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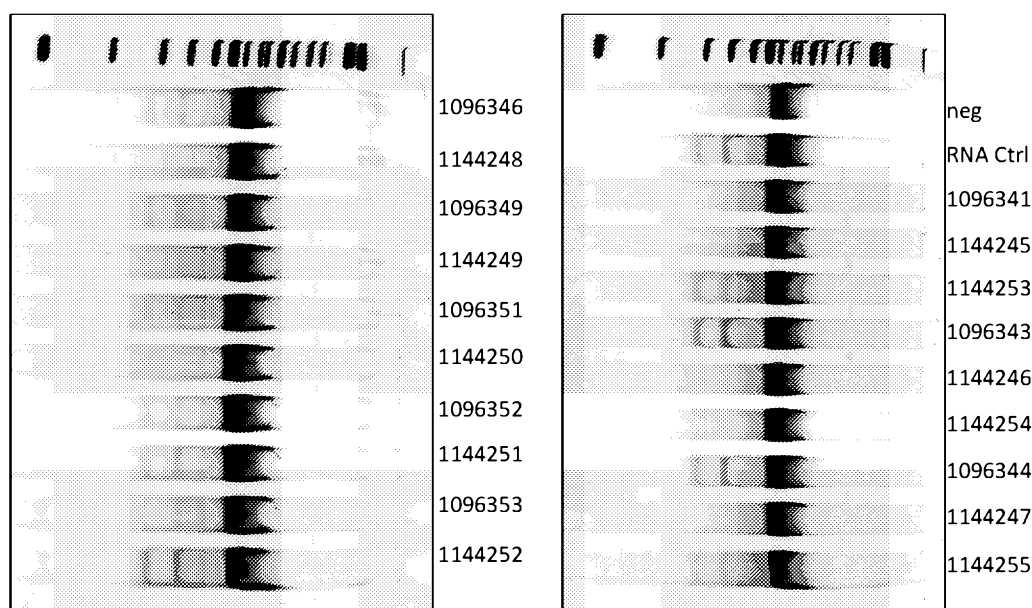
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(54) Title: MODIFIED CRISPR RNA AND USES THEREOF



(57) Abstract: The present disclosure provides compounds comprising modified oligonucleotides for use in CRISPR. In certain embodiments, such modified oligonucleotides provide improved properties of crRNA.

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MODIFIED CRISPR RNA AND USES THEREOF**SEQUENCE LISTING**

5 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled CORE0141WOSEQ_ST25.txt, created on December 15, 2017, which is 948 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND

10 Use of Cluster Regulatory Interspaced Short Palindromic Repeats (CRISPR) to edit or disable genes has been described. See for example Jinek et al., Science 337: 816-821 (2012); Mali et al. Science 339: 823-826 (2013).

SUMMARY

15 Various CRISPR systems have been described. See for example: WO2013/176772; WO2015/006747; Qi et al., Cell 152: 1 173-1 (2013); Gilbert et al., Cell 154: 1-10 (2013) Jinek et al., Science 337: 816-821 (2012); Mali et al. Science 339: 823-826 (2013); Doudna et al., Science 346: 6213 (2014). See also for example: Zetsche et al., Cell 163: 1-13 (2015). The present invention provides modified
20 oligonucleotides for use as crRNA in CRISPR systems. In certain embodiments, such modified crRNA have improved stability relative to unmodified crRNA. In certain embodiments, modified crRNA is stabilized at the 5' end and/or the 3'. In certain embodiments, such stabilized crRNA is resistant to exonuclease and/or endonuclease digestion. In certain embodiments, modified crRNA have improved affinity for target DNA or RNA relative to unmodified crRNA. In certain embodiments, modified crRNA have improved selectivity for
25 target DNA or RNA relative to unmodified crRNA. In certain embodiments, modified crRNA have improved cellular uptake relative to unmodified crRNA. In certain embodiments, modified crRNA increase gene editing activity of a CRISPR system relative to unmodified crRNA.

 In certain embodiments, the crRNA modifications increase affinity for the target DNA or RNA allowing the modified crRNA to be shortened while retaining sufficient affinity to hybridize to target DNA or
30 RNA and/or associate with other CRISPR system components. Thus, in certain embodiments, modified crRNA is shorter than unmodified crRNA. In certain embodiments, modified crRNA is 35-45 linked nucleosides in length. In certain embodiments, modified crRNA is 35-43 linked nucleosides in length. In certain embodiments, modified crRNA is 35-42 linked nucleosides in length. In certain embodiments,

modified crRNA is 36-43 linked nucleosides in length. In certain embodiments, modified crRNA is 36-42 linked nucleosides in length. In certain embodiments, modified crRNA is 36-40 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 15-23 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 15-22 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 16-22 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 17-22 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 18-22 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 16-20 linked nucleosides in length. In certain embodiments, the target recognition portion of modified crRNA is 18-20 linked nucleosides in length. In certain embodiments, the CRISPR recognition portion of modified crRNA is 17-20 linked nucleosides in length. In certain embodiments, the CRISPR recognition portion of modified crRNA is 18-20 linked nucleosides in length. In certain embodiments, such shorter crRNA have improved uptake properties and/or are easier to synthesize than longer crRNA. In certain embodiments, modified crRNA are taken into cells without transfection reagents or electroporation. In certain such embodiments, the cells are in an animal. In certain embodiments, the animal expresses a CRISPR nuclease. In certain embodiments, the animal is previously or concomitantly treated with a means of expressing a CRISPR nuclease. In certain such embodiments, such treatment comprises administration of a vector for delivering a CRISPR nuclease. In certain such embodiments, such vector is a viral vector, for example adeno-associated virus (AAV). In certain such embodiments, the viral vector expresses a bacterial derived CRISPR nuclease that fits into an AAV vector. In certain embodiments, the CRISPR nuclease is a Cpf1 nuclease.

In certain embodiments, the CRISPR system is inhibited after the target gene is edited. In certain such embodiments, the modified crRNA inside a cell is degraded after the target gene has been edited. In certain such embodiments, the CRISPR nuclease continues to be expressed in the cell but is no longer active because it requires crRNA in order to exhibit nuclease activity. In certain such embodiments, off-target effects of the CRISPR system, such as undesired cleavage of an off-target gene, are decreased relative to a CRISPR system in which all of the components necessary for nuclease activity continue to be expressed indefinitely, *e.g.* by a viral vector. In certain such embodiments, degradation of the modified crRNA is facilitated by hybridization to an oligonucleotide complementary to the crRNA. In certain embodiments, degradation of the modified crRNA is facilitated by nucleases present in the cell.

In certain embodiments, the CRISPR system is inhibited after the target gene is edited via degradation of a tracrRNA inside the cell. In certain such embodiments, degradation of the tracrRNA is facilitated by hybridization to an oligonucleotide complementary to the tracrRNA. In certain embodiments, degradation of the tracrRNA is facilitated by nucleases present in the cell.

In certain embodiments, the CRISPR system is inhibited after the target gene is edited via inhibition of the expression of the CRISPR nuclease. In certain such embodiments, the nuclease gene is edited by a

modified crRNA. In certain embodiments, the nuclease transcript is degraded following hybridization of the nuclease transcript to an oligonucleotide complementary to the nuclease transcript.

Brief Description of Figures

Figure 1 shows a gel that illustrates the extent of gene editing of DNMT1 by modified crRNAs described in Example 12.

DETAILED DESCRIPTION

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose.

DEFINITIONS

Unless otherwise indicated, the following terms have the following meanings:

As used herein, “2'-deoxynucleoside” means a nucleoside comprising 2'-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In a crRNA, a 2'-deoxynucleoside is a modified nucleoside.

As used herein, “2'-substituted nucleoside” or “2'-modified nucleoside” means a nucleoside comprising a 2'-substituted or 2'-modified sugar moiety. As used herein, “2'-substituted” or “2'-modified” in reference to a sugar moiety in a crRNA means a furanosyl sugar moiety comprising a 2'-substituent group in place of the 2'-OH of an unmodified sugar moiety.

As used herein, “3'-stabilized” in reference to a modified oligonucleotide means a modified oligonucleotide that comprises at least one stabilizing modification or is connected to a stabilizing conjugate group, wherein the at least one modification and/or the conjugate group increases the stability of the 3'-terminus of the modified oligonucleotide in cells or in an animal relative to a corresponding oligonucleotide that does not comprise the at least one stabilizing modification or is not connected to the stabilizing conjugate group. In certain embodiments, modified crRNAs are 3'-stabilized. In certain such embodiments, the 3'-

terminal nucleoside of the modified crRNA comprises the stabilizing modification. In certain embodiments, the 3'-terminal internucleoside linkage of the crRNA comprises the stabilizing modification.

As used herein, "5'-stabilized" in reference to a modified oligonucleotide means a modified oligonucleotide that comprises at least one stabilizing modification or is connected to a stabilizing conjugate group, wherein the at least one modification and/or the conjugate group increases the stability of the 5'-terminus of the modified oligonucleotide in cells or in an animal relative to a corresponding oligonucleotide that does not comprise the at least one stabilizing modification or is not connected to the stabilizing conjugate group. In certain embodiments, modified crRNAs are 5'-stabilized. In certain such embodiments, the 5'-terminal nucleoside of the modified crRNA comprises the stabilizing modification. In certain such
10 embodiments, the 5'-terminal nucleoside of the modified crRNA is a linker nucleoside.

As used herein, "bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety. As used herein, "bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.
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As used herein, "cell-targeting moiety" means a conjugate group or portion of a conjugate group that is capable of binding to a particular cell type or particular cell types.

As used herein, "complementary" in reference to an oligonucleotide means the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^mC) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. As used herein, "fully complementary" or "100% complementary" in reference to oligonucleotides means that such oligonucleotides are complementary to another oligonucleotide or nucleic acid at each
20 nucleoside. In such embodiments, mismatches are not tolerated.

As used herein, "conjugate group" means a group of atoms that is directly or indirectly attached to a parent compound, *e.g.*, an oligonucleotide.

As used herein, "conjugate linker" means a group of atoms that connects a conjugate group to a parent compound, *e.g.*, an oligonucleotide.

As used herein, "contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other
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As used herein, "crRNA" or "CRISPR RNA" means an oligonucleotide that comprises a target recognition portion and a CRISPR recognition portion. As used herein, a "target recognition portion" is a

portion of an oligonucleotide with a nucleobase sequence that is complementary to a DNA or RNA target. As used herein, "CRISPR recognition portion" is a portion of an oligonucleotide that can bind to, associate with, or contribute to the binding or association with a CRISPR nuclease or a molecule that binds to or associates with a CRISPR nuclease. The CRISPR recognition portion of a crRNA is not complementary to the DNA or RNA target of the target recognition portion of the crRNA. Thus, although the target recognition portion of a crRNA may associate with a CRISPR nuclease, the target recognition and CRISPR recognition portions of a crRNA do not overlap. A CRISPR nuclease is a protein that directly or indirectly associates with a crRNA and cleaves the target DNA or RNA. In certain embodiments, the CRISPR nuclease is a Cpf1 nuclease. In certain such embodiments, the CRISPR recognition portion of a crRNA binds to or associates with a Cpf1 nuclease. In certain embodiments, the CRISPR recognition portion of a crRNA binds to or associates with a tracrRNA. In certain embodiments, crRNAs comprise a self-complementary region. In certain such embodiments, the CRISPR recognition portion partially or completely overlaps with the self-complementary region. In certain embodiments, crRNAs comprise one or more linker nucleosides.

As used herein, "linker nucleosides" in the context of a crRNA means one or more nucleosides that are linked to the target recognition portion and/or the CRISPR recognition portion of a crRNA. Linker nucleosides are not part of either the target recognition portion or the CRISPR recognition portion of the crRNA. In certain embodiments, such linker nucleosides are located at the 5'-terminus of a crRNA, the 3'-terminus of the crRNA, and/or in between the target recognition and CRISPR recognition portions of a crRNA.

As used herein, "fully modified" in reference to an oligonucleotide means a modified oligonucleotide in which each sugar moiety is modified. "Uniformly modified" in reference to an oligonucleotide means a fully modified oligonucleotide in which each at least one modification of each sugar moiety is the same. For example, the nucleosides of a uniformly modified oligonucleotide can each have a 2'-MOE modification but different nucleobase modifications, and the internucleoside linkages may be different.

As used herein, "gene editing" means any process mediated by a complex comprising a CRISPR nuclease and a modified or unmodified crRNA, including but not limited to gene knock-down, gene knock-out, gene disruption, deletion, insertion, and gene activation.

As used herein, "hybridization" means the pairing or annealing of complementary oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

As used herein, "increases", when used in reference to an effect mediated by a modified oligonucleotide, means that the effect is greater in the presence of the oligonucleotide containing a certain modification than the effect is in the presence of a corresponding oligonucleotide that does not contain the certain modification.

As used herein, the terms "internucleoside linkage" means a group that forms a covalent linkage

between adjacent nucleosides in an oligonucleotide. As used herein “modified internucleoside linkage” means any internucleoside linkage other than a naturally occurring, phosphate internucleoside linkage. Naturally occurring, non-phosphate linkages are referred to herein as modified internucleoside linkages. “Phosphorothioate linkage” means a linkage between nucleosides wherein the phosphodiester bond of a phosphate linkage is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

As used herein, “linearly modified sugar” or “linearly modified sugar moiety” means a modified sugar moiety that comprises an acyclic or non-bridging modification. Such linear modifications are distinct from bicyclic sugar modifications.

As used herein, “linked nucleosides” are nucleosides that are connected in a continuous sequence (*i.e.* no additional nucleosides are present between those that are linked) and are linked by internucleoside linkages.

As used herein, “mismatch” or means a nucleobase of a first oligonucleotide that is not capable of pairing with the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligomeric compound are aligned.

As used herein, “MOE” means methoxyethyl. “2'-MOE” means a -OCH₂CH₂OCH₃ group at the 2' position of a furanosyl ring.

As used herein, “motif” means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

As used herein, “naturally occurring” means found in nature.

As used herein, “nucleobase” means a heterocyclic moiety capable of pairing with a second, different nucleobase. As used herein, “nucleobase sequence” means the order of contiguous nucleobases independent of any sugar or internucleoside linkage modification. As used herein, “modified nucleobase” means a nucleobase other than adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G), herein defined as the five, unmodified nucleobases. A universal base is a nucleobase that can pair with any one of the five unmodified nucleobases.

As used herein, “nucleoside” means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. As used herein, “modified nucleoside” means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides include abasic nucleosides.

As used herein, “oligonucleotide” means a strand of linked nucleosides connected via internucleoside linkages, wherein each nucleoside and internucleoside linkage may be modified or unmodified. Unless otherwise indicated, oligonucleotides consist of 8-50 linked nucleosides. As used herein, “modified oligonucleotide” means an oligonucleotide, wherein at least one nucleoside or internucleoside linkage is modified. As used herein, “unmodified oligonucleotide” means an oligonucleotide that does not comprise any nucleoside modifications or internucleoside modifications.

As used herein, “pharmaceutically acceptable carrier or diluent” means any substance suitable for use in administering to an animal. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject.

5 As used herein “pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of compounds, such as oligomeric compounds, *i.e.*, salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

As used herein “pharmaceutical composition” means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an crRNA compound and a sterile aqueous solution. In certain embodiments, a pharmaceutical composition shows activity in free uptake assay in certain cell lines.

As used herein, “phosphorus moiety” means a group of atoms comprising a phosphorus atom. In certain embodiments, a phosphorus moiety comprises a mono-, di-, or tri-phosphate, or phosphorothioate.

As used herein “prodrug” means a therapeutic agent in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or physiologic conditions.

As used herein, “self-complementary” in reference to an oligonucleotide means an oligonucleotide that is at least partially complementary to itself. In certain embodiments, a self-complementary oligonucleotide forms a hairpin when a portion of the self-complementary oligonucleotide hybridizes to itself.

As used herein, “sugar moiety” means a group of atoms that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group. In certain embodiments, a sugar moiety is attached to a nucleobase to form a nucleoside. As used herein, “unmodified sugar moiety” in the context of crRNA means a 2'-OH(H) furanosyl moiety, as found in RNA. Unmodified sugar moieties have one hydrogen at each of the 1', 3', and 4' positions, an oxygen at the 3' position, and two hydrogens at the 5' position. As used herein, “modified sugar moiety” or “modified sugar” means a sugar surrogate or a furanosyl moiety comprising any substitution relative to an unmodified sugar moiety. In certain embodiments, a modified sugar moiety is a 2'-substituted sugar moiety. Such modified sugar moieties include bicyclic sugars and linearly modified sugars.

As used herein, “sugar surrogate” means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide. In certain embodiments, such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or nucleic acids.

As used herein, “target nucleic acid,” “target RNA,” “target DNA,” “target gene” and “nucleic acid target” mean a nucleic acid to which the target recognition portion of a crRNA is complementary. In certain

such embodiments, a crRNA is designed to affect the target nucleic acid. An “off-target gene” is a gene that a crRNA is not designed to affect. In certain embodiments, the editing of an off-target gene is deleterious.

As used herein, "terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

5

CRISPR systems and certain oligonucleotides for use in CRISPR systems

I. Certain CRISPR RNA (crRNA)

In certain embodiments, the present invention provides modified oligonucleotides for use in CRISPR. Typically, CRISPR employs CRISPR RNA (crRNA), which hybridizes to target DNA or RNA and directly or indirectly recruits a nuclease that cleaves the target DNA or RNA. Thus, the crRNA in such systems has two functions: (1) recognition and hybridization to the target DNA or RNA and (2) recognition by a CRISPR nuclease or a molecule that recruits a CRISPR nuclease. Typically, in such systems, the crRNA comprises two portions which correspond to these two functions: a target recognition portion and a CRISPR recognition portion. The present invention provides modified oligonucleotides that may be used as crRNA. Such modified oligonucleotides may have modifications in the target recognition portion and/or CRISPR recognition portion.

In certain embodiments, the CRISPR recognition portion of the crRNA comprises a portion of the direct repeat sequence from a bacterial organism that has a Cpf1 nuclease or a Cpf1 ortholog. In certain such embodiments, the CRISPR recognition portion of the crRNA comprises a sequence selected from the table below. In certain embodiments, the CRISPR recognition portion of the crRNA comprises 16, 17, 18, 19, or 20 nucleobases of a sequence selected from the table below. In certain embodiments, the CRISPR recognition portion of the crRNA consists of 16, 17, 18, 19, or 20 nucleobases of a sequence selected from the table below.

