

ANTISENSE OLIGONUCLEOTIDES AND THEIR USE FOR TREATMENT OF
NEURODEGENERATIVE DISORDERS

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 63/181,023, filed April 28, 2021; U.S. Provisional Application No. 63/320,651, filed March 16, 2022; U.S. Provisional Application No. 63/334,496, filed April 25, 2022; the entire contents of each are incorporated herein by reference.

[0002] Disclosed herein are novel antisense oligonucleotides ("ASOs") that may induce exon skipping during pre-mRNA splicing, pharmaceutical compositions comprising the same, and methods of using the same.

[0003] Neurodegenerative disorders are a group of disorders characterized by the decline of central nervous system and peripheral nervous system structure and function. While neurodegenerative disorders exhibit heterogeneous symptoms, they can share similar features. One neurodegenerative disease, Alzheimer's Disease, is a neurodegenerative disorder characterized by buildup of amyloid beta plaques and neurofibrillary tangles. It is also the leading cause of dementia. Although some cases of rare familial Alzheimer's Disease involve autosomal dominant mutations to the amyloid beta precursor protein, the majority of cases are late-onset Alzheimer's Disease (LOAD), which do not follow Mendelian inheritance patterns. While the mechanics of LOAD are not completely understood, genome-wide association studies have identified genetic risk factors for LOAD. Scientists have shown the ability of these genes to impact the production, aggregation, or clearance of amyloid beta plaques. One such gene is *CD33*, also known as Siglec-3. Griciuc et al., *Alzheimer's Disease Risk Gene CD33 Inhibits Microglial Uptake of Amyloid Beta*, 78 NEURON 631 (2013).

[0004] *CD33* is expressed in myeloid-derived cells, including macrophages such as microglia, and encodes the CD33 protein. Microglia account for approximately 10% of the cells in the brain and represent the first line of immunological defense. Microglia modulate several important activities in the brain, such as homeostasis, cognition, and neurogenesis. Augusto-Oliveira et al., *What Do Microglia Really Do in Healthy Adult Brain?*, 8 CELLS 1293 (2019). Microglia cells are known to contribute to neurodegeneration by releasing proinflammatory substances in the central nervous system. Wojtera et al., *Microglial cells in neurodegenerative disorders*, 43 FOLIA NEUROPATHOLOGY 311 (2005).

[0005] CD33 is a transmembrane receptor protein that has an extracellular receptor that binds the ligand sialic acid. The intracellular immunoreceptor tyrosine-based inhibition motif recruits phosphatases upon phosphorylation of its tyrosine residues, leading to suppression of immune cell activity such as phagocytosis. CD33 has been found to inhibit microglial uptake of amyloid beta protein, which suggests that therapies targeting *CD33* could be potential LOAD treatment options. Griciuc et al., *Alzheimer's Disease Risk Gene CD33 Inhibits Microglial Uptake of Amyloid Beta*, 78 NEURON 631 (2013).

[0006] Two single nucleotide polymorphisms (SNPs) in the promoter region of the *CD33* gene are associated with LOAD: rs3826656 and rs3865444. The rs3865444 SNP comes in two forms, rs3865444-C and rs3865444-A. The first form results in normal length CD33 protein. The second form, rs3865444-A, modulates splicing of *CD33* pre-mRNA resulting in skipping of Exon-2 and a CD33 protein lacking the sialic acid binding domain. Malik et al., *CD33 Alzheimer's Risk-Altering Polymorphism, CD33 Expression, and Exon 2 Splicing*, 33 J. NEUROSCIENCE 13320 (2013).

[0007] In eukaryotic genes containing coding (exons) and noncoding (intron) sequences, the noncoding introns are excised from the pre-mRNA transcript and the coding exons are spliced together to form mRNA. If an intron is left in the final mRNA transcript or an exon is left out, the mRNA reading frame may be disrupted during translation of the mRNA. This may result in a non-functional polypeptide sequence or a premature stop codon. The splicing process is further complicated by alternative splicing, where the same pre-mRNA sequence can be spliced into different exon combinations to form multiple mRNA sequences.

