NMR spectra of compounds **24** and **30**; H−H COSY spectra of compounds **22**, **23**, **28**, and **29**; and ESI-TOF/MS spectra of photo-cross-linked products. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-58-293-2919; Fax: +81-58-293-2919; E-mail: uenoy@gifu-u.ac.jp

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was partially supported by the Ministry of Education, Culture, Sports, Science, and Technology in Japan [Grant-in-Aid for Exploratory Research, 24659045] and the C19 Kiyomi Yoshizaki research grant.

REFERENCES

(1) Winter, J.; Jung, S.; Keller, S.; Gregory, R. I.; Diederichs, S. *Nat. Cell Biol.* **2009**, *11*, 228−234.
(2) Tang, G. *Trends Biochem. Sci.* **2005**, *30*, 106−114.
(3) Pritchard, C. C.; Cheng, H. H.; Tewari, M. *Nat. Rev. Genet.* **2012**, *13*, 358−369.
(4) Meister, G. *Nat. Rev. Genet.* **2013**, *14*, 447−457.
(5) Esquela-Kerscher, A.; Slack, F. J. *Nat. Rev. Cancer* **2006**, *6*, 259−269.
(6) Iorio, M. V.; Croce, C. M. *EMBO Mol. Med.* **2012**, *4*, 143−159.
(7) Baumjohann, D.; Ansel, K. M. *Nat. Rev. Immunol.* **2013**, *13*, 666−678.
(8) Bartel, D. P. *Cell* **2009**, *136*, 215−233.
(9) Ørom, U. A.; Lund, A. H. *Gene* **2010**, *451*, 1−5.
(10) Witkos, T. M.; Koscianska, E.; Krzyzosiak, W. J. *Curr. Mol. Med.* **2011**, *11*, 93−109.
(11) Martinez-Sanchez, A.; Murphy, C. L. *Biology* **2013**, *2*, 189−205.
(12) Friedman, R. C.; Farh, K. K.; Burge, C. B.; Bartel, D. P. *Genome Res.* **2009**, *19*, 92−105.
(13) Betel, D.; Wilson, M.; Gabow, A.; Marks, D. S.; Sander, C. *Nucleic Acids Res.* **2008**, *36*, D149−D153.
(14) Griffiths-Jones, S.; Saini, H. K.; van Dongen, S.; Enright, A. J. *Nucleic Acids Res.* **2008**, *36*, D154−D158.
(15) Lall, S.; Grun, D.; Krek, A.; Chen, K.; Wang, Y. L.; Dewey, C. N.; Sood, P.; Colombo, T.; Bray, N.; Macmenamin, P.; Kao, H. L.; Gunsalus, K. C.; Pachter, L.; Piano, F.; Rajewsky, N. *Curr. Biol.* **2006**, *16*, 460−471.
(16) Baigude, H.; Ahsanullah, L. Z.; Zhou, Y.; Rana, T. M. *Angew. Chem., Int. Ed.* **2012**, *51*, 5880−5883.
(17) Smith, R. A. G.; Knowles, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 5072−5073.
(18) Dubinsky, L.; Krom, B. P.; Meijler, M. M. *Bioorg. Med. Chem.* **2012**, *20*, 554−570.
(19) Kuboe, S.; Yoda, M.; Ogata, A.; Kitade, Y.; Tomari, Y.; Ueno, Y. *Chem. Commun.* **2010**, *46*, 7367−7369.
(20) Yamaguchi, T.; Suyama, K.; Narita, K.; Kohgo, S.; Tomikawa, A.; Saneyoshi, M. *Nucleic Acids Res.* **1997**, *25*, 2352−2358.
(21) Liebmann, M.; Di Pasquale, F.; Marx, A. *ChemBioChem* **2006**, *7*, 1965−1969.
(22) Winnacker, M.; Breeger, S.; Strasser, R.; Carell, T. *ChemBioChem* **2009**, *10*, 109−118.
(23) Shigdel, U. K.; Zhang, J.; He, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 90−93.
(24) Qiu, Z.; Lu, L.; He, C. *J. Am. Chem. Soc.* **2008**, *130*, 14398−14399.
(25) Song, C.-X.; He, C. *Acc. Chem. Res.* **2011**, *44*, 709−717.
(26) Murakami, Y.; Yasuda, T.; Saigo, K.; Urashima, T.; Toyoda, H.; Okanoue, T.; Shimotohno, K. *Oncogene* **2006**, *25*, 2537−2545.
(27) Iorio, M. V.; Visone, R.; Di Leva, G.; Donati, V.; Petrocca, F.; Casalini, P.; Taccioli, C.; Volinia, S.; Liu, C.-G.; Alder, H.; Calin, G. A.; Ménard, S.; Croce, C. M. *Cancer Res.* **2007**, *67*, 8699−8707.
(28) Lee, J.-W.; Choi, C. H.; Choi, J.-J.; Park, Y.-A.; Kim, S.-J.; Hwang, S. Y.; Kim, W. Y.; Kim, T.-J.; Lee, J.-H.; Kim, B.-G.; Bae, D.-S. *Clin. Cancer Res.* **2008**, *14*, 2535−2542.
(29) Ogata, A.; Furukawa, C.; Sakurai, K.; Iba, H.; Kitade, Y.; Ueno, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7299−7302.
(30) Schwarz, D. S.; Hutvagner, G.; Du, T.; Xu, Z.; Aronin, N.; Zamore, P. D. *Cell* **2003**, *115*, 199−208.
(31) Khvorova, A.; Reynolds, A.; Jayasena, S. D. *Cell* **2003**, *115*, 209−216.
(32) Kawamata, T.; Seitz, H.; Tomari, Y. *Nat. Struct. Mol. Biol.* **2009**, *16*, 953−960.