Table A

Direct repeat sequences used in CRISPR recognition portions of crRNA

Organism	Sequence	SEQ ID NO.
Francisella novicida	UAAUUUCUACUGUUGUAGAU	3
Lachnospiraceae bacterium MC2017	AGAAAUGCAUGGUUCUCAUGC	4
Butyrivibrio proteoclasticus	AAAAUUACCUAGUAAUUAGGU	5
Peregrinibacteria bacterium	GGAUUUCUACUUUUGUAGAU	6
Parcubacteria bacterium	AAAUUUCUACUUUUGUAGAU	7
Smithella	GUUUCAAUCCACGCGCCACGCGGGGCGCGAC	8
Acidaminococcus	UAAUUUCUACUCUUGUAGAU	9
Lachnospiraceae bacterium MA2020	GAAUUUCUACU AUUGUAGAU	10
Candidatus Methanoplasma termitum	GAAUCUCUACUCUUUGUAGAU	11
Eubacterium eligens	UAAUUUCUACUUUGUAGAU	12

Moraxella bovoculi	AAAUUUCUACUGUUUGUAGAU	13
Leptospira inadai	GAAUUUCUACUUUUGUAGAU	14
Lachnospiraceae bacterium ND2006	UAAUUUCUACUAAGUGUAGAU	15
Porphyromonas crevioricanis	UAAUUUCUACUAUUGUAGAU	16
Prevotella disiens	UAAUUUCUACUUCGGUAGAU	17
Porphyromonas macacae	UAAUUUCUACUAUUGUAGAU	16

In certain embodiments, the target recognition portion of the crRNA comprises a nucleobase sequence that is complementary to a target DNA. In certain such embodiments, the entire nucleobase sequence of the target recognition portion is complementary to a target DNA. In certain embodiments, the nucleobase sequence of the target recognition portion is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to a target DNA. In certain embodiments, the target DNA is a DNMT1 gene. In certain embodiments, the nucleobase sequence of the target DNMT1 gene is GENBANK Accession Number NT_011295.10 truncated from nucleobases 1506424 to 1569013, herein referred to as SEQ ID NO: 66. In certain embodiments, the target DNA is a GRIN2B gene. In certain embodiments, the nucleobase sequence of the target GRIN2B gene is GENBANK Accession Number NC_000012.12 truncated from nucleobases 13534001 to 13985000, herein referred to as SEQ ID NO: 67. In certain embodiments, the target DNA is a LDLR gene. In certain embodiments, the nucleobase sequence of the target LDLR gene is GENBANK Accession Number NC_000019.10 truncated from nucleobases 11086001 to 11137000, herein referred to as SEQ ID NO: 68. In certain embodiments, the target DNA is a complement component 5 (C5) gene. In certain embodiments, the nucleobase sequence of the target C5 gene is GENBANK Accession Number NC_000009.12 truncated from nucleobases 120949001 to 121078000, herein referred to as SEQ ID NO: 69. In certain embodiments, the target DNA is an empty spiracles homolog1 (EMX1) gene. In certain embodiments, the nucleobase sequence of the target EMX1 gene is GENBANK Accession Number NC_000002.12 truncated from nucleobases 72908001 to 72940000, herein referred to as SEQ ID NO: 70.

In certain embodiments, modified crRNA comprises a target recognition portion, a CRISPR recognition portion, and linker nucleosides. The linker nucleosides are not part of the target recognition or CRISPR recognition portions of the crRNA. In certain embodiments, linker nucleosides are modified in order to provide nuclease stability. In certain such embodiments, the linker nucleosides provide exonuclease stability. In certain embodiments, modified crRNA contain no linker nucleosides. In certain embodiments, modified crRNA comprise 2 linker nucleosides.

In certain embodiments, modified crRNA comprise a CRISPR recognition portion, a target recognition portion, and optional, one or more linker nucleosides. The CRISPR recognition and target recognition portions and the optional linker nucleosides may be in any orientation relative to each other, as shown below, wherein “CR” is the CRISPR recognition portion, “Ta” is the target recognition portion, and

“Ln” is the linker nucleoside(s). In certain embodiments, modified crRNA are represented by one of the following formulas:

5'-CR-Ta-3'

5'-Ln-CR-Ta-3'

5'-CR-Ta-Ln-3'

5'-Ln-CR-Ta-Ln-3'

5'-CR-Ln-Ta-3'

5'-Ln-CR-Ln-Ta-3'

5'-Ln-CR-Ln-Ta-Ln-3'

5'-Ta-CR-3'

5'-Ta-CR-Ln-3'

5'-Ln-Ta-CR-3'

5'-Ln-Ta-CR-Ln-3'

5'-Ta-Ln-CR-Ln-3'

5'-Ta-Ln-CR-3'

5'-Ln-Ta-Ln-CR-Ln-3'

In certain embodiments, a compound comprising a modified crRNA comprises a conjugate group. In certain such embodiments, the conjugate group is connected to the 5'-terminus of the modified crRNA. In certain embodiments, the conjugate group is connected to the 3'-terminus of the modified crRNA. In certain embodiments, the conjugate group is connected to an internal nucleoside or internucleoside linkage of the modified crRNA.

In certain embodiments, the modified crRNA is 5'-stabilized and/or 3'-stabilized. In certain such embodiments, the 5'- or 3'-terminal nucleoside of the 5'- or 3'-stabilized crRNA comprises a stabilizing modification, respectively. In certain embodiments, the nucleoside comprising the stabilizing modification is the terminal nucleoside of the CRISPR recognition portion. In certain embodiments, the nucleoside comprising the stabilizing modification is the terminal nucleoside of the target recognition portion. In certain embodiments, the nucleoside comprising the stabilizing modification is a linker nucleoside. In certain embodiments, the 5'- or 3'-stabilized crRNA is connected to a stabilizing conjugate group at the 5'- or 3'-terminus, respectively. In certain such embodiments, the conjugate group does not comprise a cleavable moiety.

In certain embodiments, modified crRNAs comprise a modified oligonucleotide. In certain embodiments, modified crRNAs consist of a modified oligonucleotide. Modified oligonucleotides described herein are suitable for use as crRNA.

In certain embodiments, modified crRNAs comprise at least three of the following features:

- a. two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;

- b. 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;
- c. at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA
- 5 d. each nucleoside of the CRISPR recognition portion comprising an unmodified sugar moiety
- e. one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties

In certain such embodiments, the modified crRNA comprises features (b), (d), and (e). In certain embodiments, the modified crRNA comprises features (b), (c), and (e). In certain embodiments, the modified crRNA comprises features (b), (c), and (d). In certain embodiments, the modified crRNA comprises features (b), (c), (d) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), and (e). In certain embodiments, the modified crRNA comprises features (a), (c), and (e). In certain embodiments, the modified crRNA comprises features (a), (b), and (d). In certain embodiments, the modified crRNA comprises features (a), (b), (d) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), (c) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), (c), (d), and (e).

In certain embodiments, modified crRNAs comprise at least three of the following features:

- a. two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;
- b. 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;
- c. at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA
- d. at least one nucleoside at position 5, 6, 7, 8, 11, or 12 from the 3'-end of the CRISPR recognition portion comprises a modified sugar moiety
- 25 e. one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties

In certain such embodiments, the modified crRNA comprises features (b), (d), and (e). In certain embodiments, the modified crRNA comprises features (b), (c), and (e). In certain embodiments, the modified crRNA comprises features (b), (c), and (d). In certain embodiments, the modified crRNA comprises features (b), (c), (d) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), and (e). In certain embodiments, the modified crRNA comprises features (a), (c), and (e). In certain embodiments, the modified crRNA comprises features (a), (b), and (d). In certain embodiments, the modified crRNA comprises features (a), (b), (d) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), (c) and (e). In certain embodiments, the modified crRNA comprises features (a), (b), (c), (d), and (e).

In certain embodiments, the modified crRNA comprises any combination of features (a), (b), (c), (d), and (e) listed in the table below, wherein one selection is made for each of (a), (b), (c), (d), and (e).

Table B

Features of certain modified crRNAs

(a) linker nucleosides	(b) modifications of target recognition portion	(c) terminal phosphorothioate (PS) internucleoside linkages	(d) sugar moieties of CRISPR recognition portion	(e) sugar modifications of 3'-terminal nucleosides
Two modified linker nucleosides	2'-H(H) modified nucleosides at positions 1, 8, and 9	Two PS linkages at each terminus	Each nucleoside of CRISPR recognition portion comprises an unmodified sugar moiety	The two 3'-terminal nucleosides comprise modified sugar moieties
Two 2'-H(H) modified linker nucleosides	2'-H(H) modified nucleosides at positions 1 and 8	Two PS linkages at 5' terminus and six PS linkages at 3'-terminus	N/A (at least one nucleoside of CRISPR recognition portion comprises a modified sugar)	The five 3'-terminal nucleosides comprise modified sugar moieties
Two 2'-OMe modified linker nucleosides	2'-H(H) modified nucleosides at positions 1, and 9	N/A (at least one terminus has a non-PS terminal linkage)	bicyclic modified nucleoside at position 11 from the 3'-end of the CRISPR recognition portion	The two 3'-terminal nucleosides comprise 2'-OMe modified sugar moieties
Two cEt modified linker nucleosides	2'-H(H) modified nucleosides at positions 8, and 9		bicyclic modified nucleoside at position 12 from the 3'-end of the CRISPR recognition portion	The two 3'-terminal nucleosides comprise cEt modified sugar moieties
One modified linker nucleoside and one unmodified linker nucleoside	2'-H(H) modified nucleoside at position 1		bicyclic modified nucleosides at positions 11 and 12 from the 3'-end of	The five 3'-terminal nucleosides comprise 2'-OMe

			the CRISPR recognition portion	modified sugar moieties
One 2'-OMe modified linker nucleoside and one unmodified linker nucleoside	N/A (No modified nucleosides at any of positions 1, 8, or 9)			The five 3'-terminal nucleosides comprise 2'-F modified sugar moieties
One cEt modified linker nucleoside and one unmodified linker nucleoside	2'-H(H) modified nucleosides at positions 14, 15, and 16			N/A (The 3'- terminal nucleoside comprises an unmodified sugar moiety.)
N/A (zero or one linker nucleosides)	2'-H(H) modified nucleosides at positions 14, 15, 16, and at least one of 1, 8, and 9			
	2'-F modified nucleosides at positions 1, 8, and/or 9			
	2'-F modified nucleosides at positions 14, 15, and/or 16			

Certain modified oligonucleotides have one or more asymmetric center and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), as α or β such as for sugar anomers, or as (D) or (L) such as for amino acids etc. Included in the modified oligonucleotides provided herein are all such possible isomers, including their racemic and optically pure forms, unless specified otherwise. Likewise, all cis- and trans-isomers and tautomeric forms are also included.

5

In certain embodiments, such modified oligonucleotides may contain any combination of the modified sugar moieties, modified nucleobases, modified internucleoside linkages, motifs, and/or lengths described herein.

Certain methods of use comprising modified crRNA

In certain embodiments, methods comprising contacting a cell with a compound comprising a modified crRNA are *in vitro* methods. In certain embodiments, methods comprising contacting a cell with a compound comprising a modified crRNA are *ex vivo* methods. In certain embodiments, methods comprising contacting a cell with a compound comprising a modified crRNA are *in vivo* methods.

Various CRISPR nuclease variants, both naturally occurring and genetically engineered, can be used in the methods of the present invention. Such variants include but are not limited to inactive nuclease mutants that are used in applications that do not require target nucleic acid cleavage, such as gene activation; and truncated nuclease variants that are suitable for expression in certain vectors, such as AAV vectors. In certain such embodiments, the CRISPR nuclease variant is a Cpf1 nuclease variant.

In certain embodiments, methods comprising contacting a cell with a compound comprising a modified crRNA further comprise contacting the cell with a second compound to inhibit (or turn off) the CRISPR system after the target gene is edited.

In certain embodiments, gene editing methods comprising contacting a cell with a compound comprising a modified crRNA produce fewer and/or less deleterious off-target effects than gene editing methods that use of an unmodified crRNA in place of the modified crRNAs of the invention.

The disclosure includes the following numbered embodiments:

Embodiment 1. A compound comprising a modified crRNA consisting of 35-45 linked nucleosides.

Embodiment 2. A compound comprising a modified crRNA, wherein the CRISPR recognition portion of the modified crRNA consists of 17-20 linked nucleosides.

Embodiment 3. A compound comprising a modified crRNA, wherein the target recognition portion of the modified crRNA consists of 18-23 linked nucleosides.

Embodiment 4. A compound comprising a modified crRNA, wherein the modified crRNA comprises at least one linker nucleoside.

Embodiment 5. A compound comprising a 5'-stabilized modified crRNA.

Embodiment 6. The compound of any of embodiments 1-5, wherein the compound comprises a stabilizing conjugate group.

Embodiment 7. The compound of any of embodiments 1-5, wherein the crRNA comprises at least one linker nucleoside comprising a stabilizing modification.

Embodiment 8. The compound of any of embodiments 1-4, wherein the modified crRNA is 5'-stabilized.

Embodiment 9. The compound of any of embodiments 1-8, wherein the modified crRNA is 3'-stabilized.

Embodiment 10. The compound of any of embodiments 1-9, wherein the CRISPR recognition portion of the modified crRNA binds to a Cpf1 nuclease.

Embodiment 11. The compound of any of embodiments 1-10, wherein the target recognition portion of the modified crRNA comprises at least one modification that increases affinity of the crRNA for a target DNA or RNA.

Embodiment 12. The compound of any of embodiments 10-11, wherein the CRISPR recognition portion of the modified crRNA comprises at least one modification that increases affinity of the crRNA for a Cpf1 nuclease.

Embodiment 13. The compound of any of embodiments 1-12, wherein at least one nucleobase of the modified crRNA is thymine.

Embodiment 14. The compound of any of embodiments 1-13, wherein at least one nucleobase of the modified crRNA is a modified nucleobase.

Embodiment 15. The compound of embodiment 14, wherein the modified nucleobase is 5-methyl cytosine.

Embodiment 16. The compound of any of embodiments 1-15, wherein modified crRNA consists of 35-42 linked nucleosides.

Embodiment 17. The compound of any of claims 1-15, wherein the modified crRNA consists of 36-40 linked nucleosides.

Embodiment 18. The compound of any of embodiments 1-17, wherein the modified crRNA comprises at least two linker nucleosides.

Embodiment 19. The compound of embodiment 18, wherein at least two linker nucleosides are linked to the CRISPR recognition portion of the modified crRNA.

Embodiment 20. The compound of embodiment 19, wherein at least two linker nucleosides are linked to the 5'-end of the CRISPR recognition portion of the modified crRNA.

Embodiment 21. The compound of any of embodiments 1-20, wherein the CRISPR recognition portion of the modified crRNA consists of 18-20 linked nucleosides.

Embodiment 22. The compound of embodiment 21, wherein the CRISPR recognition portion of the modified crRNA consists of 18 linked nucleosides.

Embodiment 23. The compound of embodiment 21, wherein the CRISPR recognition portion of the modified crRNA consists of 19 linked nucleosides.

Embodiment 24. The compound of embodiment 21, wherein the CRISPR recognition portion of the modified crRNA consists of 20 linked nucleosides.

Embodiment 25. The compound of any of embodiments 1-24, wherein the target recognition portion of the modified crRNA consists of 18-22 linked nucleosides.

Embodiment 26. The compound of any of embodiments 1-24, wherein the target recognition portion of the modified crRNA consists of 18-20 linked nucleosides.

Embodiment 27. The compound of embodiment 26, wherein the target recognition portion of the modified crRNA consists of 18 linked nucleosides.

Embodiment 28. The compound of embodiment 26, wherein the target recognition portion of the modified crRNA consists of 19 linked nucleosides.

5 Embodiment 29. The compound of embodiment 26, wherein the target recognition portion of the modified crRNA consists of 20 linked nucleosides.

Embodiment 30. The compound of any of embodiments 1-29, wherein at least one internucleoside linkage of the modified crRNA is a modified internucleoside linkage.

10 Embodiment 31. The compound of embodiment 30, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 32. The compound of embodiment 30 or 31, wherein each internucleoside linkage of the modified crRNA is a modified internucleoside linkage.

Embodiment 33. The compound of any of embodiments 30-32, wherein at least one internucleoside linkage is a neutral internucleoside linkage.

15 Embodiment 34. The compound of embodiment 33, wherein at least one modified internucleoside linkage comprises a methoxypropyl group.

Embodiment 35. The compound of embodiment 33, wherein at least one modified internucleoside linkage comprises a phosphonoacetate.

20 Embodiment 36. The compound of embodiment 33, wherein at least one modified internucleoside linkage comprises a methylphosphonate.

Embodiment 37. The compound of any of embodiments 1-31, wherein each internucleoside linkage of the modified crRNA is a phosphodiester internucleoside linkage or a phosphorothioate internucleoside linkage.

25 Embodiment 38. The compound of any of embodiments 30, 31, or 33-37, wherein at least two internucleoside linkages of the modified crRNA are modified internucleoside linkages.

Embodiment 39. The compound of embodiment 38, wherein at least two modified internucleoside linkages of the modified crRNA are the same as one another.

30 Embodiment 40. The compound of any of embodiments 1-39, wherein the modified crRNA comprises one to five contiguous phosphorothioate internucleoside linkages at the 5'-end of the modified crRNA.

Embodiment 41. The compound of embodiment 40, wherein the modified crRNA comprises one phosphorothioate internucleoside linkage at the 5'-end of the modified crRNA.

Embodiment 42. The compound of embodiment 40, wherein the modified crRNA comprises two contiguous phosphorothioate internucleoside linkages at the 5'-end of the modified crRNA.

Embodiment 43. The compound of any of embodiments 1-42, wherein the modified crRNA comprises at least one linker nucleoside that is linked to the CRISPR recognition portion of the modified crRNA by a modified internucleoside linkage.

Embodiment 44. The compound of embodiment 43, wherein the modified internucleoside linkage that links the at least one linker nucleoside to the CRISPR recognition portion of the modified crRNA is a phosphorothiaote internucleoside linkage.

Embodiment 45. The compound of embodiment 44, wherein the modified crRNA comprises two linker nucleosides.

Embodiment 46. The compound of embodiment 45, wherein the linker nucleosides are linked to each other by a modified internucleoside linkage.

Embodiment 47. The compound of embodiment 46, wherein the modified internucleoside that links the linker nucleosides to each other is a phosphorothioate internucleoside linkage.

Embodiment 48. The compound of any embodiments 43-44, wherein the modified crRNA comprises more than two linker nucleosides.

Embodiment 49. The compound of any of embodiments 1-48, wherein the modified crRNA comprises one to six modified internucleoside linkages within the target recognition portion of the modified crRNA.

Embodiment 50. The compound of embodiment 49, wherein the one to six modified internucleoside linkages within the target recognition portion of the modified crRNA are contiguous.

Embodiment 51. The compound of embodiment 49, wherein the one to six modified internucleoside linkages within the target recognition portion of the modified crRNA alternate with unmodified internucleoside linkages.

Embodiment 52. The compound of any of embodiments 49-51, wherein the 3'-end of the target recognition portion of the modified crRNA contains the one to six modified internucleoside linkages.

Embodiment 53. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises one modified internucleoside linkage.

Embodiment 54. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises two modified internucleoside linkages.

Embodiment 55. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises three modified internucleoside linkages.

Embodiment 56. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises four modified internucleoside linkages.

Embodiment 57. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises five modified internucleoside linkages.

Embodiment 58. The compound of any of embodiments 50-52, wherein the target recognition portion of the modified crRNA comprises six modified internucleoside linkages.

Embodiment 59. The compound of any of embodiments 49-58, wherein at least one internucleoside linkage within the target recognition portion of the modified crRNA is a phosphorothioate internucleoside linkage.

Embodiment 60. The compound of any of embodiments 49-58, wherein all of the modified internucleoside linkages within the target recognition portion of the modified crRNA are phosphorothioate internucleoside linkages.

Embodiment 61. The compound of any of embodiments 1-60, wherein the target recognition portion of the modified crRNA is directly or indirectly linked to the 3' end of the CRISPR recognition portion of the modified crRNA.

Embodiment 62. The compound of any of embodiments 1-61, wherein at least one nucleoside of the modified crRNA comprises a modified sugar moiety.

Embodiment 63. The compound of embodiment 62, wherein the 5'-terminal nucleoside of the crRNA comprises a modified sugar moiety.

Embodiment 64. The compound of embodiment 63, wherein the 5'-terminal nucleoside comprises a linearly modified sugar moiety.

Embodiment 65. The compound of embodiment 64, wherein the 5'-terminal nucleoside comprises a 2'-modified sugar moiety.

Embodiment 66. The compound of embodiment 63, wherein the 5'-terminal nucleoside comprises a bicyclic sugar moiety.

Embodiment 67. The compound of embodiment 63, wherein the 5'-terminal nucleoside comprises a modified sugar moiety selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.