[0008] Splicing of pre-mRNA is an intricate process involving a multi-megadalton ribonucleoprotein complex called the spliceosome. The spliceosome recognizes specific sequences in pre-mRNA to precisely excise introns and ligate exons. The spliceosome catalyzes intron excision in two transesterification reactions using three conserved RNA sequences. These RNA sequences are the 5' splice site, 3' splice site, and the branch site. Will & Luhrmann, *Spliceosome Structure and Function*, 3 COLD SPRING HARB. PERSPECT. BIOL. 1 (2011).

[0009] Splicing begins with the 2' OH group of the branch site binding to the 5' splice site via a nucleophilic attack, causing cleavage of the 5' exon at the 5' splice site and forming a lariat. Then the 3' OH group of the 5' exon attacks the 3' exon at the 3' splice site, ligating the 5' and 3' exons and cleaving the intron lariat. Will & Luhrmann, *Spliceosome Structure and Function*, 3 COLD SPRING HARB. PERSPECT. BIOL. 1 (2011). Because the splicing process involves spliceosome recognition sites, 5' and 3' splice sites, and the branch site, a mutation in any one of these sites can disrupt the splicing process.

[0010] ASOs are polynucleotides designed to bind with specificity to a target nucleotide sequence, thereby affecting one or more aspects of gene expression, such as, transcription, splicing, stability, and/or translation. ASOs may be directed to either RNA or DNA. ASOs directed to RNA can bind to target mRNA sequences, effecting mRNA stability or translation at the ribosome.

[0011] ASOs that bind to target sequences in pre-mRNA transcripts can affect the splicing process. In some cases, ASOs may be used to induce exon skipping during pre-mRNA splicing. For example, Duchenne Muscular Dystrophy (DMD) is caused by a mutation that alters the reading frame of dystrophin mRNA during translation, resulting in a premature stop codon and truncated dystrophin protein. ASOs may be utilized to correct the reading frame by inducing

skipping of an exon during splicing. Removing an exon of the correct number of base pairs results in a shorter mRNA transcript, but the reading frame may be corrected. Because dystrophin RNA consists of 79 exons, skipping one or several exons during splicing still results in a partly functional protein. Echigoya et al., *Multiple Exon Skipping in the Duchenne Muscular Dystrophy Hot Spots: Prospects and Challenges*, 8 J. PERS. MED. 41 (2018). The FDA approved an exon-skipping drug called Exondys 51 (eteplirsen) for treatment of DMD in 2016. Dowling, *Eteplirsen therapy for Duchenne muscular dystrophy: skipping to the front of the line*, 12 NATURE REV. NEUROLOGY 675 (2016).

[0012] In other cases, ASOs may be used to prevent or reduce exon skipping during pre-mRNA splicing. As an example, the ASO drug nusinersen (Spinraza®) reduces Exon-7 skipping during splicing of the *SMN2* gene to treat spinal muscular atrophy. Son & Yokota, *Recent Advances and Clinical Applications of Exon Inclusion for Spinal Muscular Atrophy*, in EXON SKIPPING & INCLUSION THERAPIES, 57-68 (2018). The rs3865444-A variant that induces Exon-2 skipping of *CD33* conveys protection against LOAD. Malik et al., *CD33 Alzheimer's Risk-Altering Polymorphism, CD33 Expression, and Exon 2 Splicing*, 33 J. NEUROSCIENCE 13320 (2013). There remains a need, however, for ASOs that successfully induce Exon-2 skipping during pre-mRNA splicing of *CD33* and for their use in treating neurodegenerative diseases.

[0013] Disclosed herein are ASOs, methods of using such ASOs to induce exon skipping during pre-mRNA splicing, pharmaceutical compositions that comprise such ASOs, and methods of using such compositions to treat neurodegenerative disease.

[0014] In some embodiments, disclosed herein is an antisense oligonucleotide of 16-30, such as 18-30, nucleotides in length, which is complementary to a portion of SEQ ID NO:1. In some embodiments, the antisense oligonucleotide is complementary to a portion of: SEQ ID NO:213; SEQ ID NO:214; SEQ ID NO:215; SEQ ID NO:216; SEQ ID NO:217; SEQ ID NO:218; SEQ ID NO:219; and/or SEQ ID NO:220. In some embodiments, the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 35% or greater. In some embodiments, the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater. In some embodiments, the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater according to a Standard Exon-Skipping Efficiency Assay for ASOs. In some embodiments, the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises phosphorodiamidate morpholino oligomers or according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs when the antisense oligonucleotide comprises methoxyethyl ribose oligomers.