Embodiment 68. The compound of any of embodiments 1-67, wherein the 5'-terminal nucleoside is a linker nucleoside.

Embodiment 69. The compound of any of embodiments 62-68, wherein the 5th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.

Embodiment 70. The compound of any of embodiments 62-69, wherein the 6th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.

Embodiment 71. The compound of any of embodiments 62-70, wherein the 7th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.

Embodiment 72. The compound of any of embodiments 62-71, wherein the 10th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.

Embodiment 73. The compound of any of embodiments 62-72, wherein the 14th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.

Embodiment 74. The compound of any of embodiments 62-73, wherein the 1st nucleoside from the 3'-end of the CRISPR recognition portion comprises a modified sugar moiety.

- Embodiment 75. The compound of any of embodiments 69-74, wherein at least one modified sugar moiety selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
- Embodiment 76. The compound of any of embodiments 69-74, wherein each modified sugar moiety is independently selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
- 5 Embodiment 77. The compound of embodiment 62, wherein the 3'-terminal nucleoside of the modified crRNA comprises a modified sugar moiety.
- Embodiment 78. The compound of embodiment 77, wherein the 3'-terminal nucleoside comprises a linearly modified sugar moiety.
- Embodiment 79. The compound of embodiment 78, wherein the 3'-terminal nucleoside comprises a
10 2'-modified sugar moiety.
- Embodiment 80. The compound of embodiment 77, wherein the 3'-terminal nucleoside comprises a bicyclic sugar moiety.
- Embodiment 81. The compound of embodiment 77, wherein the 3'-terminal nucleoside comprises a modified sugar moiety selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
- 15 Embodiment 82. The compound of any of embodiments 62-81, wherein the 1st nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
- Embodiment 83. The compound of any of embodiments 62-82, wherein the 8th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
- Embodiment 84. The compound of any of embodiments 62-83, wherein the 9th nucleoside from the
20 5'-end of the target recognition portion comprises a modified sugar moiety.
- Embodiment 85. The compound of any of embodiments 62-84, wherein one to five 3'-terminal nucleosides of the target recognition portion of the modified crRNA each comprise a modified sugar moiety.
- Embodiment 86. The compound of embodiment 85, wherein the one to five 3'-terminal nucleosides of
25 the target recognition portion of the modified crRNA each comprise the same modified sugar moiety.
- Embodiment 87. The compound of embodiment 84 or 85, wherein the modified sugar moieties of the one to five 3'-terminal nucleosides of the target recognition portion are each independently selected from among 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
- Embodiment 88. The compound of any of embodiments 1-87, wherein the target recognition portion
30 of the modified crRNA comprises at least one unmodified sugar moiety.
- Embodiment 89. The compound of any of embodiments 1-88, wherein the CRISPR recognition portion of the modified crRNA comprises at least one unmodified sugar moiety.
- Embodiment 90. The compound of any of embodiments 1-89, wherein the modified crRNA comprises at least one linker nucleoside that comprises an unmodified sugar moiety.
- 35 Embodiment 91. The compound of any of embodiments 1-90, wherein the compound consists of the modified crRNA.

Embodiment 92. The compound of any of embodiments 1-91, wherein the nucleobase sequence of the target recognition portion of the modified crRNA is at least 90% complementary to a target DNA or RNA.

Embodiment 93. The compound of embodiment 92, wherein the nucleobase sequence of the target recognition portion of the modified crRNA is 100% complementary to a target DNA or RNA.

Embodiment 94. The compound of any of embodiments 1-93, wherein the modified crRNA comprises a self-complementary region.

Embodiment 95. The compound of embodiment 94, wherein the self-complementary region is within the CRISPR recognition portion of the modified crRNA.

Embodiment 96. The compound of embodiment 94 or 95, wherein the self-complementary region can form a hairpin.

Embodiment 97. The compound of any of embodiments 94-96, wherein the self-complementary region comprises at least one modification that increases the stability of the self-complementary region.

Embodiment 98. The compound of any of embodiments 94-97, wherein the self-complementary region comprises at least one modification that increases the hybridization affinity of the self-complementary region.

Embodiment 99. The compound of any of embodiments 1-98, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises at least 12 contiguous nucleobases of a sequence selected from Table A.

Embodiment 100. The compound of any of embodiments 1-98, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA consists of a sequence or a portion of a sequence selected from Table A.

Embodiment 101. The compound of any of embodiments 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence XCXACX, wherein each X is, independently, a U nucleobase or a T nucleobase.

Embodiment 102. The compound of any of embodiments 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence GXAGAX, wherein each X is, independently, a U nucleobase or a T nucleobase.

Embodiment 103. The compound of any of embodiments 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence XCXACX and the sequence GXAGAX, wherein each X is, independently, a U nucleobase or a T nucleobase.

Embodiment 104. The compound of any of embodiments 1-90 or 92-103, wherein the compound comprises a conjugate group.

Embodiment 105. The compound of embodiment 104, wherein the conjugate group comprises GalNAc.

Embodiment 106. The compound of embodiment 104, wherein the conjugate group is lipophilic.

Embodiment 107. The compound of embodiment 106, wherein the conjugate group comprises a lipid.

Embodiment 108. A pharmaceutical composition comprising the compound of any of embodiments 1-107.

Embodiment 109. A method comprising contacting a cell with the compound or composition of any of
5 embodiments 1-108.

Embodiment 110. The method of embodiment 109, wherein the cell expresses a Cpf1 nuclease.

Embodiment 111. A method comprising contacting a cell with the compound or composition of any of
 embodiments 1-108 and a plasmid that encodes a Cpf1 nuclease.

Embodiment 112. A method comprising contacting a cell with the compound or composition of any of
10 embodiments 1-108 and an mRNA that encodes a Cpf1 nuclease.

Embodiment 113. The method of any of embodiments 109-112, wherein the modified crRNA is taken
 up by the cell in the absence of a transfection reagent.

Embodiment 114. The method of any of embodiments 109-113, wherein the cell is in an animal.

Embodiment 115. A method comprising administering to an animal the compound or composition of
15 any of embodiments 1-108.

Embodiment 116. The method of embodiment 115, wherein the administration is subcutaneous.

Embodiment 117. The method of embodiment 115, wherein the administration is intrathecal.

Embodiment 118. The method of any of embodiments 115-117 comprising administering a plasmid
 that encodes a Cpf1 nuclease.

Embodiment 119. The method of any of embodiments 115-117 wherein the animal expresses a Cpf1
20 nuclease.

Embodiment 120. The method of embodiment 111 or 118, wherein the plasmid is delivered to cells
 within the animal via an adeno-associated virus (AAV).

Embodiment 121. The method of embodiment 111 or 118, wherein the plasmid is delivered to cells
25 within the animal via a lentivirus.

Embodiment 122. The method of any of embodiments 109-121, wherein a target gene is edited.

Embodiment 123. The method of embodiment 122, wherein the modified crRNA is degraded in a cell
 after the target gene is edited in the cell.

Embodiment 124. The method of any of embodiments 110-112 or 118-123, wherein the Cpf1 nuclease
30 does not exhibit nuclease activity in the absence of the modified crRNA.

Embodiment 125. The method of any of embodiments 109-124 comprising contacting the cell with a
 second compound that degrades or inhibits the activity or expression of the modified crRNA or a
 Cpf1 nuclease.

Embodiment 126. The method of embodiment 125, wherein the cell is contacted with the second
35 compound after a target gene has been edited.

Embodiment 127. The method of embodiment 125 or 126, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.

Embodiment 128. The method of embodiment 125 or 126, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.

5 Embodiment 129. The method of embodiment 125 or 126, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.

Embodiment 130. The method of embodiment 128 or 129, wherein the expression of the Cpf1 nuclease is inhibited.

Embodiment 131. The method of any of embodiments 114-130, wherein the animal is a human.

10 Embodiment 132. The method of any of embodiments 109-131, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.

Embodiment 133. The method of any of embodiments 115 or 118-132, wherein the administration is intravitreal.

15 Embodiment 134. The method of any of embodiments 109-113, wherein the cell is a plant cell.

Embodiment 135. The method of any of embodiments 109-114, wherein the cell is a T-cell.

Embodiment 136. A method of treating a disease in an individual comprising administering the compound of any of embodiments 1-107 or the composition of embodiment 108 to the individual, thereby treating the disease in the individual.

20 Embodiment 137. Use of the compound of any of embodiments 1-107 or the composition of embodiment 108 for the treatment of a disease.

Embodiment 138. Use of the compound of any of embodiments 1-107 or the composition of embodiment 108 for preparation of a medicament.

25 Embodiment 139. A method of administering the compound of any of embodiments 1-107 or the composition of embodiment 108 to an animal, and harvesting an organ from the animal for transplantation into a human.

Embodiment 140. The compound of any of embodiments 90-107, wherein the 5'-terminal nucleoside comprises a cEt modified sugar moiety.

30 Embodiment 141. The compound of embodiment 140, wherein the 3'-end of the target recognition portion of the modified crRNA contains two contiguous phosphorothioate internucleoside linkages.

Embodiment 142. The compound of any of embodiments 140-141, wherein each internucleoside linkage of the CRISPR recognition portion of the modified crRNA is phosphorothioate.

35 Embodiment 143. The compound of any of embodiments 140-142, wherein the two 3'-terminal nucleosides of the target recognition portion of the modified crRNA each comprise a 2'-O-methyl modified sugar moiety.

Embodiment 144. The compound of any of embodiments 140-143, wherein the 1st nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 145. The compound of any of embodiments 140-144, wherein the modified crRNA comprises 30-38 unmodified sugar moieties.

5 Embodiment 146. The compound of embodiment 145, wherein the modified crRNA comprises 36 unmodified sugar moieties.

Embodiment 147. A pharmaceutical composition comprising the compound of any of embodiments 140-146.

10 Embodiment 148. A method comprising contacting a cell with the compound or composition of any of embodiments 140-147.

Embodiment 149. The method of embodiment 148, wherein the cell expresses a Cpf1 nuclease.

Embodiment 150. A method comprising contacting a cell with the compound or composition of any of embodiments 140-147 and a plasmid that encodes a Cpf1 nuclease.

15 Embodiment 151. A method comprising contacting a cell with the compound or composition of any of embodiments 140-147 and an mRNA that encodes a Cpf1 nuclease.

Embodiment 152. The method of any of embodiments 148-151, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.

Embodiment 153. The method of any of embodiments 148-152, wherein the cell is in an animal.

20 Embodiment 154. A method comprising administering to an animal the compound or composition of any of embodiments 140-147.

Embodiment 155. The method of embodiment 154, wherein the administration is subcutaneous.

Embodiment 156. The method of embodiment 154, wherein the administration is intrathecal.

Embodiment 157. The method of any of embodiments 154-156 comprising administering a plasmid that encodes a Cpf1 nuclease.

25 Embodiment 158. The method of any of embodiments 154-156 wherein the animal expresses a Cpf1 nuclease.

Embodiment 159. The method of embodiment 150 or 157, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).

30 Embodiment 160. The method of embodiment 150 or 157, wherein the plasmid is delivered to cells within the animal via a lentivirus.

Embodiment 161. The method of any of embodiments 148-160, wherein a target gene is edited.

Embodiment 162. The method of embodiment 161, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.

35 Embodiment 163. The method of any of embodiments 149-151 or 157-162, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.

Embodiment 164. The method of any of embodiments 148-163 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.

Embodiment 165. The method of embodiment 164, wherein the cell is contacted with the second compound after a target gene has been edited.

Embodiment 166. The method of embodiment 164 or 165, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.

Embodiment 167. The method of embodiment 164 or 165, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.

Embodiment 168. The method of embodiment 164 or 165, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.

Embodiment 169. The method of embodiment 167 or 168, wherein the expression of the Cpf1 nuclease is inhibited.

Embodiment 170. The method of any of embodiments 153-169, wherein the animal is a human.

Embodiment 171. The method of any of embodiments 148-170, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.

Embodiment 172. The method of any of embodiments 154 or 157-171, wherein the administration is intravitreal.

Embodiment 173. The method of any of embodiments 148-152, wherein the cell is a plant cell.

Embodiment 174. The method of any of embodiments 148-153, wherein the cell is a T-cell.

Embodiment 175. A method of treating a disease in an individual comprising administering the compound of any of embodiments 140-146 or the composition of embodiment 147 to the individual.

Embodiment 176. A method of treating a disease in an individual comprising administering the compound of any of embodiments 140-146 or the composition of embodiment 147 to the individual, thereby treating the disease in the individual.

Embodiment 177. Use of the compound of any of embodiments 140-146 or the composition of embodiment 147 for the treatment of a disease.

Embodiment 178. Use of the compound of any of embodiments 140-146 or the composition of embodiment 147 for preparation of a medicament.

Embodiment 179. A method of administering the compound of any of embodiments 140-146 or the composition of embodiment 147 to an animal, and harvesting an organ from the animal for transplantation into a human.

Embodiment 180. The compound of any of embodiments 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA is a 2'-deoxynucleoside.

Embodiment 181. The compound of any of embodiments 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA comprises a linearly modified sugar moiety having a 2'-H substitution.

Embodiment 182. The compound of any of embodiments 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA comprises a modified 2'-H(H) sugar moiety as found in naturally occurring DNA.

Embodiment 183. The compound of any of embodiments 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 40 linked nucleosides.

Embodiment 184. The compound of any of embodiments 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 43 linked nucleosides.

Embodiment 185. The compound of any of embodiments 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 45 linked nucleosides.

Embodiment 186. The compound of any of embodiments 91-107, 140-146, or 180-185, wherein the target recognition portion of the modified crRNA is at least 90% complementary to a DNMT1 nucleic acid.

Embodiment 187. The compound of embodiment 186, wherein the target recognition portion is 100% complementary to a DNMT1 nucleic acid.

Embodiment 188. The compound of embodiment 186 or 187, wherein the DNMT1 nucleic acid is a deoxyribonucleic acid.

Embodiment 189. The compound of embodiment 188, wherein the DNMT1 nucleic acid is a human deoxyribonucleic acid.

Embodiment 190. The compound of any of embodiments 91-107, 140-146, or 180-185, wherein the target recognition portion of the modified crRNA is at least 90% complementary to a LDLR nucleic acid.

Embodiment 191. The compound of embodiment 190, wherein the target recognition portion is 100% complementary to a LDLR nucleic acid. The compound of embodiment 190 or 191, wherein the LDLR nucleic acid is a deoxyribonucleic acid.

Embodiment 192. The compound of embodiment 191, wherein the LDLR nucleic acid is a human deoxyribonucleic acid.

Embodiment 193. The compound of any of embodiments 91-107, 140-146, or 180-192, wherein the two 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.

Embodiment 194. The compound of any of embodiments 91-107, 140-146, or 180-192, wherein the three 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.

Embodiment 195. The compound of any of embodiments 91-107, 140-146, or 180-192, wherein the four 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.

Embodiment 196. The compound of any of embodiments 91-107, 140-146, or 180-192, wherein the five 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.

Embodiment 197. The compound of any of embodiments 77 or 193-196, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H), 2'-O-methyl, 2'-F, cEt, and LNA modified sugar moieties.

Embodiment 198. The compound of embodiment 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H), 2'-O-methyl, and cEt modified sugar moieties.

Embodiment 199. The compound of embodiment 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H) and 2'-O-methyl modified sugar moieties.

Embodiment 200. The compound of embodiment 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among cEt and LNA modified sugar moieties.

Embodiment 201. The compound of any of embodiments 82-107, 140-146, or 180-200, wherein the 1st nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.

Embodiment 202. The compound of any of embodiments 82-107, 140-146, or 180-201, wherein the 8th nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.

Embodiment 203. The compound of any of embodiments 82-107, 140-146, or 180-202, wherein the 9th nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.

Embodiment 204. The compound of any of embodiments 91-107, 140-146, or 180-203, wherein the modified crRNA comprises at least three of the following features:

- a. two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;
- b. 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;
- c. at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA
- d. each nucleoside of the CRISPR recognition portion comprising an unmodified sugar moiety

- e. one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties

Embodiment 205. The compound of any of embodiments 1-107, 140-146, or 180-204, wherein the modified crRNA is a salt.

Embodiment 206. A pharmaceutical composition comprising the compound of any of embodiments 180-205.

Embodiment 207. The pharmaceutical composition of any of embodiments 108, 147, or 206, wherein the pharmaceutical composition comprises a ribonucleoprotein complex.

Embodiment 208. The pharmaceutical composition of embodiment 207, wherein the ribonucleoprotein complex comprises a Cpf1 nuclease and the compound comprising the modified crRNA.

Embodiment 209. A method comprising contacting a cell with the compound or composition of any of embodiments 180-208.

Embodiment 210. A method comprising contacting a cell with the compound or composition of any of embodiments 180-207, wherein the cell expresses a Cpf1 nuclease.

Embodiment 211. A method comprising contacting a cell with the compound or composition of any of embodiments 180-207 and a plasmid that encodes a Cpf1 nuclease.

Embodiment 212. A method comprising contacting a cell with the compound or composition of any of embodiments 180-207 and an mRNA that encodes a Cpf1 nuclease.

Embodiment 213. The method of any of embodiments 209-212, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.

Embodiment 214. The method of any of embodiments 209-213, wherein the cell is in an animal.

Embodiment 215. A method comprising administering to an animal the compound or composition of any of embodiments 180-208.

Embodiment 216. The method of embodiment 215, wherein the administration is subcutaneous.

Embodiment 217. The method of embodiment 215, wherein the administration is intrathecal.

Embodiment 218. The method of any of embodiments 215-217 comprising administering a plasmid that encodes a Cpf1 nuclease.

Embodiment 219. The method of any of embodiments 215-217 wherein the animal expresses a Cpf1 nuclease.

Embodiment 220. The method of embodiment 211 or 218, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).

Embodiment 221. The method of embodiment 211 or 218, wherein the plasmid is delivered to cells within the animal via a lentivirus.

Embodiment 222. The method of any of embodiments 209-221, wherein a target gene is edited.

Embodiment 223. The method of embodiment 222, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.

Embodiment 224. The method of any of embodiments 210-212 or 215-223, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.

Embodiment 225. The method of any of embodiments 209-224 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.

Embodiment 226. The method of embodiment 225, wherein the cell is contacted with the second compound after a target gene has been edited.

Embodiment 227. The method of embodiment 225 or 226, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.

Embodiment 228. The method of embodiment 225 or 226, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.

Embodiment 229. The method of embodiment 225 or 226, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.

Embodiment 230. The method of embodiment 228 or 229, wherein the expression of the Cpf1 nuclease is inhibited.

Embodiment 231. The method of any of embodiments 214-230, wherein the animal is a human.

Embodiment 232. The method of any of embodiments 209-231, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.

Embodiment 233. The method of any of embodiments 215 or 218-232, wherein the administration is intravitreal.

Embodiment 234. The method of any of embodiments 209-213, wherein the cell is a plant cell.

Embodiment 235. The method of any of embodiments 209-214, wherein the cell is a T-cell.

Embodiment 236. A method of treating a disease in an individual comprising administering the compound of any of embodiments 180-205 or the composition of any of embodiments 206-208 to the individual.

Embodiment 237. A method of treating a disease in an individual comprising administering the compound of any of embodiments 180-205 or the composition any of embodiments 206-208 to the individual, thereby treating the disease in the individual.

Embodiment 238. Use of the compound of any of embodiments 180-205 or the composition of any of embodiments 206-208 for the treatment of a disease.

Embodiment 239. Use of the compound of any of embodiments 180-205 or the composition of any of embodiments 206-208 for preparation of a medicament.

Embodiment 240. A method of administering the compound of any of embodiments 180-205 or the composition of any of embodiments 206-208 to an animal, and harvesting an organ from the animal for transplantation into a human.