[0015] In some embodiments, the antisense oligonucleotide comprises all or a portion of:

- a. PMO-002 (5'-CCTCACCTGTACATGCACAGAGAG-3') (SEQ ID NO:2);
- b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);

- c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
- d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
- e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTAAGTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
- 5 g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
- j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- 10 l. PMO-096 (5'-ACTTGCAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:96);
- m. PMO-007 (5'-CAGCCAGAAATTTGGATCCATAGCC-3') (SEQ ID NO:7);
- n. PMO-097 (5'-AGAAATTTGGATCCATAGCCAGGGC-3') (SEQ ID NO:97);
- o. PMO-008 (5'-CCCTGTGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:8);
- p. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- 15 q. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- r. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- s. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- t. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- u. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- 20 v. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- w. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
- x. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- y. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- z. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO: 15);
- 25 aa. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- bb. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- cc. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- dd. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- ee. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).
- 30 [0016] In some embodiments, the antisense oligonucleotide comprises all or a portion of:
 - a. PMO-221 (5'- CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);
 - b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
 - c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
 - d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);
 - 35 e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
 - f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
 - g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
 - h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);

- i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
- j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
- k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
- l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
- 5 m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
- n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:234);
- o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
- p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
- q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
- 10 r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
- s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
- t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
- u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
- v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
- 15 w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
- x. PMO-244 (5'-TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
- y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
RRRRRRRRRRRRRRRRRRRRRR
- z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
20 SSSSSSSSSSSSSSSSSSSSS
- aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRR; or
- bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSSSS.
- 25 [0017] In some embodiments, the antisense oligonucleotide comprises all or a portion of:
- a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
- b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
- c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
- d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);
- 30 e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- 35 j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);

- n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- 5 r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- t. MOE-264 (5'- ATCC-gAAAGAAgTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- 10 w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- 15 bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID
- 20 NO:012);
- gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
RRRRRRRRRRRRRRRRRRRR;
- 25 ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSSS;
- jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSSS;
- kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
30 SSSRSRSRSRSRSRSRSSS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSRRRRRRRRSSSSSSS;
- mm. MOE-283 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRSRRRSRRRSRRSSS;
- 35 nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRSRRRSRRRSRRSSS;
- oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSRRRRRRRRRRSSSSS;

- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRRRRRRRRRRRRSSSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRSSSSSSSSRSRSSSSS;
- 5 rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRRR;
- tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
10 SSSRRRRRRRRRRRRSSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRSSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSRRRRRRRRR;
- 15 ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSSRSSSRSSRSSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRSRSRSRSRSRSSS;
- yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SRSSSRSSSRSSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSRSRSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSOSRSRSRSSOSSS;
- 25 bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSSRSSSRSSOSSS;
- ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 RRRORRRROSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRORRRROSSSSSSSSS;
- fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOOOSSSSSSSSSSSSS;
- 35 ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
OOOOOSSSSSSSSSSSSS;
- hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSSOSSOSSOSSS;

- iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSSSSSSSSSOSSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSOSSS;
- 5 kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSSOSSS;
- III. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSOSSOSSSSOSSS;
- mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSSOSSS; or
- 10 nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRRROSSSSSSSSOSSS.
- [0018] In some embodiments, the antisense oligonucleotide comprises modified sugar moieties. In some embodiments, the modified sugar moieties comprise 2'-O-methoxyethyl ribose (2'-O-
- 15 MOE). In some embodiments, the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs). In some embodiments, the antisense oligonucleotide comprises non-natural internucleotide linkages. In some embodiments, the non-natural internucleotide linkages are stereopure. In some embodiments, the non-natural internucleotide linkages are all Sp. In some embodiments, the non-natural internucleotide linkages are all Rp. In some
- 20 embodiments, the non-natural internucleotide linkages are independently selected from Sp and Rp, i.e., each internucleotide linkage is independently selected to be Sp or Rp. In some embodiments, the non-natural internucleotide linkages are stereorandom. In some embodiments, the antisense oligonucleotide comprises modified nucleobases.
- [0019] Also provided herein is a composition comprising an antisense oligonucleotide and
- 25 optionally a pharmaceutically acceptable carrier or excipient.
- [0020] In some embodiments, the present disclosure provides a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide complementary to a portion of SEQ ID NO:1, wherein the oligonucleotide hybridizes to a target
- 30 region of the *CD33* gene, wherein the oligonucleotide induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene. In some embodiments, the antisense oligonucleotide is complementary to a portion of: SEQ ID NO:213; SEQ ID NO:214; SEQ ID NO:215; SEQ ID NO:216; SEQ ID NO:217; SEQ ID NO:218; SEQ ID NO:219; and/or SEQ ID NO:220. In some
- embodiments, the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater.
- 35 In some embodiments, the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater according to a Standard Exon-Skipping Efficiency Assay for ASOs. In some embodiments, the Standard Exon-Skipping Efficiency Assay is a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises

phosphorodiamidate morpholino oligomers or a Standard Exon-Skipping Efficiency Assay for MOE ASOs when the antisense oligonucleotide comprises methoxyethyl ribose oligomers.

[0021] In some embodiments, the present disclosure provides a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises

- 5 all or a portion of:
 - a. PMO-002 (5'-CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:2);
 - b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);
 - c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
 - d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
 - 10 e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
 - f. PMO-038 (5'-GTACTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
 - g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
 - h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
 - i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
 - 15 j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
 - k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
 - l. PMO-096 (5'-ACTTGCAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:96);
 - m. PMO-007 (5'-CAGCCAGAAATTTGGATCCATAGCC-3') (SEQ ID NO:7);
 - n. PMO-097 (5'-AGAAATTTGGATCCATAGCCAGGGC-3') (SEQ ID NO:97);
 - 20 o. PMO-008 (5'-CCCTGTGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:8);
 - p. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
 - q. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
 - r. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
 - s. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
 - 25 t. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
 - u. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO: 11);
 - v. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
 - w. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
 - x. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
 - 30 y. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
 - z. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO:15);
 - aa. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
 - bb. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
 - cc. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
 - 35 dd. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
 - ee. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).

[0022] In some embodiments, the present disclosure provides a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- a. PMO-221 (5'- CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);
- 5 b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
- c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
- d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);
- e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
- f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
- 10 g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
- h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
- i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
- j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
- k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
- 15 l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
- m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
- n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGGG-3') (SEQ ID NO:234);
- o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
- p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
- 20 q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
- r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
- s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
- t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
- u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
- 25 v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
- w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
- x. PMO-244 (5'- TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
- y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
RRRRRRRRRRRRRRRRRRRRRR
- 30 z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSS
- aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRR; or
- bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
- 35 Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSS.

[0023] In some embodiments, the present disclosure provides a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
- b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
- c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
- d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);
- 5 e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- 10 j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- 15 o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- 20 t. MOE-264 (5'- ATCC-gAAAGAAAGTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- 25 y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- 30 dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID NO:012);
- gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
35 SSSSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
RRRRRRRRRRRRRRRRRRRR;

- ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSS;
- jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSS;
- 5 kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSRRRRRRRRSSSSSS;
- mm. MOE-283 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
10 SSSRRSRRSRRSRRSRRSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRRSRRSRRSRRSRRSS;
- oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSRRRRRRRRRRSSSSSS;
- 15 pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRRRRRRRRRRRRRRSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRSSSSSSSSSRSSSSSS;
- rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SSSSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRRR;
- tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRRRRRRRRRRRRSSS;
- 25 uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRSSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSRRRRRRRR;
- ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SSSRSSRSSSRSSSRSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSSRSSSRSSSRSS;
- yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRSSSRSSSRSSSRSS;
- 35 zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSOSSRSSSRSSSOSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSOSRSRSSSRSSSOSS;

- bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSRSSSRSSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSRSSSRSSSOSSS;
- 5 ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: RRRORRRROSSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SRRORRRROSSSSSSSSSS;
- fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOOSSSSSSSSSSSSSS;
- 10 ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: OOOOSSSSSSSSSSSSSS;
- hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSOSSSOSSSOSSS;
- 15 iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSSSOSSSSSSSOSSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSSSOSSS;
- kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSSOSSS;
- 20 III. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSOSSSOSSSOSSS;
- mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSOSSS; or
- 25 nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRRROSSSSSSSSSOSSS.

[0024] In some embodiments, the present disclosure provides a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the cell is an animal cell. In some embodiments, the cell is a human cell.

- 30 [0025] In some embodiments, the present disclosure provides a method of treating a subject having a neurodegenerative disease comprising administering a therapeutically effective amount of an antisense oligonucleotide of 16-30 nucleotides in length, wherein the antisense oligonucleotide is complementary to a portion of SEQ ID NO:1, and wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a
- 35 Standard Exon-Skipping Efficiency Assay for the antisense oligonucleotide.

[0026] In some embodiments, the present disclosure provides a method of treating a subject having a neurodegenerative disease mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- a. PMO-002 (5'-CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:2);
- b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);
- c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36)
- d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37)
- 5 e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTACTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38)
- g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39)
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82)
- 10 j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83)
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- l. PMO-096 (5'-ACTTGCAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:96);
- m. PMO-007 (5'-CAGCCAGAAATTTGGATCCATAGCC-3') (SEQ ID NO:7);
- n. PMO-097 (5'-AGAAATTTGGATCCATAGCCAGGGC-3') (SEQ ID NO:97);
- 15 o. PMO-008 (5'-CCCTGTGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:8);
- p. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- q. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- r. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- s. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- 20 t. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- u. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- v. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- w. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
- x. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- 25 y. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- z. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO: 15);
- aa. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- bb. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- cc. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- 30 dd. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- ee. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).

[0027] In some embodiments, the present disclosure provides a method of treating a subject having a neurodegenerative disease mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- 35 a. PMO-221 (5'- CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);
- b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
- c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
- d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);

- e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
- f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
- g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
- h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
- 5 i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
- j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
- k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
- l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
- m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
- 10 n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGGG-3') (SEQ ID NO:234);
- o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
- p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
- q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
- r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
- 15 s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
- t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
- u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
- v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
- w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
- 20 x. PMO-244 (5'-TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
- y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
RRRRRRRRRRRRRRRRRRRRRR
- z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSSSS
- 25 aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRRRR; or
- bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSSSS.
- [0028] In some embodiments, the present disclosure provides a method of treating a
- 30 subject having a neurodegenerative disease mentioned above, wherein the antisense
oligonucleotide comprises all or a portion of:
- a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
- b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
- c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
- 35 d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);
- e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);

- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- 5 l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- 10 q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- t. MOE-264 (5'- ATCC-gAAAGAAgTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- 15 v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- 20 aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- 25 ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID NO:012);
- gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRRR;
- 30 ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;
- jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;
- 35 kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSRSRSRSRSRSRSRS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSSSRRRRRRRRSSSSSS;

- mm. MOE-283 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- 5 oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSRRRRRRRRRRSSSSS;
- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRRRRRRRRRRRRSSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
10 SSRSSSSSSSSSRSSSSS;
- rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRR;
- 15 tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRRRRRRRRRRRRSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SSSSSSSSSRRRRRRRR;
- ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSSRSSSRSSRSSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRSRSRSRSRSRSSS;
- 25 yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRSSSRSSSRSSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSOSSRSSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SSSOSRSRSRSSSOSSS;
- bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSSRSSSRSSSOSSS;
- 35 ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRORRRROSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRRORRRROSSSSSSSSS;

- fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOOOSSSSSSSSSSSSS;
- ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: OOOOOSSSSSSSSSSSSS;
- 5 hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSOSSSOSSSOSSS;
- iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSSOSSSSSSSOSSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSSSOSSS;
- 10 kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSSOSSS;
- III. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSOSSSOSSSOSSS;
- 15 mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSOSSS; or
- nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRRROSSSSSSSOSSS.
- [0029] In some embodiments, the present disclosure provides a method of treating a subject having a neurodegenerative disease mentioned above, wherein the neurodegenerative disease is Alzheimer's Disease.
- [0030] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that is complementary to a portion of SEQ ID NO:1, that hybridizes to a target region of the *CD33* gene, and that induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene. In some embodiments, the antisense oligonucleotide is complementary to a portion of: SEQ ID NO:213; SEQ ID NO:214; SEQ ID NO:215; SEQ ID NO:216; SEQ ID NO:217; SEQ ID NO:218; SEQ ID NO:219; and/or SEQ ID NO:220. In some embodiments, the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater. In some embodiments, the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater according to a Standard Exon-Skipping Efficiency Assay for ASOs. In some embodiments, the Standard Exon-Skipping Efficiency Assay is a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises phosphorodiamidate morpholino oligomers or a Standard Exon-Skipping Efficiency Assay for MOE ASOs when the antisense oligonucleotide comprises methoxyethyl ribose oligomers.