- Embodiment 241. The compound of any of embodiments 1-107, 140-146, or 180-205, wherein the CRISPR recognition portion of the modified crRNA comprises at least one modified sugar moiety.
- Embodiment 242. The compound of embodiment 241, wherein the at least one modified sugar moiety of the CRISPR recognition portion is a linearly modified sugar moiety.
- 5 Embodiment 243. The compound of embodiment 241, wherein the at least one modified sugar moiety of the CRISPR recognition portion is a bicyclic sugar moiety.
- Embodiment 244. The compound of embodiment 243, wherein the bicyclic sugar moiety is cEt or LNA.
- Embodiment 245. The compound of embodiment 243, wherein the bicyclic sugar moiety is cEt.
- 10 Embodiment 246. The compound of any of claims 241-245, wherein the 2nd nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 247. The compound of any of claims 241-245, wherein the 3rd nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 248. The compound of any of claims 241-245, wherein the 4th nucleoside from the 3'-end
15 of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 249. The compound of any of claims 241-245, wherein the 5th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 250. The compound of any of claims 241-245, wherein the 6th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- 20 Embodiment 251. The compound of any of claims 241-245, wherein the 7th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 252. The compound of any of claims 241-245, wherein the 8th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 253. The compound of any of claims 241-245, wherein the 9th nucleoside from the 3'-end
25 of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 254. The compound of any of claims 241-245, wherein the 11th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 255. The compound of any of claims 241-245, wherein the 12th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- 30 Embodiment 256. The compound of any of claims 241-245, wherein the 13th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 257. The compound of any of claims 241-245, wherein the 18th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
- Embodiment 258. The compound of any of claims 241-245, wherein the 11th and 12th nucleosides from
35 the 3'-end of the CRISPR recognition portion each comprise a modified sugar moiety.

Embodiment 259. The compound of any of claims 241-258, wherein the 1st nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 260. The compound of any of claims 241-259, wherein the 10th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

5 Embodiment 261. The compound of any of claims 241-260, wherein the 14th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 262. The compound of any of claims 241-261, wherein the 15th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

10 Embodiment 263. The compound of any of claims 241-262, wherein the 16th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 264. The compound of any of claims 241-263, wherein the 17th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 265. The compound of any of claims 241-264, wherein the 1st nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

15 Embodiment 266. The compound of any of claims 241-265, wherein the 2nd nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

Embodiment 267. The compound of any of claims 241-266, wherein the 3rd nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.

20 Embodiment 268. The compound of any of claims 1-107, 140-146, 180-205, or 241-267, wherein the 14th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.

Embodiment 269. The compound of any of claims 1-107, 140-146, 180-205, or 241-268, wherein the 15th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.

Embodiment 270. The compound of any of claims 1-107, 140-146, 180-205, or 241-269, wherein the 16th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.

25 Embodiment 271. The compound of any of claims 268-270, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is a linearly modified sugar moiety.

Embodiment 272. The compound of claim 271, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is independently selected from 2'-H(H) and 2'-F modified sugar moieties.

30 Embodiment 273. The compound of claim 272, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is a 2'-H(H) modified sugar moiety.

Embodiment 274. The compound of any of claims 1-107, 140-146, 180-205, or 241-273, wherein the modified crRNA comprises at least three of the following features:

(a) two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;

(b) 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;

5 (c) at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA

(d) at least one nucleoside at position 5, 6, 7, 8, 11, or 12 from the 3'-end of the CRISPR recognition portion comprises a modified sugar moiety

10 (e) one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties

Embodiment 275. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), and (e).

Embodiment 276. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), and (d).

15 Embodiment 277. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), and (c).

Embodiment 278. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), (c), and (e).

20 Embodiment 279. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), (d), and (e).

Embodiment 280. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), (c), (d), and (e).

Embodiment 281. A pharmaceutical composition comprising the compound of any of claims 241-280.

25 Embodiment 282. The pharmaceutical composition of claim 281, wherein the pharmaceutical composition comprises a ribonucleoprotein complex.

Embodiment 283. The pharmaceutical composition of claim 282, wherein the ribonucleoprotein complex comprises a Cpf1 nuclease and the compound comprising the modified crRNA.

Embodiment 284. A method comprising contacting a cell with the compound or composition of any of claims 241-283.

30 Embodiment 285. A method comprising contacting a cell with the compound or composition of any of claims 241-283, wherein the cell expresses a Cpf1 nuclease.

Embodiment 286. A method comprising contacting a cell with the compound or composition of any of claims 241-283 and a plasmid that encodes a Cpf1 nuclease.

Embodiment 287. A method comprising contacting a cell with the compound or composition of any of claims 241-283 and an mRNA that encodes a Cpf1 nuclease.

Embodiment 288. The method of any of claims 284-287, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.

5 Embodiment 289. The method of any of claims 284-288, wherein the cell is in an animal.

Embodiment 290. A method comprising administering to an animal the compound or composition of any of claims 241-283.

Embodiment 291. The method of claim 290, wherein the administration is subcutaneous.

Embodiment 292. The method of claim 290, wherein the administration is intrathecal.

10 Embodiment 293. The method of any of claims 290-292 comprising administering a plasmid that encodes a Cpf1 nuclease.

Embodiment 294. The method of any of claims 290-292 wherein the animal expresses a Cpf1 nuclease.

Embodiment 295. The method of claim 286 or 293, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).

15 Embodiment 296. The method of claim 286 or 293, wherein the plasmid is delivered to cells within the animal via a lentivirus.

Embodiment 297. The method of any of claims 284-296, wherein a target gene is edited.

Embodiment 298. The method of claim 297, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.

20 Embodiment 299. The method of any of claims 285-287 or 293-298, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.

Embodiment 300. The method of any of claims 284-299 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.

25 Embodiment 301. The method of claim 300, wherein the cell is contacted with the second compound after a target gene has been edited.

Embodiment 302. The method of claim 300 or 301, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.

30 Embodiment 303. The method of claim 300 or 301, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.

Embodiment 304. The method of claim 300 or 301, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.

Embodiment 305. The method of claim 303 or 304, wherein the expression of the Cpf1 nuclease is inhibited.

35 Embodiment 306. The method of any of claims 289-305, wherein the animal is a human.

Embodiment 307. The method of any of claims 284-306, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.

Embodiment 308. The method of any of claims 290 or 293-297, wherein the administration is intravitreal.

Embodiment 309. The method of any of claims 284-288, wherein the cell is a plant cell.

Embodiment 310. The method of any of claims 284-289, wherein the cell is a T-cell.

Embodiment 311. A method of treating a disease in an individual comprising administering the compound of any of claims 241-280 or the composition of any of claims 281-283 to the individual.

Embodiment 312. A method of treating a disease in an individual comprising administering the compound of any of claims 241-280 or the composition any of claims 281-283 to the individual, thereby treating the disease in the individual.

Embodiment 313. Use of the compound of any of claims 241-280 or the composition of any of claims 281-283 for the treatment of a disease.

Embodiment 314. Use of the compound of any of claims 241-280 or the composition of any of claims 281-283 for preparation of a medicament.

Embodiment 315. A method of administering the compound of any of claims 241-280 or the composition of any of claims 281-283 to an animal, and harvesting an organ from the animal for transplantation into a human.

Embodiment 316. The pharmaceutical composition of any of embodiments 108, 147, 206, or 281 comprising a liposome or lipid nanoparticle.

Embodiment 317. The pharmaceutical composition of any of embodiments 108, 147, 206, 281, or 316 comprising mRNA that encodes a CpfI nuclease.

Embodiment 318. The pharmaceutical composition of embodiment 317, wherein the compound comprising the modified crRNA and the mRNA encoding a CpfI nuclease are contained with a liposome or lipid nanoparticle.

Embodiment 319. The method of any of embodiments 212-214, 151-153, 212-214, or 287-289, wherein the mRNA encoding the CpfI nuclease and the compound comprising the modified crRNA are contained within a liposome or lipid nanoparticle.

Embodiment 320. A method of treating a disease in an individual comprising administering the pharmaceutical composition of any of embodiments 316-318 to the individual.

Embodiment 321. A method of treating a disease in an individual comprising administering the pharmaceutical composition of any of embodiments 316-318 to the individual, thereby treating the disease in the individual.

A. Certain Modified Nucleosides

Certain compounds of the present invention incorporate modified nucleosides. Unless otherwise provided, the following modified nucleosides, without limitation, are suitable for such incorporation into modified oligonucleotides for use as crRNA. In certain embodiments, modified oligonucleotides comprise at least one modified nucleoside. Such modified nucleosides comprise a modified sugar moiety or a modified nucleobase or both a modified sugar moiety and a modified nucleobase.

1. Certain Sugar Moieties

In certain embodiments, modified oligonucleotides, such as modified crRNAs, comprise one or more modified nucleosides comprising a modified sugar moiety. Such modified oligonucleotides comprising one or more sugar-modified nucleosides may have desirable properties, such as enhanced nuclease stability or increased binding affinity with a target nucleic acid relative to oligonucleotides lacking such sugar-modified nucleosides. In certain embodiments, modified sugar moieties are linearly modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of substituted sugar moieties.

In certain embodiments, modified sugar moieties are linearly modified sugar moieties comprising a furanosyl ring with one or more acyclic substituent, including but not limited to substituents at the 2' and/or 5' positions. Examples of 2'-substituent groups suitable for linearly modified sugar moieties for use in modified crRNA include but are not limited to: 2'-H, 2'-F, 2'-OCH₃ ("OMe" or "O-methyl"), and 2'-O(CH₂)₂OCH₃ ("MOE"). The 2'-substituent groups of such linearly modified sugar moieties replace the 2'-OH group that is present in unmodified sugar moieties. In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF₃, OCF₃, O-C₁-C₁₀ alkoxy, O-C₁-C₁₀ substituted alkoxy, O-C₁-C₁₀ alkyl, O-C₁-C₁₀ substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(R_m)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, O(CH₂)₂SCH₃, O(CH₂)₂ON(R_m)(R_n) or OCH₂C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C₁-C₁₀ alkyl. Certain embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO₂), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 5'-substituent groups suitable for linearly modified sugar moieties include but are not limited to: 5'-methyl (R or S), 5'-vinyl, and 5'-methoxy. In certain embodiments, linearly modified sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties (*see*, e.g., PCT International Application WO 2008/101157, for additional 2', 5'-bis substituted sugar moieties and nucleosides).

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: H, F, NH₂, N₃, OCF₃, OCH₃, O(CH₂)₃NH₂, CH₂CH=CH₂, OCH₂CH=CH₂, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O(CH₂)₂ON(R_m)(R_n),

$O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)-N(R_m)(R_n)$), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1 - C_{10} alkyl.

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: H, F, OCF_3 , OCH_3 , $OCH_2CH_2OCH_3$,
 5 $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)-N(H)CH_3$ ("NMA").

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: H, F, OCH_3 , and $OCH_2CH_2OCH_3$.

Nucleosides comprising modified sugar moieties, such as linearly modified sugar moieties, are referred to by the position(s) of the substitution(s) on the sugar moiety of the nucleoside. For example,
 10 nucleosides comprising 2'-substituted or 2'-modified sugar moieties are referred to as 2'-substituted nucleosides or 2'-modified nucleosides.

Certain modified sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' bridging sugar substituents
 15 include but are not limited to: 4'- CH_2 -2', 4'-(CH_2)₂-2', 4'-(CH_2)₃-2', 4'- CH_2 -O-2' ("LNA"), 4'- CH_2 -S-2', 4'-(CH_2)₂-O-2' ("ENA"), 4'-CH(CH_3)-O-2' (referred to as "constrained ethyl" or "cEt" when in the *S* configuration), 4'- CH_2 -O- CH_2 -2', 4'- CH_2 -N(R)-2', 4'-CH(CH_2OCH_3)-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.*, U.S. Patent 7,399,845), 4'-C(CH_3)(CH_3)-O-2' and analogs thereof (*see, e.g.*, WO2009/006478), 4'- CH_2 -N(OCH_3)-2' and analogs thereof (*see, e.g.*, WO2008/150729), 4'- CH_2 -O-N(CH_3)-
 20 2' (*see, e.g.*, US2004/0171570), 4'- CH_2 -C(H)(CH_3)-2' (*see, e.g.*, Chattopadhyaya, *et al.*, *J. Org. Chem.*, 2009, 74, 118-134), 4'- CH_2 -C($=CH_2$)-2' and analogs thereof (*see*, published PCT International Application WO 2008/154401), 4'-C(R_aR_b)-N(R)-O-2', 4'-C(R_aR_b)-O-N(R)-2', 4'- CH_2 -O-N(R)-2', and 4'- CH_2 -N(R)-O-2', wherein each R, R_a , and R_b is, independently, H, a protecting group, or C_1 - C_{12} alkyl (*see, e.g.* U.S. Patent 7,427,672).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from: $-[C(R_a)(R_b)]_n$ -, $-[C(R_a)(R_b)]_n-O$ -, $-C(R_a)=C(R_b)$ -, $-C(R_a)=N$ -, $-C(=NR_a)$ -, $-C(=O)$ -, $-C(=S)$ -, $-O$ -, $-Si(R_a)_2$ -, $-S(=O)_x$ -, and $-N(R_a)$ -,
 25

wherein:

x is 0, 1, or 2;

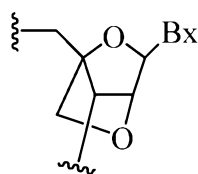
30 n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , $COOJ_1$, acyl ($C(=O)-H$), substituted acyl, CN, sulfonyl ($S(=O)_2-J_1$), or sulfoxyl ($S(=O)-J_1$); and
 35

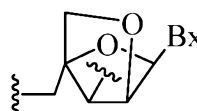
each J₁ and J₂ is, independently, H, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkyl, or a protecting group.

Additional bicyclic sugar moieties are known in the art, for example: Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443; Albaek *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740; Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 20017, 129, 8362-8379; Elayadi *et al.*, *Curr. Opinion Invens. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos. 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 6,670,461, and 7,399,845; WO 2004/106356, WO 1994/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Applications Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922.

In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, an LNA nucleoside (described above) may be in the α -L configuration or in the β -D configuration.



LNA (β -D-configuration)
bridge = 4'-CH₂-O-2'



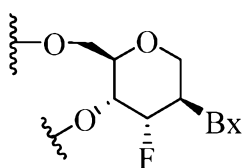
α -L-LNA (α -L-configuration)
bridge = 4'-CH₂-O-2'

α -L-methyleneoxy (4'-CH₂-O-2') or α -L-LNA bicyclic nucleosides have been incorporated into oligonucleotides (Frieden *et al.*, *Nucleic Acids Research*, 2003, 31, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β -D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (*e.g.*, 5'-substituted and 4'-2' bridged sugars). (*see, e.g.*, WO 2007/134181, wherein LNA nucleosides are further substituted with, for example, a 5'-methyl or a 5'-vinyl group, and *see, e.g.*, U.S. Patents 7,547,684; 7,750,131; 8,030,467; 8,268,980; 7,666, 854; and 8,088,746).

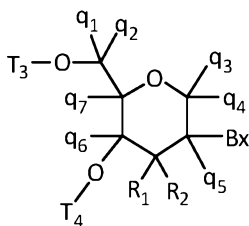
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.*, with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described above. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-
 5 position (*see, e.g.*, US2005/0130923) and/or the 5' position.

In certain embodiments, sugar surrogates comprise rings having other than 5 atoms. For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran ("THP"). Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include but are not limited to hexitol nucleic acid ("HNA"), anitol nucleic acid ("ANA"),
 10 manitol nucleic acid ("MNA") (*see* Leumann, CJ. *Bioorg. & Med. Chem.* 2002, 10, 841-854), fluoro HNA:



F-HNA

("F-HNA", *see e.g.*, US Patents 8,088,904; 8,440,803; and 8,796,437, F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran), and nucleosides comprising additional modified THP compounds having the formula:



wherein, independently, for each of said modified THP nucleoside:

Bx is a nucleobase moiety;

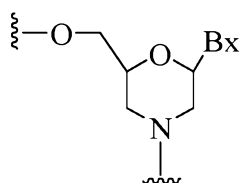
T₃ and T₄ are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T₃ and T₄ is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T₃ and T₄ is H, a
 20 hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

each of R_1 and R_2 is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ_1J_2 , SJ_1 , N_3 , $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN , wherein X is O , S or NJ_1 , and each J_1 , J_2 , and J_3 is, independently, H or C_1 - C_6 alkyl.

In certain embodiments, modified THP nucleosides are provided wherein q_1 , q_2 , q_3 , q_4 , q_5 , q_6 and q_7 are each H . In certain embodiments, at least one of q_1 , q_2 , q_3 , q_4 , q_5 , q_6 and q_7 is other than H . In certain embodiments, at least one of q_1 , q_2 , q_3 , q_4 , q_5 , q_6 and q_7 is methyl. In certain embodiments, modified THP nucleosides are provided wherein one of R_1 and R_2 is F . In certain embodiments, R_1 is F and R_2 is H , in certain embodiments, R_1 is methoxy and R_2 is H , and in certain embodiments, R_1 is methoxyethoxy and R_2 is H .

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in oligonucleotides have been reported (*see, e.g.*, Braasch et al., *Biochemistry*, 2002, 41, 4503-4510 and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term “morpholino” means a sugar surrogate having the following structure:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as “modified morpholinos.”

In certain embodiments, sugar surrogates comprise acyclic moieties. Examples of nucleosides and oligonucleotides comprising such acyclic sugar surrogates include but are not limited to: peptide nucleic acid (“PNA”), acyclic butyl nucleic acid (*see, e.g.*, Kumar et al., *Org. Biomol. Chem.*, 2013, 11, 5853-5865), and nucleosides and oligonucleotides described in WO2011/133876.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides (*see, e.g.*, Leumann, J. C., *Bioorganic & Medicinal Chemistry*, 2002, 10, 841-854).

2. Certain Modified Nucleobases

In certain embodiments, modified oligonucleotides, such as modified crRNAs, comprise one or more nucleoside comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside that does not comprise a nucleobase, referred to as an abasic nucleoside.

In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6

substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch *et al.*, *Angewandte Chemie*, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, *Antisense Drug Technology*, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, US2003/0158403, U.S. 3,687,808; 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,434,257; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,645,985; 5,681,941; 5,750,692; 5,763,588; 5,830,653 and 6,005,096.

B. Certain Modified Internucleoside Linkages

In certain embodiments, nucleosides of modified oligonucleotides, such as modified crRNAs, may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include but are not limited to phosphates, which contain a phosphodiester bond ($"P=O"$) (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, phosphoramidates, and phosphorothioates ($"P=S"$), and phosphorodithioates ($"HS-P=S"$). Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino ($-CH_2-N(CH_3)-O-CH_2-$), thiodiester ($-O-C(=O)-S-$), thionocarbamate ($-O-C(=O)(NH)-S-$); siloxane ($-O-SiH_2-O-$); and N,N'-dimethylhydrazine ($-CH_2-N(CH_3)-N(CH_3)-$). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having

a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral internucleoside linkages include but are not limited to alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH₂-N(CH₃)-O-5'), amide-3 (3'-CH₂-C(=O)-N(H)-5'), amide-4 (3'-CH₂-N(H)-C(=O)-5'), formacetal (3'-O-CH₂-O-5'), methoxypropyl, and thioformacetal (3'-S-CH₂-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: *Carbohydrate Modifications in Antisense Research*; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH₂ component parts.

C. Certain Conjugate Groups and Terminal Groups

In certain embodiments, oligonucleotides for use as crRNA further comprise conjugate groups and/or terminal groups. In certain embodiments, compounds comprising oligonucleotides for use as crRNA further comprise a conjugate group or terminal group. In certain such embodiments, oligonucleotides are covalently attached to one or more conjugate group. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, charge and clearance. In certain embodiments, conjugate groups impart a new property on the attached oligonucleotide, e.g., fluorophores or reporter groups that enable detection of the oligonucleotide. Conjugate groups and/or terminal groups may be added to oligonucleotides having any of the modifications or motifs described above.