[0031] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- a. PMO-002 (5'-CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:2);
- 5 b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);
- c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
- d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
- e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTAATTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
- 10 g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
- j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- 15 l. PMO-096 (5'-ACTTGCAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:96);
- m. PMO-007 (5'-CAGCCAGAAATTTGGATCCATAGCC-3') (SEQ ID NO:7);
- n. PMO-097 (5'-AGAAATTTGGATCCATAGCCAGGGC-3') (SEQ ID NO:97);
- o. PMO-008 (5'-CCCTGTGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:8);
- p. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- 20 q. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- r. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- s. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- t. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- u. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- 25 v. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- w. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
- x. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- y. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- z. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO:15);
- 30 aa. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- bb. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- cc. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- dd. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- ee. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).

35 [0032] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:

- a. PMO-221 (5'-CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);

- b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
- c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
- d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);
- e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
- 5 f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
- g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
- h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
- i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
- j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
- 10 k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
- l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
- m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
- n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGGG-3') (SEQ ID NO:234);
- o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
- 15 p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
- q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
- r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
- s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
- t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
- 20 u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
- v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
- w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
- x. PMO-244 (5'- TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
- y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
- 25 RRRRRRRRRRRRRRRRRRRRR
- z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSS
- aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRR; or
- 30 bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSS.
- [0033] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of inducing Exon-2 skipping in the *CD33* gene mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:
- 35 a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
- b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
- c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
- d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);

- e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- 5 i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- 10 n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- 15 s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- t. MOE-264 (5'- ATCC-gAAAGAAgTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- 20 x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- 25 cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID NO:012);
- 30 gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRR;
- ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;
- 35 jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;

- kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSRSRSRSRSRSSSS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSRRRRRRRSSSSSS;
- 5 mm. MOE-283 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
10 SSSSRRRRRRRRRSSSSS;
- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRRRRRRRRRRSSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRSSSSSSSRSSSSS;
- 15 rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRR;
- tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SSSRRRRRRRRRRSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSRRRRRRRRR;
- 25 ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRRSSRRSSRRSSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSRSRSRSRSSS;
- yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SRSSSRSSSRSSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSRSSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSRSRSRSSOSSS;
- 35 bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSOSSSRSSSRSSOSSS;

- ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: RRRORRRROSSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SRRORRRROSSSSSSSSSS;
- 5 fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOOSSSSSSSSSSSSSS;
- ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: OOOOSSSSSSSSSSSSSS;
- hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSOSSSOSSSOSS;
- 10 iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSSSOSSSSSSSOSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSSSOSS;
- 15 kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSSOSS;
- lll. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSOSSSOSSSOSS;
- mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSSSOSS; or
- 20 nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRROSSSSSSSSSOSS.
- [0034] In some embodiments, the cell is an animal cell. In some embodiments, the animal cell is a human cell.
- 25 [0035] In some embodiments, the method of inducing Exon-2 skipping is performed *in vitro*. In some embodiments, the method of inducing Exon-2 skipping is performed *in vivo*.
- [0036] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of treating a subject having a neurodegenerative disease comprising administering a therapeutically effective amount of an antisense oligonucleotide of
- 30 16-30 nucleotides in length, wherein the antisense oligonucleotide is complementary to a portion of SEQ ID NO:1, and wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for the antisense oligonucleotide.
- [0037] In some embodiments, the present disclosure provides an antisense oligonucleotide
- 35 mentioned above for use in a method of treating a subject having a neurodegenerative disease mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:
- a. PMO-002 (5'-CCTCACCTGTACATGCACAGAGAG-3') (SEQ ID NO:2);
- b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);