Conjugate groups include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates, vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterol, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes. Certain conjugate groups have been described previously, for example: cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, e.g., do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene

glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, 4, e220; doi:10.1038/mtna.2014.72 and Nishina et al., *Molecular Therapy*, 2008, 16, 734-740), or a GalNAc cluster (e.g., WO2014/179620).

In certain embodiments, a conjugate group comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, fingolimod, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepam, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

Conjugate groups are attached directly or via an optional conjugate linker to a parent compound, such as a crRNA oligonucleotide. In certain embodiments, conjugate groups are directly attached to oligonucleotides. In certain embodiments, conjugate groups are indirectly attached to oligonucleotides via conjugate linkers. In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units such as ethylene glycol or amino acid units. In certain embodiments, conjugate groups comprise a cleavable moiety. In certain embodiments, conjugate groups are attached to oligonucleotides via a cleavable moiety. In certain embodiments, conjugate linkers comprise a cleavable moiety. In certain such embodiments, conjugate linkers are attached to oligonucleotides via a cleavable moiety.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, conjugate linkers, including the conjugate linkers described above, are bifunctional linking moieties, e.g., those known in the art to be useful for attaching conjugate groups to parent compounds, such as the crRNA oligonucleotides provided herein. In general, a bifunctional linking moiety comprises at least two functional groups. One of the functional groups is selected to bind to a particular site on a parent compound and the other is selected to bind to a conjugate group. Examples of functional groups used in a bifunctional linking moiety include but are not limited to electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In certain embodiments, bifunctional linking moieties comprise one or more groups selected from amino, hydroxyl, carboxylic acid,

thiol, alkyl, alkenyl, and alkynyl.

Examples of conjugate linkers include but are not limited to pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl or substituted or unsubstituted C₂-C₁₀ alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

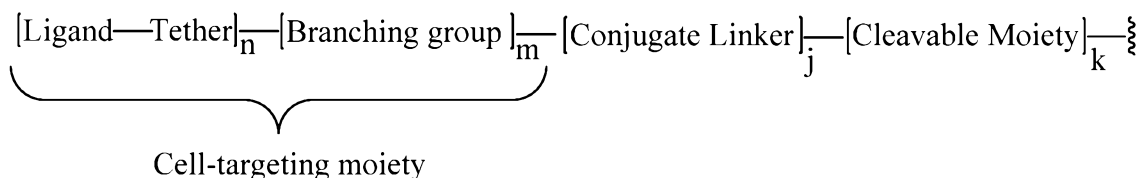
In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety comprises a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

In certain embodiments, a cleavable bond is selected from among: an amide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, or a disulfide. In certain embodiments, a cleavable bond is one or both of the esters of a phosphodiester. In certain embodiments, a cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a phosphate linkage between an oligonucleotide and a conjugate linker or conjugate group.

Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

Examples of terminal groups include but are not limited to conjugate groups, capping groups, phosphate moieties, protecting groups, modified or unmodified nucleosides, and two or more nucleosides that are independently modified or unmodified.

In certain embodiments, a conjugate group is a cell-targeting moiety. In certain embodiments, a conjugate group, optional conjugate linker, and optional cleavable moiety have the general formula:



wherein n is from 1 to about 3, m is 0 when n is 1, m is 1 when n is 2 or greater, j is 1 or 0, and k is 1 or 0.

5 In certain embodiments, n is 1, j is 1 and k is 0. In certain embodiments, n is 1, j is 0 and k is 1. In certain embodiments, n is 1, j is 1 and k is 1. In certain embodiments, n is 2, j is 1 and k is 0. In certain embodiments, n is 2, j is 0 and k is 1. In certain embodiments, n is 2, j is 1 and k is 1. In certain embodiments, n is 3, j is 1 and k is 0. In certain embodiments, n is 3, j is 0 and k is 1. In certain embodiments, n is 3, j is 1 and k is 1.

10 In certain embodiments, conjugate groups comprise cell-targeting moieties that have at least one tethered ligand. In certain embodiments, cell-targeting moieties comprise two tethered ligands covalently attached to a branching group. In certain embodiments, cell-targeting moieties comprise three tethered ligands covalently attached to a branching group.

In certain embodiments, the cell-targeting moiety comprises a branching group comprising one or
 15 more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain such embodiments, the branched aliphatic group
 20 comprises groups selected from alkyl, amino and ether groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl and ether groups. In certain embodiments, the branching group comprises a mono or polycyclic ring system.

In certain embodiments, each tether of a cell-targeting moiety comprises one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amino, oxo, amide, phosphodiester, and polyethylene
 25 glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amino, oxo, amide, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, phosphodiester, ether, amino, oxo, and amide, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl,
 30 ether, amino, oxo, and amid, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, amino, and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or

more groups selected from alkyl and phosphodiester, in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group. In certain embodiments, each tether comprises a chain from about 6 to about 20 atoms in length. In certain embodiments, each tether comprises a chain from about 10 to about 18 atoms in length. In certain embodiments, each tether comprises about 10
5 atoms in chain length.

In certain embodiments, each ligand of a cell-targeting moiety has an affinity for at least one type of receptor on a target cell. In certain embodiments, each ligand has an affinity for at least one type of receptor on the surface of a mammalian liver cell. In certain embodiments, each ligand has an affinity for the hepatic asialoglycoprotein receptor (ASGP-R). In certain embodiments, each ligand is a carbohydrate. In certain
10 embodiments, each ligand is, independently selected from galactose, N-acetyl galactoseamine (GalNAc), mannose, glucose, glucoseamine and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the cell-targeting moiety comprises 3 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 2 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 1 GalNAc ligand.

15 **Certain Pharmaceutical Compositions**

In certain embodiments, the present invention provides pharmaceutical compositions comprising one or more crRNA. In certain embodiments, such pharmaceutical composition comprises a tracrRNA. In certain embodiments, the pharmaceutical composition comprises a means of expressing a CRISPR nuclease. In certain embodiments, such means of expressing the CRISPR nuclease is a plasmid or a viral vector. In certain
20 such embodiments, the pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a modified crRNA. In certain such embodiments, the modified crRNA is a component of a ribonucleoprotein particle or or complex (RNP). In certain such embodiments, the RNP also comprises a nuclease. In certain such embodiments, the nuclease is a Cpf1 nuclease. In certain embodiments, a pharmaceutical composition comprises a liposome or lipid
25 nanoparticle. In certain such embodiments, the liposome or lipid nanoparticle contains the modified crRNA. In certain such embodiments, the liposome or lipid nanoparticle contains an mRNA encoding a Cpf1 nuclease. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more antisense compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more antisense compound. In certain embodiments, the sterile saline is
30 pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one antisense compound and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one
35 or more antisense compound and sterile PBS. In certain embodiments, the sterile PBS is pharmaceutical grade PBS.

Nonlimiting disclosure and incorporation by reference

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence “ATCGATCG” encompasses any oligomeric compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” and those having some DNA bases and some RNA bases such as “AUCGATCG” and oligomeric compounds having other modified or naturally occurring bases, such as “AT^mCGAUCG,” wherein ^mC indicates a cytosine base comprising a methyl group at the 5-position.

Examples

The following examples illustrate certain embodiments of the present invention and are not limiting. Moreover, where specific embodiments are provided, the inventors have contemplated generic application of those specific embodiments. For example, disclosure of an oligonucleotide having a particular motif provides reasonable support for additional oligonucleotides having the same or similar motif. And, for example, where a particular high-affinity modification appears at a particular position, other high-affinity modifications at the same position are considered suitable, unless otherwise indicated.

Example 1: Gene editing effects of truncated crRNAs

Truncated crRNAs comprising a target recognition portion that is complementary to DNA (cytosine-5)-methyltransferase 1 (DNMT1) were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected with a plasmid encoding Cpf1 and a double-stranded gblock (IDT,

Coralville, Iowa) encoding a crRNA listed in the table below. 48 hours later, genomic DNA was isolated from cells and used in a SURVEYOR assay (Integrated DNA Technologies) according to the manufacturer's directions. The PCR primers used to amplify the crRNA target site in the DNMT1 gene were forward: 5'-CTGGGACTCAGGCGGGTCAC-3' (SEQ ID NO: 1) and reverse: 5'-

- 5 CCTCACACAACAGCTTCATGTCAGC-3' (SEQ ID NO: 2). Following Cell cleavage, the DNA was run on a gel. Gene editing of DNMT1 was evaluated by measuring the extent of non-homologous end joining (NHEJ) within DNMT1. Quantification of the bands in the gel was performed using Image J software, and the NHEJ incidence percentage was calculated using the following formula: $NHEJ (\%) = 100 \times (1 - (\text{fraction cut of target gene})^{0.5})$, wherein the fraction cut of the target gene was determined by dividing the fluorescent
- 10 signal of the cut target gene fragment(s) by the total fluorescent signal of the cut and intact target gene fragment(s). The NHEJ incidence for each truncated crRNA was normalized to the NHEJ incidence of the positive control, full-length crRNA 002, and the normalized value was referred to as the gene disruption percentage. The results, shown in the table below, indicate that multiple truncated crRNAs edited the target gene. An entry of "n.d." indicates no data due to the lack of detectable cleavage bands in the gel.

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Table 1
crRNAs targeting DNMT1

Name		Sequence (5' to 3')	Length	Normalized gene disruption (%)	SEQ ID NO.
002	1090808	UAAUUUCUACUCUUGUAGAUC <u>UGAUGGUCCA</u> <u>UGUCUGUUACUC</u>	43	100	18
005	1091140	UUCUACUCUUGUAGAUC <u>UGAUGGUCCAUGUC</u> <u>UGUUACUC</u>	39	n.d.	19
006	1091141	UAAUUUCUACUCUUGUAGAUC <u>UGAUGGUCCA</u> <u>UGUCUGUUA</u>	40	102	20
007	1091142	UUCUACUCUUGUAGAUC <u>UGAUGGUCCAUGUC</u> <u>UGU</u>	34	n.d.	21
008	1090812	UAAUUUCUACUCUUGUAGAUC <u>UGAUGGUCCA</u> <u>UGUCUGU</u>	38	90	22
009	1091143	UUCUACUCUUGUAGAUC <u>UGAUGGUCCAUGUC</u> <u>UGUUA</u>	36	n.d.	23
010	1090813	AAUUUCUACUCUUGUAGAUC <u>UGAUGGUCCA</u> <u>GUCUGU</u>	37	84	24
1034621	1034621	AUUUCUACUCUUGUAGAUC <u>UGAUGGUCCAUG</u> <u>UCUGU</u>	36	60	25

012	1090814	UUUCUACUCUUGUAGAUC <u>CUGAUGGUCCAUG</u> <u>CUGU</u>	35	17	26
013	1090809	AAUUUCUACUCUUGUAGAUC <u>CUGAUGGUCCA</u> <u>GUCUGUUACUC</u>	42	99	27
014	1090810	AUUUCUACUCUUGUAGAUC <u>CUGAUGGUCCAUG</u> <u>UCUGUUACUC</u>	41	65	28
015	1090811	UUUCUACUCUUGUAGAUC <u>CUGAUGGUCCAUG</u> <u>CUGUUACUC</u>	40	20	29

All of the nucleosides in the table above are unmodified ribonucleosides comprising 2'-hydroxy sugar moieties, and all of the internucleoside linkages in the table above are phosphate internucleoside linkages.

The underlined portion of each crRNA is the target recognition portion, and the portion that is not underlined is the CRISPR recognition portion of each crRNA. In Table 1, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 2: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected with a plasmid encoding Cpf1 using Lipofectamine 3000 (Life Technologies, Carlsbad, CA). The next morning, the cells were transfected with a modified crRNA listed in the table below using Lipofectamine RNAi max (Life Technologies). 24 hours later, genomic DNA was isolated from cells and analyzed as described in Example 1 in order to determine the extent of gene editing of DNMT1. The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 1034621, which was also tested in Example 1. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that multiple modified crRNAs edited the target gene. An entry of "n.d." indicates no data due to the lack of detectable cleavage bands in the gel.

Table 2
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	(Normalized) gene disruption (%)	SEQ ID NO.
1034621	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> <u>U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U₁₀G₁₀U₁₀</u>	36	100	25
1038257	A ₁₅ U ₁₅ U ₁₅ U ₁₅ C ₁₅ U ₁₅ A ₁₅ C ₁₅ U ₁₅ C ₁₅ U ₁₅ U ₁₅ G ₁₅ U ₁₅ A ₁₅ G ₁₅ A ₁₅ U ₁₅ <u>C₁₅U₁₅G₁₅A₁₅U₁₅</u> <u>G₁₅G₁₅U₁₅C₁₅C₁₅A₁₅U₁₅G₁₅U₁₅C₁₅U₁₅G₁₅U₁₅</u>	36	n.d.	25
1038259	A ₁₀₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> <u>U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U₁₀G₁₀U₁₀</u>	36	n.d.	25

1038260	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	108	25
1038261	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> oU ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	n.d.	25

A subscript “m” indicates a 2’-O-methyl modification. A subscript “r” indicates an unmodified, 2’-hydroxy sugar moiety. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the portion that is not underlined is the CRISPR recognition portion of the crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 3: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected as described in Example 2, except the modified crRNAs are listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 1034621, which was also tested in Examples 1 and 2. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that modified crRNAs edited the target gene. Nearly all of the modified crRNAs in the table below edited the target gene with greater efficacy than the unmodified control (crRNA 1034621).

Table 3
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	(Normalized) gene disruption (%)	SEQ ID NO.
1034621	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	100	25
1038268	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	141	25
1038269	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	148	25
1038270	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	109	25
1038271	A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀G₁₀A₁₀</u> U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	36	141	25
1038272	U ₁₀ A ₁₀ A ₁₀ U ₁₀ U ₁₀ U ₁₀ C ₁₀ U ₁₀ A ₁₀ C ₁₀ U ₁₀ C ₁₀ U ₁₀ U ₁₀ G ₁₀ U ₁₀ A ₁₀ G ₁₀ A ₁₀ U ₁₀ <u>C₁₀U₁₀</u> G ₁₀ A ₁₀ U ₁₀ G ₁₀ G ₁₀ U ₁₀ C ₁₀ C ₁₀ A ₁₀ U ₁₀ G ₁₀ U ₁₀ C ₁₀ U ₁₀ G ₁₀ U ₁₀	38	152	22

1038273	$\text{U}_{\text{ms}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oG}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{m}}$	38	88	22
1038274	$\text{U}_{\text{ms}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oG}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{m}}$	38	109	22
1038275	$\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oC}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{m}}$	40	169	30
1038276	$\text{C}_{\text{ms}}\text{U}_{\text{rs}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oC}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{m}}$	40	144	30
990509	$\text{C}_{\text{ms}}\text{U}_{\text{rs}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oC}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{rs}}\text{G}_{\text{rs}}\text{U}_{\text{m}}$	40	165	30

A subscript “m” indicates a 2’-O-methyl modification. A subscript “r” indicates an unmodified, 2’-hydroxy sugar moiety. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1. Modified crRNAs 1038273, 1038274, 1038276 and 990509 are 5’-stabilized. The CRISPR recognition portions of crRNAs 1038273 and 1038274 comprise one or more 5’-stabilizing modifications. The linker nucleosides of crRNAs 1038276 and 990509 comprise one or more 5’-stabilizing modifications.

Example 4: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected as described in Example 2, except the modified crRNAs are listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 1038299, which had the highest activity of those tested in this experiment. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that multiple modified crRNAs edited the target gene.

Table 4
crRNAs targeting DNMT1

Name	Sequence (5’ to 3’)	Length	(Normalized) gene disruption (%)	SEQ ID NO.
1038292	$\text{C}_{\text{ms}}\text{U}_{\text{ms}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{r}}$ $\text{roC}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{C}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{rs}}\text{G}_{\text{ms}}\text{U}_{\text{m}}$	40	39	30
1038293	$\text{C}_{\text{ms}}\text{U}_{\text{rs}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{C}_{\text{ro}}\text{U}_{\text{ro}}\text{U}_{\text{ro}}\text{G}_{\text{ro}}\text{U}_{\text{ro}}\text{A}_{\text{ro}}\text{G}_{\text{ro}}\text{A}_{\text{ro}}\text{U}_{\text{r}}$ $\text{oC}_{\text{rs}}\text{U}_{\text{rs}}\text{G}_{\text{rs}}\text{A}_{\text{rs}}\text{U}_{\text{rs}}\text{G}_{\text{rs}}\text{G}_{\text{rs}}\text{U}_{\text{rs}}\text{C}_{\text{rs}}\text{C}_{\text{rs}}\text{A}_{\text{rs}}\text{U}_{\text{rs}}\text{G}_{\text{rs}}\text{U}_{\text{rs}}\text{C}_{\text{rs}}\text{U}_{\text{rs}}\text{G}_{\text{ms}}\text{U}_{\text{m}}$	40	n.d.	30

1038294	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{ms}U_m</u>	40	n.d.	30
1038295	Cms U _{ms} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>roC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{ms}U_m</u>	40	n.d.	30
1038296	Cms U _{ms} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>roC_{rs}U_{rs}G_{rs}A_{rs}U_{rs}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{ms}U_m</u>	40	n.d.	30
1038297	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{rs}U_f</u>	40	80	30
1038298	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}C_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{rs}U_f</u>	40	86	30
1038299	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{rs}U_{rs}U_{rs}C_{rs}C_{ro}A_{rs}U_{ro}G_{rs}U_{ro}C_{rs}U_{ro}C_{rs}U_{ro}G_{rs}U_f</u>	40	100	30
1038300	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	49	30
1038301	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	44	30
1038302	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	58	30
1038303	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	52	30
1038304	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	37	30
1038305	Cms U _{rs} U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _f <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	n.d.	30

A subscript “m” indicates a 2'-O-methyl modification. A subscript “r” indicates an unmodified, 2'-hydroxy sugar moiety. A subscript “f” indicates a 2'-F modification. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1. The modified crRNAs in the table above are 5'-stabilized, and the linker nucleosides comprise the 5'-stabilizing modifications.

Example 5: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected as described in Example 2, except the modified crRNAs are listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA was

normalized to the NHEJ incidence observed for crRNA 1034621, which was also tested in Examples 1-3. The normalized values are referred to as the gene disruption percentages. An entry of “n.d.” indicates no data due to the lack of detectable cleavage bands in the gel. The results, shown in the table below, indicate that multiple modified crRNAs edited the target gene, and, in many cases, were more efficacious than the unmodified crRNA 1034621. The results also indicated that certain positions that tolerate nucleobase changes in the target recognition portion (see, *e.g.*, Kleinstiver et al. in *Nature Biotechnology*, 34, 869 (2016)), also tolerate modified sugars.

Table 5
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	(Normalized) gene disruption (%)	SEQ ID NO.
1034621	<u>A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_{ro}U_{ro}G_{ro}A_{ro}</u> <u>U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ro}U_r</u></u>	36	100	25
991458	C_{ms}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r</u> <u>_oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	77	30
991461	C_{ms}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r</u> <u>_oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{rs}T_k</u>	40	124	31
991462	C_{ms}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r</u> <u>_oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ks}T_k</u>	40	168	31
991775	^m C_{ks}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U</u> <u>_{ro}C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{rs}U_m</u>	40	147	30
991776	^m C_{ks}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U</u> <u>_{ro}C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ks}T_k</u>	40	159	31
991777	C_{ms}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r</u> <u>_oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ks}T_k</u>	40	138	31
991783	^m C_{ks}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U</u> <u>_{ro}C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}^mC_{ko}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	176	30
991784	^m C_{ks}U_{rs} <u>U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U</u> <u>_{ro}^mC_{ko}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}T_{ko}^mC_{ko}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	n.d.	32

A subscript “m” indicates a 2'-O-methyl modification. A superscript “m” adjacent to a “C” indicates a 5-methyl cytosine. A subscript “r” indicates an unmodified, 2'-hydroxy sugar moiety. A subscript “f” indicates a 2'-F modification. A subscript “k” indicates a cEt modification. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1. Other than

crRNA 1034621, the modified crRNAs in the table above are 5'-stabilized, and the linker nucleosides comprise the 5'-stabilizing modifications.

Example 6: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected as described in Example 2, except the modified crRNAs are listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 989549. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that modified crRNAs edited the target gene.

Table 6
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	(Normalized) gene disruption (%)	SEQ ID NO.
989549	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ro}U_r</u>	40	100	30
1038293	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r <u>oC_{rs}U_{rs}G_{rs}A_{rs}U_{rs}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{ms}U_m</u>	40	26	30
1038294	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{ms}U_m</u>	40	38	30
1086669	C_{rs}U_{rs}U_{rs}A_{rs}A_{rs}U_{rs}U_{rs}U_{rs}C_{rs}U_{rs}A_{rs}C_{rs}U_{rs}C_{rs}U_{rs}U_{rs}G_{rs}U_{rs}A_{rs}G_{rs}A_{rs}U_{rs}C_{rs} <u>U_{rs}G_{rs}A_{rs}U_{rs}G_{rs}G_{rs}U_{rs}C_{rs}C_{rs}A_{rs}U_{rs}G_{rs}U_{rs}C_{rs}U_{rs}G_{rs}U_r</u>	40	26	30
1086670	C_{ms}U_{rs}U_{rs}A_{rs}A_{rs}U_{rs}U_{rs}U_{rs}C_{rs}U_{rs}A_{rs}C_{rs}U_{rs}C_{rs}U_{rs}U_{rs}G_{rs}U_{rs}A_{rs}G_{rs}A_{rs}U_{ro}C_r <u>oU_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{rs}G_{ms}U_m</u>	40	68	30
1086671	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_r <u>oC_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{rs}C_{ro}A_{rs}U_{ro}G_{rs}U_{ro}C_{rs}U_{ro}G_{ms}U_m</u>	40	77	30

A subscript "m" indicates a 2'-O-methyl modification. A subscript "r" indicates an unmodified, 2'-hydroxy sugar moiety. A subscript "o" indicates a phosphate internucleoside linkage, and a subscript "s" indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1. The modified crRNAs in the table above are 5'-stabilized, and the linker nucleosides comprise the 5'-stabilizing modifications.

Example 7: Gene editing effects of modified crRNAs

Modified crRNAs described below and in Examples 4, 5, and 6 were tested for their effects on gene editing of DNMT1 relative to crRNA 989549. HEK293T cells were transfected as described in Example 2, with 3 μ L of 100 μ M of a crRNA listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 989549. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that multiple modified crRNAs edited the target gene.

Table 7
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	SEQ ID No.
1120133	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_{ro}U_{ro}</u> <u>G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ms}U_m</u>	40	30
1120139	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_{ro}U_{ro}</u> <u>G_{ro}A_{ro}U_{ro}G_{ro}G_{rs}U_{ro}C_{rs}C_{ro}A_{rs}U_{ro}G_{rs}U_{ro}C_{fs}U_{ro}U_{ro}G_{ms}U_m</u>	40	30
1120140	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_{ro}U_{ro}</u> <u>G_{ro}A_{ro}U_{ro}G_{ro}G_{rs}U_{ro}C_{ms}C_{ro}A_{rs}U_{ro}G_{rs}U_{ro}C_{fs}U_{ro}U_{ro}G_{ms}U_m</u>	40	30
1120141	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_{ro}U_{ro}</u> <u>G_{ro}A_{ro}U_{ro}G_{ro}G_{rs}U_{ro}C_{fs}C_{ro}A_{rs}U_{ro}G_{rs}U_{ro}C_{fs}U_{ro}U_{ro}G_{ms}U_m</u>	40	30

A subscript “m” indicates a 2'-O-methyl modification. A subscript “r” indicates an unmodified, 2'-hydroxy sugar moiety. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. A subscript “f” indicates a 2'-F modification. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides.

The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1. The modified crRNAs in the table above are 5'-stabilized, and the linker nucleosides comprise the 5'-stabilizing modifications.

Table 8a
Gene disruption

Name	(Normalized) gene disruption (%)
989549	100
1038297	110
1038298	160
1038299	140
1120133	60
1120139	50
991461	90
991462	90
991777	80
991775	80
991776	90
991783	130
991784	10

Table 8b
Gene disruption

Name	(Normalized) gene disruption (%)
989549	100
1120140	10
1120141	70

5 Example 8: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1. HEK293T cells were transfected as described in Example 2, with 3 μ L of 100 μ M of a crRNA listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1. The NHEJ incidence for each modified crRNA in Table 9 was normalized to the NHEJ incidence observed for modified crRNA 1090626. The normalized values are referred to as the gene disruption percentages. The NHEJ incidence for each modified crRNA in Table 10 was not normalized, the absolute percentages of gene disruption observed are listed. The results, shown in the tables below, indicate that multiple modified crRNAs edited the target gene.

Table 9
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	Norm. gene disruption (%)	SEQ ID NO.
1038306	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{ro} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} U _{ro} C _{ro} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	120	33
1038307	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} U _{ro} C _{ro} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	143	33
1038308	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{ro} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} T _{do} C _{ro} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	135	34
1038309	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{ro} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} U _{ro} C _{do} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	169	33
1038335	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} T _{do} C _{ro} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	198	34
1038310	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} U _{ro} C _{do} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	181	33
1038311	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{ro} U _{ro} G _{ro} A _{ro} U _{ro} G _{ro} G _{ro} T _{do} C _{do} C _{ro} A _{ro} U _{ro} G _{ro} U _{ro} C _{ro} U _{ro} G _{ro} U _{ro} T _{do} A _{do} C _{do} T _{do} C _d	43	181	34

1038312	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	184	34
1090623	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_{do}C_{do}T_d</u>	42	105	35
1090624	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_{do}C_d</u>	41	120	36
1090625	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_d</u>	40	105	37
1090626	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_d</u>	39	100	38
1090627	<u>U_{r0}A_{r0}A_{r0}U_{r0}U_{r0}U_{r0}C_{r0}U_{r0}A_{r0}C_{r0}U_{r0}C_{r0}U_{r0}U_{r0}G_{r0}U_{r0}A_{r0}G_{r0}A_{r0}U_{r0}C_{do}U_{r0}G_{r0}</u> <u>A_{r0}U_{r0}G_{r0}G_{r0}T_{do}C_{do}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}T_d</u>	38	11	39
1038313	C_{do}T_{do} U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _r <u>oU_{r0}G_{r0}A_{r0}U_{r0}G_{r0}G_{r0}U_{r0}C_{r0}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_{do}C_{do}T_{do}C_d</u>	45	161	40

Table 10
crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	Abs. Gene disruption (%)	SEQ ID NO.
1038313	C_{do}T_{do} U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _r <u>oU_{r0}G_{r0}A_{r0}U_{r0}G_{r0}G_{r0}U_{r0}C_{r0}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}U_{r0}G_{r0}U_{r0}T_{do}A_{do}C_{do}T_{do}C_d</u>	45	21	40
1090628	U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _{r0} U _{r0} G _{r0} <u>A_{r0}U_{r0}G_{r0}G_{r0}U_{r0}C_{r0}C_{r0}A_{r0}U_{r0}G_{r0}U_{r0}C_{r0}T_{do}G_{do}T_{do}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	n.d.	41
1090629	U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _{r0} U _{r0} G _{r0} <u>A_{r0}U_{r0}G_{r0}G_{r0}U_{r0}C_{r0}C_{do}A_{do}T_{do}G_{do}T_{do}C_{do}T_{do}G_{do}T_{do}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	n.d.	42
1090630	U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _{r0} U _{r0} G _{r0} <u>A_{r0}U_{r0}G_{r0}G_{do}T_{do}C_{do}C_{do}A_{do}T_{do}G_{do}T_{do}C_{do}T_{do}G_{do}T_{do}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	n.d.	43
1090631	U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _{r0} U _{r0} G _{r0} <u>A_{r0}U_{r0}G_{do}G_{do}T_{do}C_{do}C_{do}A_{do}T_{do}G_{do}T_{do}C_{do}T_{do}G_{do}T_{do}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	n.d.	43
1090632	U _{r0} A _{r0} A _{r0} U _{r0} U _{r0} U _{r0} C _{r0} U _{r0} A _{r0} C _{r0} U _{r0} C _{r0} U _{r0} U _{r0} G _{r0} U _{r0} A _{r0} G _{r0} A _{r0} U _{r0} C _{do} T _{do} G _d <u>oA_{do}T_{do}G_{do}G_{do}T_{do}C_{do}C_{do}A_{do}T_{do}G_{do}T_{do}C_{do}T_{do}G_{do}T_{do}T_{do}A_{do}C_{do}T_{do}C_d</u>	43	n.d.	44
1090633	T _{do} A _{do} A _{do} T _{do} T _{do} T _{do} ^m C _{do} T _{do} A _{do} ^m C _{do} T _{do} ^m C _{do} T _{do} T _{do} G _{do} T _{do} A _{do} G _{do} A _{do} T _{do} ^m C _d <u>doT_{do}G_{do}A_{do}T_{do}G_{do}G_{do}T_{do}^mC_{do}^mC_{do}A_{do}T_{do}G_{do}T_{do}^mC_{do}T_{do}G_{do}T_{do}T_{do}A_{do}^mC_d</u> <u>oT_{do}^mC_d</u>	43	n.d.	45

In the tables above, a subscript “r” indicates an unmodified, 2'-hydroxy sugar moiety. A subscript “d”

5 indicates a modified, 2'-deoxy sugar moiety. A subscript “o” indicates a phosphate internucleoside linkage. A “C” following a superscript “m” indicates a 5-methyl cytosine. The underlined portion of each crRNA is the

target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. The CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 9: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to Low Density Lipoprotein Receptor (LDLR) were designed and synthesized to test their effects on gene editing of LDLR. HEK293T cells were transfected as described in Example 2, with 3 μ L of 100 μ M of a crRNA listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1 except that the PCR primers used to amplify the crRNA target site in the LDLR gene were forward: 5'-GGAGACCCAAATACAACAAATC - 3' (SEQ ID NO: 56) and reverse: 5'-CTAGACTCCGTCTCAAAGAAG - 3' (SEQ ID NO: 57). The NHEJ incidence for each modified crRNA was normalized to the NHEJ incidence observed for crRNA 1091152. The normalized values are referred to as the gene disruption percentages. The results, shown in the table below, indicate that modified crRNAs edited the target gene.

Table 11a
crRNAs targeting LDLR

Name	Sequence (5' to 3')	Length	Norm. gene disruption (%)	SEQ ID NO.
1091152	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_r <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_r</u>	40	100	46
1091153	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_d <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{rs}G_{ms}G_m</u>	40	n.d.	46
1091154	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} A _{ro} G _{ro} <u>C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}T_{do}C_{do}G_{do}T_{do}G_d</u>	43	120	47
1091155	U _{ro} A _{ro} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} A _{ro} G _{ro} <u>C_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}T_{do}C_{do}G_{do}T_{do}G_d</u>	43	100	47
1091156	U _{ms} A _{rs} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} A _{ro} G _r <u>oC_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}U_{ro}C_{ro}G_{rs}U_{ms}G_m</u>	43	120	48
1091157	U _{ms} A _{rs} A _{ro} U _{ro} U _{ro} U _{ro} C _{ro} U _{ro} A _{ro} C _{ro} U _{ro} C _{ro} U _{ro} U _{ro} G _{ro} U _{ro} A _{ro} G _{ro} A _{ro} U _{ro} C _{do} A _{ro} G _r <u>oC_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}U_{ro}C_{ro}G_{rs}U_{ms}G_m</u>	43	110	48
1091158	C_{do}T_{do}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_d <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}T_{do}C_{do}G_{do}T_{do}G_d</u>	45	120	49
1091159	C_{do}T_{do}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_d <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}T_{do}C_{do}G_{do}T_{do}G_d</u>	45	100	49
1091160	C_{ms}U_{ms}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_r <u>doA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}U_{ro}C_{ro}G_{rs}U_{ms}G_m</u>	45	110	50

1091161	CmsU_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_d</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_{ro}U_{ro}C_{ro}G_{rs}U_{ms}G_m</u>	45	120	50

Table 11b
crRNAs targeting LDLR

Name	Sequence (5' to 3')	Length	Norm. gene disruption (%)	SEQ ID NO.
1091152	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_r</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_r</u>	40	100	51
1120137	CmsU_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C</u> <u>moA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{rs}G_{mo}A_{ms}C_{ro}A_{rs}C_{ro}A_{rs}G_{fo}C_{fs}A_{fs}G_{ms}G_m</u>	40	10	51
1120138	CmsU_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_f</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{rs}G_{fo}A_{fs}C_{ro}A_{rs}C_{ro}A_{rs}G_{fo}C_{fs}A_{fs}G_{ms}G_m</u>	40	60	51

A subscript “m” indicates a 2'-O-methyl modification. A subscript “r” indicates an unmodified, 2'-hydroxy sugar moiety. A subscript “d” indicates a modified, 2'-deoxy sugar moiety. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 10: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to LDLR were designed and synthesized to test their effects on gene editing of LDLR as described in Example 9. The NHEJ incidence for each crRNA was not normalized. The values in the table below are the absolute gene disruption percentages. The results indicate that modified crRNAs edited the target gene.

Table 12
crRNAs targeting LDLR

Name	Sequence (5' to 3')	Length	Abs. Gene disruption (%)	SEQ ID NO.
1091152	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_r</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{ro}G_{ro}G_r</u>	40	26	51
1091153	CmsU_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_d</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{do}A_{do}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{rs}G_{ms}G_m</u>	40	4	51
1091162	CmsU_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_r</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{rs}G_{ms}G_m</u>	40	18	51

1091164	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>C_f</u> <u>oA_{ro}G_{ro}C_{ro}U_{ro}A_{ro}G_{ro}G_{ro}A_{ro}C_{ro}A_{ro}C_{ro}A_{ro}G_{ro}C_{ro}A_{rs}G_{ms}G_m</u>	40	18	51

A subscript “m” indicates a 2’-O-methyl modification. A subscript “r” indicates an unmodified, 2’-hydroxy sugar moiety. A subscript “d” indicates a modified, 2’-deoxy sugar moiety. A subscript “f” indicates a 2’-F modification. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 11: Gene editing effects of crRNAs

crRNAs comprising target recognition portions complementary to various targets were designed and synthesized to test their effects on gene editing. HEK293T cells were transfected as described in Example 2, with 3 µL of 100 µM of a crRNA listed in the table below. Genomic DNA was isolated and analyzed as described in Example 1 except that the PCR primers used to amplify the crRNA target site were one of the following: for the Complement 5 gene (C5, Table 13), forward: 5’- CATGGGGTAACCCAGCAAAC -3’ (SEQ ID NO: 58) and reverse: 5’- GGAAATAAGTGATGGGGCAGG -3’ (SEQ ID NO: 59); for the Empty Spiracles Homeobox 1 gene (EMX1, Table 14), forward: 5’- CCATCCCCTTCTGTGAATGT -3’ (SEQ ID NO: 60) and reverse: 5’- GGAGATTGGAGACACGGAGA -3’ (SEQ ID NO: 61); for the Glutamate Ionotropic Receptor NMDA Type Subunit 2B gene (GRIN2b, Table 15), forward: 5’- GCATACTCGCATGGCTACCT -3’ (SEQ ID NO: 62) and reverse: 5’- CTCCTGCAGCCCCCTTTTTA -3’ (SEQ ID NO: 63); for the Transthyretin gene (TTR, Table 16), forward: 5’- CAGAATCAGCAGGTTTGCAG -3’ (SEQ ID NO: 64) and reverse: 5’- CAAACCTAATGCACCAAAGC -3’ (SEQ ID NO: 65). The NHEJ incidence for each crRNA was not normalized. The values in the tables below are the absolute gene disruption percentages. The results, shown in the tables below, indicate that most crRNAs edited the corresponding target genes and that there is some variability among different targets.

Table 13
crRNA targeting C5

Name	Sequence (5’ to 3’)	Length	Abs. Gene disruption (%)	SEQ ID NO.
1091478	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}<u>U_r</u> <u>oA_{ro}C_{ro}U_{ro}C_{ro}C_{ro}A_{ro}G_{ro}A_{ro}C_{ro}C_{ro}A_{ro}G_{ro}U_{ro}C_{ro}A_{ro}G_{ro}G_r</u>	40	< 5	52

Table 14
crRNA targeting EMX1

Name	Sequence (5' to 3')	Length	Abs. Gene disruption (%)	SEQ ID NO.
1091480	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}U_r _oG_{ro}G_{ro}U_{ro}U_{ro}G_{ro}C_{ro}C_{ro}C_{ro}A_{ro}C_{ro}C_{ro}C_{ro}U_{ro}A_{ro}G_{ro}U_{ro}C_r	40	16	53

5

Table 15
crRNA targeting GRIN2b

Name	Sequence (5' to 3')	Length	Abs. Gene disruption (%)	SEQ ID NO.
1091484	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}G_r _oU_{ro}G_{ro}C_{ro}U_{ro}C_{ro}A_{ro}A_{ro}U_{ro}G_{ro}A_{ro}A_{ro}A_{ro}G_{ro}G_{ro}A_{ro}G_{ro}A_r	40	19	54

Table 16
crRNA targeting TTR

Name	Sequence (5' to 3')	Length	Abs. Gene disruption (%)	SEQ ID NO.
1091482	C_{ro}U_{ro}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}U_r _oG_{ro}U_{ro}C_{ro}U_{ro}G_{ro}A_{ro}G_{ro}G_{ro}C_{ro}U_{ro}G_{ro}G_{ro}C_{ro}C_{ro}C_{ro}U_{ro}A_r	40	n.d.	55

10 The legend for Table 12 applies to Tables 13-16.

Example 12: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed and synthesized to test their effects on gene editing of DNMT1 relative to unmodified crRNA 989549 (see Table 6). HEK293T cells were transfected as described in Example 2, with 3 μ L of 100 μ M of a modified crRNA listed in the table below, crRNA 989549 ("RNA Ctrl"), or no crRNA ("neg"). Genomic DNA was isolated and analyzed as described in Example 1 except that NHEJ incidence was not quantified. The resulting DNA gel is shown in Figure 1 and indicates that multiple modified crRNAs comprising at least one modified sugar moiety in the CRISPR recognition portion edited the target gene.

crRNAs targeting DNMT1

Name	Sequence (5' to 3')	Length	SEQ ID NO.
1096341	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀^mC_{ko}U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096343	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A_{ko}C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096344	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀^mC_{ko}U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096346	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀^mC_{ko}U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096349	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G_{ko}U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096351	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A_{ko}G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096352	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G_{ko}A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1096353	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A_{ko}U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	30
1144245	C_{ms}U_{rs}U₁₀A₁₀A_{ko}U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀T_{ko}C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	71
1144246	C_{ms}U_{rs}U₁₀A₁₀A₁₀T_{ko}U₁₀U₁₀C₁₀T_{ko}A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	72
1144247	C_{ms}U_{rs}U₁₀A₁₀A₁₀T_{ko}U₁₀U₁₀C₁₀U₁₀A_{ko}C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	73
1144248	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀T_{ko}U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	74
1144249	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀T_{ko}C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀ <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	75
1144250	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀T_{ko}C₁₀U₁₀U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U_r <u>C₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	71
1144251	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀T_{ko}U₁₀G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀C_r <u>o₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	76
1144252	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀T_{ko}G₁₀U₁₀A₁₀G₁₀A₁₀U₁₀C_r <u>o₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	77
1144253	C_{ms}U_{rs}U₁₀A₁₀A₁₀U₁₀U₁₀U₁₀C₁₀U₁₀A₁₀C₁₀U₁₀C₁₀U₁₀U₁₀G₁₀T_{ko}A₁₀G₁₀A₁₀U₁₀C_r <u>o₁₀U₁₀G₁₀A₁₀U₁₀G₁₀G₁₀U₁₀C₁₀C₁₀A₁₀U₁₀G₁₀U₁₀C₁₀U_{rs}G_{ms}U_m</u>	40	78

1144254	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}T_{ko}C_{ro} <u>o_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	79
1144255	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}T_{ko}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_{ro} <u>o_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	80

A subscript “m” indicates a 2’-O-methyl modification. A subscript “r” indicates an unmodified, 2’-hydroxy sugar moiety. A subscript “k” indicates a cEt modification. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Example 13: Gene editing effects of modified crRNAs

Modified crRNAs comprising a target recognition portion that is complementary to DNMT1 were designed to test their effects on gene editing of DNMT1. HEK293T cells are transfected as described in Example 2. Genomic DNA is isolated and analyzed as described in Example 1.

Table 18
crRNAs targeting DNMT1

Name	Sequence (5’ to 3’)	Length	SEQ ID NO.
1186843	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro}C_{ro} <u>o_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	30
1186844	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}^mC_{ko}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro} <u>C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	30
1186845	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro} <u>C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	30
1186846	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}^mC_{ko}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro} <u>C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	30
1186847	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}^mC_{ko}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro} <u>C_{ro}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}U_{ro}C_{ro}C_{ro}A_{ro}U_{ro}G_{ro}U_{ro}C_{ro}U_{ro}G_{ms}U_m</u>	40	30
1186848	C_{ms}U_{rs}U_{ro}A_{ro}A_{ro}U_{ro}U_{ro}U_{ro}C_{ro}U_{ro}A_{ro}C_{ro}U_{ro}C_{ro}U_{ro}U_{ro}G_{ro}U_{ro}A_{ro}G_{ro}A_{ro}U_{ro} <u>C_{do}U_{ro}G_{ro}A_{ro}U_{ro}G_{ro}G_{ro}T_{do}C_{do}C_{ro}A_{ro}U_{ro}G_{ro}T_{do}C_{do}T_{do}G_{ms}U_m</u>	40	81

A subscript “m” indicates a 2’-O-methyl modification. A subscript “r” indicates an unmodified, 2’-hydroxy sugar moiety. A subscript “d” indicates a modified, 2’-deoxy sugar moiety. A subscript “k” indicates a cEt modification. A subscript “f” indicates a 2’-F modification. A subscript “o” indicates a phosphate internucleoside linkage, and a subscript “s” indicates a phosphorothioate internucleoside linkage. The underlined portion of each crRNA is the target recognition portion, and the bolded nucleosides are linker

nucleosides. The portion that is neither bold nor underlined is the CRISPR recognition portion of each crRNA. In the table above, the CRISPR recognition portions of the crRNAs recognize Cpf1.

Claims

1. A compound comprising a modified crRNA consisting of 35-45 linked nucleosides.
2. A compound comprising a modified crRNA, wherein the CRISPR recognition portion of the modified crRNA consists of 17-20 linked nucleosides.
3. A compound comprising a modified crRNA, wherein the target recognition portion of the modified crRNA consists of 18-23 linked nucleosides.
4. A compound comprising a modified crRNA, wherein the modified crRNA comprises at least one linker nucleoside.
5. A compound comprising a 5'-stabilized modified crRNA.
6. The compound of any of claims 1-5, wherein the compound comprises a stabilizing conjugate group.
7. The compound of any of claims 1-5, wherein the crRNA comprises at least one linker nucleoside comprising a stabilizing modification.
8. The compound of any of claims 1-4, wherein the modified crRNA is 5'-stabilized.
9. The compound of any of claims 1-8, wherein the modified crRNA is 3'-stabilized.
10. The compound of any of claims 1-9, wherein the CRISPR recognition portion of the modified crRNA binds to a Cpf1 nuclease.
11. The compound of any of claims 1-10, wherein the target recognition portion of the modified crRNA comprises at least one modification that increases affinity of the crRNA for a target DNA or RNA.
12. The compound of any of claims 10-11, wherein the CRISPR recognition portion of the modified crRNA comprises at least one modification that increases affinity of the crRNA for a Cpf1 nuclease.
13. The compound of any of claims 1-12, wherein at least one nucleobase of the modified crRNA is thymine.
14. The compound of any of claims 1-13, wherein at least one nucleobase of the modified crRNA is a modified nucleobase.
15. The compound of claim 14, wherein the modified nucleobase is 5-methyl cytosine.
16. The compound of any of claims 1-15, wherein modified crRNA consists of 35-42 linked nucleosides.
17. The compound of any of claims 1-15, wherein the modified crRNA consists of 36-40 linked nucleosides.
18. The compound of any of claims 1-17, wherein the modified crRNA comprises at least two linker nucleosides.
19. The compound of claim 18, wherein at least two linker nucleosides are linked to the CRISPR recognition portion of the modified crRNA.
20. The compound of claim 19, wherein at least two linker nucleosides are linked to the 5'-end of the CRISPR recognition portion of the modified crRNA.
21. The compound of any of claims 1-20, wherein the CRISPR recognition portion of the modified crRNA consists of 18-20 linked nucleosides.

22. The compound of claim 21, wherein the CRISPR recognition portion of the modified crRNA consists of 18 linked nucleosides.
23. The compound of claim 21, wherein the CRISPR recognition portion of the modified crRNA consists of 19 linked nucleosides.
24. The compound of claim 21, wherein the CRISPR recognition portion of the modified crRNA consists of 20 linked nucleosides.
25. The compound of any of claims 1-24, wherein the target recognition portion of the modified crRNA consists of 18-22 linked nucleosides.
26. The compound of any of claims 1-24, wherein the target recognition portion of the modified crRNA consists of 18-20 linked nucleosides.
27. The compound of claim 26, wherein the target recognition portion of the modified crRNA consists of 18 linked nucleosides.
28. The compound of claim 26, wherein the target recognition portion of the modified crRNA consists of 19 linked nucleosides.
29. The compound of claim 26, wherein the target recognition portion of the modified crRNA consists of 20 linked nucleosides.
30. The compound of any of claims 1-29, wherein at least one internucleoside linkage of the modified crRNA is a modified internucleoside linkage.
31. The compound of claim 30, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.
32. The compound of claim 30 or 31, wherein each internucleoside linkage of the modified crRNA is a modified internucleoside linkage.
33. The compound of any of claims 30-32, wherein at least one internucleoside linkage is a neutral internucleoside linkage.
34. The compound of claim 33, wherein at least one modified internucleoside linkage comprises a methoxypropyl group.
35. The compound of claim 33, wherein at least one modified internucleoside linkage comprises a phosphonoacetate.
36. The compound of claim 33, wherein at least one modified internucleoside linkage comprises a methylphosphonate.
37. The compound of any of claims 1-31, wherein each internucleoside linkage of the modified crRNA is a phosphodiester internucleoside linkage or a phosphorothioate internucleoside linkage.
38. The compound of any of claims 30, 31, or 33-37, wherein at least two internucleoside linkages of the modified crRNA are modified internucleoside linkages.
39. The compound of claim 38, wherein at least two modified internucleoside linkages of the modified crRNA are the same as one another.

40. The compound of any of claims 1-39, wherein the modified crRNA comprises one to five contiguous phosphorothioate internucleoside linkages at the 5'-end of the modified crRNA.
41. The compound of claim 40, wherein the modified crRNA comprises one phosphorothioate internucleoside linkage at the 5'-end of the modified crRNA.
42. The compound of claim 40, wherein the modified crRNA comprises two contiguous phosphorothioate internucleoside linkages at the 5'-end of the modified crRNA.
43. The compound of any of claims 1-42, wherein the modified crRNA comprises at least one linker nucleoside that is linked to the CRISPR recognition portion of the modified crRNA by a modified internucleoside linkage.
44. The compound of claim 43, wherein the modified internucleoside linkage that links the at least one linker nucleoside to the CRISPR recognition portion of the modified crRNA is a phosphorothioate internucleoside linkage.
45. The compound of claim 44, wherein the modified crRNA comprises two linker nucleosides.
46. The compound of claim 45, wherein the linker nucleosides are linked to each other by a modified internucleoside linkage.
47. The compound of claim 46, wherein the modified internucleoside that links the linker nucleosides to each other is a phosphorothioate internucleoside linkage.
48. The compound of any claims 43-44, wherein the modified crRNA comprises more than two linker nucleosides.
49. The compound of any of claims 1-48, wherein the modified crRNA comprises one to six modified internucleoside linkages within the target recognition portion of the modified crRNA.
50. The compound of claim 49, wherein the one to six modified internucleoside linkages within the target recognition portion of the modified crRNA are contiguous.
51. The compound of claim 49, wherein the one to six modified internucleoside linkages within the target recognition portion of the modified crRNA alternate with unmodified internucleoside linkages.
52. The compound of any of claims 49-51, wherein the 3'-end of the target recognition portion of the modified crRNA contains the one to six modified internucleoside linkages.
53. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises one modified internucleoside linkage.
54. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises two modified internucleoside linkages.
55. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises three modified internucleoside linkages.
56. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises four modified internucleoside linkages.

57. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises five modified internucleoside linkages.
58. The compound of any of claims 50-52, wherein the target recognition portion of the modified crRNA comprises six modified internucleoside linkages.
59. The compound of any of claims 49-58, wherein at least one internucleoside linkage within the target recognition portion of the modified crRNA is a phosphorothioate internucleoside linkage.
60. The compound of any of claims 49-58, wherein all of the modified internucleoside linkages within the target recognition portion of the modified crRNA are phosphorothioate internucleoside linkages.
61. The compound of any of claims 1-60, wherein the target recognition portion of the modified crRNA is directly or indirectly linked to the 3' end of the CRISPR recognition portion of the modified crRNA.
62. The compound of any of claims 1-61, wherein at least one nucleoside of the modified crRNA comprises a modified sugar moiety.
63. The compound of claim 62, wherein the 5'-terminal nucleoside of the crRNA comprises a modified sugar moiety.
64. The compound of claim 63, wherein the 5'-terminal nucleoside comprises a linearly modified sugar moiety.
65. The compound of claim 64, wherein the 5'-terminal nucleoside comprises a 2'-modified sugar moiety.
66. The compound of claim 63, wherein the 5'-terminal nucleoside comprises a bicyclic sugar moiety.
67. The compound of claim 63, wherein the 5'-terminal nucleoside comprises a modified sugar moiety selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
68. The compound of any of claims 1-67, wherein the 5'-terminal nucleoside is a linker nucleoside.
69. The compound of any of claims 62-68, wherein the 5th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.
70. The compound of any of claims 62-69, wherein the 6th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.
71. The compound of any of claims 62-70, wherein the 7th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.
72. The compound of any of claims 62-71, wherein the 10th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.
73. The compound of any of claims 62-72, wherein the 14th nucleoside from the 5'-end of the CRISPR recognition portion comprises a modified sugar moiety.
74. The compound of any of claims 62-73, wherein the 1st nucleoside from the 3'-end of the CRISPR recognition portion comprises a modified sugar moiety.

75. The compound of any of claims 69-74, wherein at least one modified sugar moiety is selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
76. The compound of any of claims 69-74, wherein each modified sugar moiety is independently selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
77. The compound of claim 62, wherein the 3'-terminal nucleoside of the modified crRNA comprises a modified sugar moiety.
78. The compound of claim 77, wherein the 3'-terminal nucleoside comprises a linearly modified sugar moiety.
79. The compound of claim 78, wherein the 3'-terminal nucleoside comprises a 2'-modified sugar moiety.
80. The compound of claim 77, wherein the 3'-terminal nucleoside comprises a bicyclic sugar moiety.
81. The compound of claim 77, wherein the 3'-terminal nucleoside comprises a modified sugar moiety selected from among: 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
82. The compound of any of claims 62-81, wherein the 1st nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
83. The compound of any of claims 62-82, wherein the 8th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
84. The compound of any of claims 62-83, wherein the 9th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
85. The compound of any of claims 62-84, wherein one to five 3'-terminal nucleosides of the target recognition portion of the modified crRNA each comprise a modified sugar moiety.
86. The compound of claim 85, wherein the one to five 3'-terminal nucleosides of the target recognition portion of the modified crRNA each comprise the same modified sugar moiety.
87. The compound of claim 84 or 85, wherein the modified sugar moieties of the one to five 3'-terminal nucleosides of the target recognition portion are each independently selected from among 2'-O-methyl, 2'-MOE, 2'-F, cEt, and LNA.
88. The compound of any of claims 1-87, wherein the target recognition portion of the modified crRNA comprises at least one unmodified sugar moiety.
89. The compound of any of claims 1-88, wherein the CRISPR recognition portion of the modified crRNA comprises at least one unmodified sugar moiety.
90. The compound of any of claims 1-89, wherein the modified crRNA comprises at least one linker nucleoside that comprises an unmodified sugar moiety.
91. The compound of any of claims 1-90, wherein the compound consists of the modified crRNA.
92. The compound of any of claims 1-91, wherein the nucleobase sequence of the target recognition portion of the modified crRNA is at least 90% complementary to a target DNA or RNA.

93. The compound of claim 92, wherein the nucleobase sequence of the target recognition portion of the modified crRNA is 100% complementary to a target DNA or RNA.
94. The compound of any of claims 1-93, wherein the modified crRNA comprises a self-complementary region.
95. The compound of claim 94, wherein the self-complementary region is within the CRISPR recognition portion of the modified crRNA.
96. The compound of claim 94 or 95, wherein the self-complementary region can form a hairpin.
97. The compound of any of claims 94-96, wherein the self-complementary region comprises at least one modification that increases the stability of the self-complementary region.
98. The compound of any of claims 94-97, wherein the self-complementary region comprises at least one modification that increases the hybridization affinity of the self-complementary region.
99. The compound of any of claims 1-98, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises at least 12 contiguous nucleobases of a sequence selected from Table A.
100. The compound of any of claims 1-98, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA consists of a sequence or a portion of a sequence selected from Table A.
101. The compound of any of claims 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence XCXACX, wherein each X is, independently, a U nucleobase or a T nucleobase.
102. The compound of any of claims 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence GXAGAX, wherein each X is, independently, a U nucleobase or a T nucleobase.
103. The compound of any of claims 1-100, wherein the nucleobase sequence of the CRISPR recognition portion of the modified crRNA comprises the sequence XCXACX and the sequence GXAGAX, wherein each X is, independently, a U nucleobase or a T nucleobase.
104. The compound of any of claims 1-90 or 92-103, wherein the compound comprises a conjugate group.
105. The compound of claim 104, wherein the conjugate group comprises GalNAc.
106. The compound of claim 104, wherein the conjugate group is lipophilic.
107. The compound of claim 106, wherein the conjugate group comprises a lipid.
108. A pharmaceutical composition comprising the compound of any of claims 1-107.
109. A method comprising contacting a cell with the compound or composition of any of claims 1-108.
110. The method of claim 109, wherein the cell expresses a Cpf1 nuclease.

111. A method comprising contacting a cell with the compound or composition of any of claims 1-108 and a plasmid that encodes a Cpf1 nuclease.
112. A method comprising contacting a cell with the compound or composition of any of claims 1-108 and an mRNA that encodes a Cpf1 nuclease.
113. The method of any of claims 109-112, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.
114. The method of any of claims 109-113, wherein the cell is in an animal.
115. A method comprising administering to an animal the compound or composition of any of claims 1-108.
116. The method of claim 115, wherein the administration is subcutaneous.
117. The method of claim 115, wherein the administration is intrathecal.
118. The method of any of claims 115-117 comprising administering a plasmid that encodes a Cpf1 nuclease.
119. The method of any of claims 115-117 wherein the animal expresses a Cpf1 nuclease.
120. The method of claim 111 or 118, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).
121. The method of claim 111 or 118, wherein the plasmid is delivered to cells within the animal via a lentivirus.
122. The method of any of claims 109-121, wherein a target gene is edited.
123. The method of claim 122, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.
124. The method of any of claims 110-112 or 118-123, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.
125. The method of any of claims 109-124 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.
126. The method of claim 125, wherein the cell is contacted with the second compound after a target gene has been edited.
127. The method of claim 125 or 126, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.
128. The method of claim 125 or 126, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.
129. The method of claim 125 or 126, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.
130. The method of claim 128 or 129, wherein the expression of the Cpf1 nuclease is inhibited.
131. The method of any of claims 114-130, wherein the animal is a human.

132. The method of any of claims 109-131, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.
133. The method of any of claims 115 or 118-132, wherein the administration is intravitreal.
134. The method of any of claims 109-113, wherein the cell is a plant cell.
135. The method of any of claims 109-114, wherein the cell is a T-cell.
136. A method of treating a disease in an individual comprising administering the compound of any of claims 1-107 or the composition of claim 108 to the individual, thereby treating the disease in the individual.
137. Use of the compound of any of claims 1-107 or the composition of claim 108 for the treatment of a disease.
138. Use of the compound of any of claims 1-107 or the composition of claim 108 for preparation of a medicament.
139. A method of administering the compound of any of claims 1-107 or the composition of claim 108 to an animal, and harvesting an organ from the animal for transplantation into a human.
140. The compound of any of claims 90-107, wherein the 5'-terminal nucleoside comprises a cEt modified sugar moiety.
141. The compound of claim 140, wherein the 3'-end of the target recognition portion of the modified crRNA contains two contiguous phosphorothioate internucleoside linkages.
142. The compound of any of claims 140-141, wherein each internucleoside linkage of the CRISPR recognition portion of the modified crRNA is phosphorothioate.
143. The compound of any of claims 140-142, wherein the two 3'-terminal nucleosides of the target recognition portion of the modified crRNA each comprise a 2'-O-methyl modified sugar moiety.
144. The compound of any of claims 140-143, wherein the 1st nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
145. The compound of any of claims 140-144, wherein the modified crRNA comprises 30-38 unmodified sugar moieties.
146. The compound of claim 145, wherein the modified crRNA comprises 36 unmodified sugar moieties.
147. A pharmaceutical composition comprising the compound of any of claims 140-146.
148. A method comprising contacting a cell with the compound or composition of any of claims 140-147.
149. The method of claim 148, wherein the cell expresses a Cpf1 nuclease.
150. A method comprising contacting a cell with the compound or composition of any of claims 140-147 and a plasmid that encodes a Cpf1 nuclease.

151. A method comprising contacting a cell with the compound or composition of any of claims 140-147 and an mRNA that encodes a Cpf1 nuclease.
152. The method of any of claims 148-151, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.
153. The method of any of claims 148-152, wherein the cell is in an animal.
154. A method comprising administering to an animal the compound or composition of any of claims 140-147.
155. The method of claim 154, wherein the administration is subcutaneous.
156. The method of claim 154, wherein the administration is intrathecal.
157. The method of any of claims 154-156 comprising administering a plasmid that encodes a Cpf1 nuclease.
158. The method of any of claims 154-156 wherein the animal expresses a Cpf1 nuclease.
159. The method of claim 150 or 157, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).
160. The method of claim 150 or 157, wherein the plasmid is delivered to cells within the animal via a lentivirus.
161. The method of any of claims 148-160, wherein a target gene is edited.
162. The method of claim 161, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.
163. The method of any of claims 149-151 or 157-162, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.
164. The method of any of claims 148-163 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.
165. The method of claim 164, wherein the cell is contacted with the second compound after a target gene has been edited.
166. The method of claim 164 or 165, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.
167. The method of claim 164 or 165, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.
168. The method of claim 164 or 165, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.
169. The method of claim 167 or 168, wherein the expression of the Cpf1 nuclease is inhibited.
170. The method of any of claims 153-169, wherein the animal is a human.

171. The method of any of claims 148-170, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.
172. The method of any of claims 154 or 157-171, wherein the administration is intravitreal.
173. The method of any of claims 148-152, wherein the cell is a plant cell.
174. The method of any of claims 148-153, wherein the cell is a T-cell.
175. A method of treating a disease in an individual comprising administering the compound of any of claims 140-146 or the composition of claim 147 to the individual.
176. A method of treating a disease in an individual comprising administering the compound of any of claims 140-146 or the composition of claim 147 to the individual, thereby treating the disease in the individual.
177. Use of the compound of any of claims 140-146 or the composition of claim 147 for the treatment of a disease.
178. Use of the compound of any of claims 140-146 or the composition of claim 147 for preparation of a medicament.
179. A method of administering the compound of any of claims 140-146 or the composition of claim 147 to an animal, and harvesting an organ from the animal for transplantation into a human.
180. The compound of any of claims 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA is a 2'-deoxynucleoside.
181. The compound of any of claims 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA comprises a linearly modified sugar moiety having a 2'-H substitution.
182. The compound of any of claims 91-107 or 140-146, wherein at least one modified nucleoside of the modified crRNA comprises a modified 2'-H(H) sugar moiety as found in naturally occurring DNA.
183. The compound of any of claims 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 40 linked nucleosides.
184. The compound of any of claims 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 43 linked nucleosides.
185. The compound of any of claims 91-107, 140-146, or 180-182, wherein the modified crRNA consists of 45 linked nucleosides.
186. The compound of any of claims 91-107, 140-146, or 180-185, wherein the target recognition portion of the modified crRNA is at least 90% complementary to a DNMT1 nucleic acid.
187. The compound of claim 186, wherein the target recognition portion is 100% complementary to a DNMT1 nucleic acid.
188. The compound of claim 186 or 187, wherein the DNMT1 nucleic acid is a deoxyribonucleic acid.

189. The compound of claim 188, wherein the DNMT1 nucleic acid is a human deoxyribonucleic acid.
190. The compound of any of claims 91-107, 140-146, or 180-185, wherein the target recognition portion of the modified crRNA is at least 90% complementary to a LDLR nucleic acid.
191. The compound of claim 190, wherein the target recognition portion is 100% complementary to a LDLR nucleic acid. The compound of claim 190 or 191, wherein the LDLR nucleic acid is a deoxyribonucleic acid.
192. The compound of claim 191, wherein the LDLR nucleic acid is a human deoxyribonucleic acid.
193. The compound of any of claims 91-107, 140-146, or 180-192, wherein the two 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.
194. The compound of any of claims 91-107, 140-146, or 180-192, wherein the three 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.
195. The compound of any of claims 91-107, 140-146, or 180-192, wherein the four 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.
196. The compound of any of claims 91-107, 140-146, or 180-192, wherein the five 3'-terminal nucleosides of the modified crRNA comprise independently selected modified sugar moieties.
197. The compound of any of claims 77 or 193-196, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H), 2'-O-methyl, 2'-F, cEt, and LNA modified sugar moieties.
198. The compound of claim 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H), 2'-O-methyl, and cEt modified sugar moieties.
199. The compound of claim 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among 2'-H(H) and 2'-O-methyl modified sugar moieties.
200. The compound of claim 197, wherein the modified sugar moieties of the 3'-terminal modified nucleosides are selected from among cEt and LNA modified sugar moieties.
201. The compound of any of claims 82-107, 140-146, or 180-200, wherein the 1st nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.
202. The compound of any of claims 82-107, 140-146, or 180-201, wherein the 8th nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.
203. The compound of any of claims 82-107, 140-146, or 180-202, wherein the 9th nucleoside from the 5'-end of the target recognition portion comprises a 2'-H(H) or 2'-F modified sugar moiety.
204. The compound of any of claims 91-107, 140-146, or 180-203, wherein the modified crRNA comprises at least three of the following features:

- a. two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;
 - b. 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;
 - c. at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA
 - d. each nucleoside of the CRISPR recognition portion comprising an unmodified sugar moiety
 - e. one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties
205. The compound of any of claims 1-107, 140-146, or 180-204, wherein the modified crRNA is a salt.
206. A pharmaceutical composition comprising the compound of any of claims 180-205.
207. The pharmaceutical composition of any of claims 108, 147, or 206, wherein the pharmaceutical composition comprises a ribonucleoprotein complex.
208. The pharmaceutical composition of claim 207, wherein the ribonucleoprotein complex comprises a Cpf1 nuclease and the compound comprising the modified crRNA.
209. A method comprising contacting a cell with the compound or composition of any of claims 180-208.
210. A method comprising contacting a cell with the compound or composition of any of claims 180-207, wherein the cell expresses a Cpf1 nuclease.
211. A method comprising contacting a cell with the compound or composition of any of claims 180-207 and a plasmid that encodes a Cpf1 nuclease.
212. A method comprising contacting a cell with the compound or composition of any of claims 180-207 and an mRNA that encodes a Cpf1 nuclease.
213. The method of any of claims 209-212, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.
214. The method of any of claims 209-213, wherein the cell is in an animal.
215. A method comprising administering to an animal the compound or composition of any of claims 180-208.
216. The method of claim 215, wherein the administration is subcutaneous.
217. The method of claim 215, wherein the administration is intrathecal.
218. The method of any of claims 215-217 comprising administering a plasmid that encodes a Cpf1 nuclease.
219. The method of any of claims 215-217 wherein the animal expresses a Cpf1 nuclease.
220. The method of claim 211 or 218, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).

221. The method of claim 211 or 218, wherein the plasmid is delivered to cells within the animal via a lentivirus.
222. The method of any of claims 209-221, wherein a target gene is edited.
223. The method of claim 222, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.
224. The method of any of claims 210-212 or 215-223, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.
225. The method of any of claims 209-224 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.
226. The method of claim 225, wherein the cell is contacted with the second compound after a target gene has been edited.
227. The method of claim 225 or 226, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.
228. The method of claim 225 or 226, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.
229. The method of claim 225 or 226, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.
230. The method of claim 228 or 229, wherein the expression of the Cpf1 nuclease is inhibited.
231. The method of any of claims 214-230, wherein the animal is a human.
232. The method of any of claims 209-231, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.
233. The method of any of claims 215 or 218-232, wherein the administration is intravitreal.
234. The method of any of claims 209-213, wherein the cell is a plant cell.
235. The method of any of claims 209-214, wherein the cell is a T-cell.
236. A method of treating a disease in an individual comprising administering the compound of any of claims 180-205 or the composition of any of claims 206-208 to the individual.
237. A method of treating a disease in an individual comprising administering the compound of any of claims 180-205 or the composition any of claims 206-208 to the individual, thereby treating the disease in the individual.
238. Use of the compound of any of claims 180-205 or the composition of any of claims 206-208 for the treatment of a disease.
239. Use of the compound of any of claims 180-205 or the composition of any of claims 206-208 for preparation of a medicament.

240. A method of administering the compound of any of claims 180-205 or the composition of any of claims 206-208 to an animal, and harvesting an organ from the animal for transplantation into a human.
241. The compound of any of claims 1-107, 140-146, or 180-205, wherein the CRISPR recognition portion of the modified crRNA comprises at least one modified sugar moiety.
242. The compound of claim 241, wherein the at least one modified sugar moiety of the CRISPR recognition portion is a linearly modified sugar moiety.
243. The compound of claim 241, wherein the at least one modified sugar moiety of the CRISPR recognition portion is a bicyclic sugar moiety.
244. The compound of claim 243, wherein the bicyclic sugar moiety is cEt or LNA.
245. The compound of claim 243, wherein the bicyclic sugar moiety is cEt.
246. The compound of any of claims 241-245, wherein the 2nd nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
247. The compound of any of claims 241-245, wherein the 3rd nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
248. The compound of any of claims 241-245, wherein the 4th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
249. The compound of any of claims 241-245, wherein the 5th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
250. The compound of any of claims 241-245, wherein the 6th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
251. The compound of any of claims 241-245, wherein the 7th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
252. The compound of any of claims 241-245, wherein the 8th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
253. The compound of any of claims 241-245, wherein the 9th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
254. The compound of any of claims 241-245, wherein the 11th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
255. The compound of any of claims 241-245, wherein the 12th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
256. The compound of any of claims 241-245, wherein the 13th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.
257. The compound of any of claims 241-245, wherein the 18th nucleoside from the 3'-end of the CRISPR recognition portion comprises the at least one modified sugar moiety.

258. The compound of any of claims 241-245, wherein the 11th and 12th nucleosides from the 3'-end of the CRISPR recognition portion each comprise a modified sugar moiety.
259. The compound of any of claims 241-258, wherein the 1st nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
260. The compound of any of claims 241-259, wherein the 10th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
261. The compound of any of claims 241-260, wherein the 14th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
262. The compound of any of claims 241-261, wherein the 15th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
263. The compound of any of claims 241-262, wherein the 16th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
264. The compound of any of claims 241-263, wherein the 17th nucleoside from the 3'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
265. The compound of any of claims 241-264, wherein the 1st nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
266. The compound of any of claims 241-265, wherein the 2nd nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
267. The compound of any of claims 241-266, wherein the 3rd nucleoside from the 5'-end of the CRISPR recognition portion comprises an unmodified sugar moiety.
268. The compound of any of claims 1-107, 140-146, 180-205, or 241-267, wherein the 14th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
269. The compound of any of claims 1-107, 140-146, 180-205, or 241-268, wherein the 15th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
270. The compound of any of claims 1-107, 140-146, 180-205, or 241-269, wherein the 16th nucleoside from the 5'-end of the target recognition portion comprises a modified sugar moiety.
271. The compound of any of claims 268-270, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is a linearly modified sugar moiety.
272. The compound of claim 271, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is independently selected from 2'-H(H) and 2'-F modified sugar moieties.
273. The compound of claim 272, wherein each modified sugar moiety at position 14, 15, and/or 16 from the 5'-end of the target recognition portion is a 2'-H(H) modified sugar moiety.
274. The compound of any of claims 1-107, 140-146, 180-205, or 241-273, wherein the modified crRNA comprises at least three of the following features:

(a) two linker nucleosides linked to the 5'-end of the CRISPR recognition portion of the modified crRNA;

(b) 1st, 8th, and/or 9th nucleoside from the 5'-end of the target recognition portion of the modified crRNA independently comprising 2'-F or 2'-H(H) modified sugar moiety;

(c) at least one terminal phosphorothioate internucleoside linkage at each of the 3' and 5' termini of the modified crRNA

(d) at least one nucleoside at position 5, 6, 7, 8, 11, or 12 from the 3'-end of the CRISPR recognition portion comprises a modified sugar moiety

(e) one to five 3'-terminal nucleosides of the modified crRNA comprising independently selected modified sugar moieties

275. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), and (e).

276. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), and (d).

277. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), and (c).

278. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), (c), and (e).

279. The compound of claim 274, wherein the modified crRNA comprises features (a), (c), (d), and (e).

280. The compound of claim 274, wherein the modified crRNA comprises features (a), (b), (c), (d), and (e).

281. A pharmaceutical composition comprising the compound of any of claims 241-280.

282. The pharmaceutical composition of claim 281, wherein the pharmaceutical composition comprises a ribonucleoprotein complex.

283. The pharmaceutical composition of claim 282, wherein the ribonucleoprotein complex comprises a Cpf1 nuclease and the compound comprising the modified crRNA.

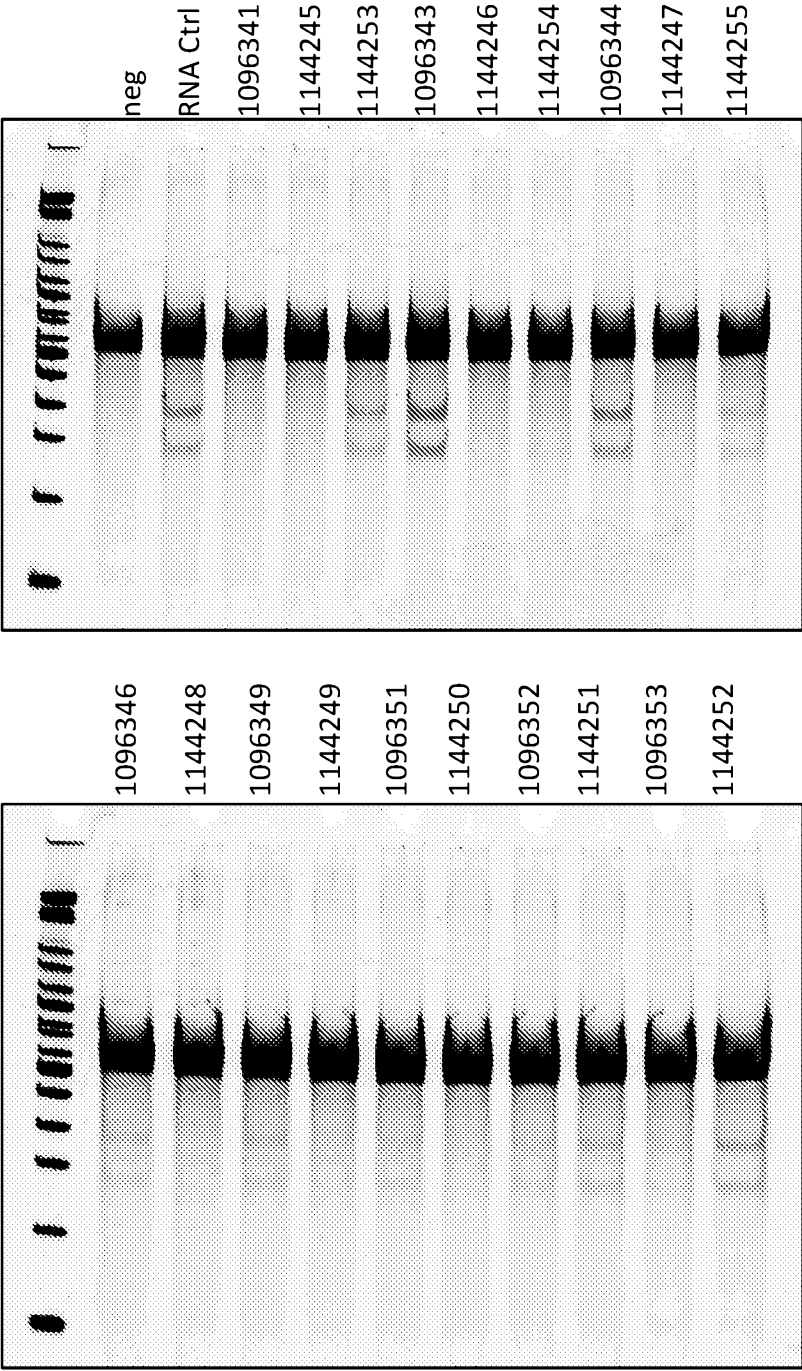
284. A method comprising contacting a cell with the compound or composition of any of claims 241-283.

285. A method comprising contacting a cell with the compound or composition of any of claims 241-283, wherein the cell expresses a Cpf1 nuclease.

286. A method comprising contacting a cell with the compound or composition of any of claims 241-283 and a plasmid that encodes a Cpf1 nuclease.

287. A method comprising contacting a cell with the compound or composition of any of claims 241-283 and an mRNA that encodes a Cpf1 nuclease.
288. The method of any of claims 284-287, wherein the modified crRNA is taken up by the cell in the absence of a transfection reagent.
289. The method of any of claims 284-288, wherein the cell is in an animal.
290. A method comprising administering to an animal the compound or composition of any of claims 241-283.
291. The method of claim 290, wherein the administration is subcutaneous.
292. The method of claim 290, wherein the administration is intrathecal.
293. The method of any of claims 290-292 comprising administering a plasmid that encodes a Cpf1 nuclease.
294. The method of any of claims 290-292 wherein the animal expresses a Cpf1 nuclease.
295. The method of claim 286 or 293, wherein the plasmid is delivered to cells within the animal via an adeno-associated virus (AAV).
296. The method of claim 286 or 293, wherein the plasmid is delivered to cells within the animal via a lentivirus.
297. The method of any of claims 284-296, wherein a target gene is edited.
298. The method of claim 297, wherein the modified crRNA is degraded in a cell after the target gene is edited in the cell.
299. The method of any of claims 285-287 or 293-298, wherein the Cpf1 nuclease does not exhibit nuclease activity in the absence of the modified crRNA.
300. The method of any of claims 284-299 comprising contacting the cell with a second compound that degrades or inhibits the activity or expression of the modified crRNA or a Cpf1 nuclease.
301. The method of claim 300, wherein the cell is contacted with the second compound after a target gene has been edited.
302. The method of claim 300 or 301, wherein the second compound comprises an oligonucleotide that is complementary to the modified crRNA.
303. The method of claim 300 or 301, wherein the second compound comprises a crRNA that targets a Cpf1 nuclease gene.
304. The method of claim 300 or 301, wherein the second compound comprises an oligonucleotide that is complementary to a Cpf1 transcript.
305. The method of claim 303 or 304, wherein the expression of the Cpf1 nuclease is inhibited.
306. The method of any of claims 289-305, wherein the animal is a human.

307. The method of any of claims 284-306, wherein editing of at least one off-target gene is reduced relative to editing the at least one off-target gene when unmodified crRNA or a compound comprising more than 45 nucleosides is used in place of the modified crRNA.
308. The method of any of claims 290 or 293-297, wherein the administration is intravitreal.
309. The method of any of claims 284-288, wherein the cell is a plant cell.
310. The method of any of claims 284-289, wherein the cell is a T-cell.
311. A method of treating a disease in an individual comprising administering the compound of any of claims 241-280 or the composition of any of claims 281-283 to the individual.
312. A method of treating a disease in an individual comprising administering the compound of any of claims 241-280 or the composition any of claims 281-283 to the individual, thereby treating the disease in the individual.
313. Use of the compound of any of claims 241-280 or the composition of any of claims 281-283 for the treatment of a disease.
314. Use of the compound of any of claims 241-280 or the composition of any of claims 281-283 for preparation of a medicament.
315. A method of administering the compound of any of claims 241-280 or the composition of any of claims 281-283 to an animal, and harvesting an organ from the animal for transplantation into a human.
316. The pharmaceutical composition of any of claims 108, 147, 206, or 281 comprising a liposome or lipid nanoparticle.
317. The pharmaceutical composition of any of claims 108, 147, 206, 281, or 316 comprising mRNA that encodes a Cpf1 nuclease.
318. The pharmaceutical composition of claim 317, wherein the compound comprising the modified crRNA and the mRNA encoding a Cpf1 nuclease are contained with a liposome or lipid nanoparticle.
319. The method of any of claims 212-214, 151-153, 212-214, or 287-289, wherein the mRNA encoding the Cpf1 nuclease and the compound comprising the modified crRNA are contained within a liposome or lipid nanoparticle.
320. A method of treating a disease in an individual comprising administering the pharmaceutical composition of any of claims 316-318 to the individual.
321. A method of treating a disease in an individual comprising administering the pharmaceutical composition of any of claims 316-318 to the individual, thereby treating the disease in the individual.



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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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CORE0141WSEQ_ST25.txt

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CORE0141WOSEQ_ST25.txt

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