- c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
- d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
- e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTAAGTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
- 5 g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
- j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- 10 l. PMO-096 (5'-ACTTGCAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:96);
- m. PMO-007 (5'-CAGCCAGAAATTTGGATCCATAGCC-3') (SEQ ID NO:7);
- n. PMO-097 (5'-AGAAATTTGGATCCATAGCCAGGGC-3') (SEQ ID NO:97);
- o. PMO-008 (5'-CCCTGTGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:8);
- p. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- 15 q. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- r. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- s. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- t. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- u. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- 20 v. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- w. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
- x. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- y. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- z. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO:15);
- 25 aa. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- bb. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- cc. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- dd. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- ee. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).
- 30 [0038] In some embodiments, the present disclosure provides an antisense oligonucleotide mentioned above for use in a method of treating a subject having a neurodegenerative disease mentioned above, wherein the antisense oligonucleotide comprises all or a portion of:
 - a. PMO-221 (5'- CCTCACCTGTACATGCACAGAG-3') (SEQ ID NO:221);
 - b. PMO-222 (5'- TCACCTGTACATGCACAGAGAG-3') (SEQ ID NO:222);
 - 35 c. PMO-223 (5'- CTCACCTGTACATGCACAGAGA-3') (SEQ ID NO:223);
 - d. PMO-224 (5'- CCTCACCTGTACATGCACAG-3') (SEQ ID NO:224);
 - e. PMO-225 (5'- ACCTGTACATGCACAGAGAG-3') (SEQ ID NO:225);
 - f. PMO-226 (5'- TCACCTGTACATGCACAGAG-3') (SEQ ID NO:226);

- g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
5 k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGGG-3') (SEQ ID NO:234);
o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
10 p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
15 u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
x. PMO-244 (5'- TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
20 RRRRRRRRRRRRRRRRRRRRRR
z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSS
aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRRRR; or
25 bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSSSS.
[0039] In some embodiments, the present disclosure provides an antisense
oligonucleotide mentioned above for use in a method of treating a subject having a
neurodegenerative disease mentioned above, wherein the antisense oligonucleotide comprises
30 all or a portion of:
a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);
35 e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);

- i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- 5 m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- 10 r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- t. MOE-264 (5'- ATCC-gAAAGAAgTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- 15 w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- 20 bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID
- 25 NO:012);
- gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
RRRRRRRRRRRRRRRRRRRR;
- 30 ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSS;
- jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSSSRSSSRSSSRSS;
- kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
35 SSSRSRSRSRSRSRSRS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSSRRRRRRRRSSSSSS;

- mm. MOE-283 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- 5 oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSRRRRRRRRRRSSSSS;
- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRRRRRRRRRRRRSSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
10 SSRSSSSSSSSSRSSSSS;
- rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRR;
- 15 tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRRRRRRRRRRRRSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SSSSSSSSSRRRRRRRR;
- ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRRSSRRSSRRSSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRSRSRSRSRSRSSS;
- 25 yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRSSSRSSSRSSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSRSSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SSSOSRSRSRSSOSSS;
- bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSSRSSSRSSOSSS;
- 35 ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRORRRROSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRRORRRROSSSSSSSSS;

- fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOOOSSSSSSSSSSSSSS;
- ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
OOOOOSSSSSSSSSSSSSS;
- 5 hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSSOSSSOSSSOSSS;
- iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSSOSSSSSSSOSSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
10 SSOSSSSSSSSSSSOSSS;
- kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSSSSSSSSSSOSSS;
- III. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSSSSOSSSOSSSOSSS;
- 15 mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSORRRRRSSSSSOSSS; or
- nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern:
RRRRROSSSSSSSOSSS.
- [0040] In some embodiments, the present disclosure provides a method of treating a subject
20 having a neurodegenerative disease mentioned above, wherein the neurodegenerative disease
is Alzheimer's Disease.

Brief Description of the Figures

- [0041] This application file contains figures in color. Copies of this patent application publication
with color drawing(s) will be provided by the Office upon request and payment of the necessary
25 fee.
- [0042] Fig. 1 shows the levels of *CD33* mRNA in plasma and cerebral spinal fluid in patients
relative to the rs3865444 SNP. C = rs3865444-C, A = rs3865444-A.
- [0043] Fig. 2 shows various cognitive results in patients with the rs3865444-A allele vs. patients
with the rs201074739 indel frameshift allele.
- 30 [0044] Fig. 3 shows various physiological results in patients with the rs3865444-A allele vs.
patients with the rs201074739 indel allele.
- [0045] Fig. 4 shows the levels of *CD33* mRNA in plasma and cerebral spinal fluid in patients
relative to the rs201074739 indel.
- [0046] Fig. 5 shows the exon skipping efficiencies of several PMO sequences at different
35 concentrations.
- [0047] Fig. 6 shows the exon skipping efficiencies of several MOE sequences at different
concentrations.

- [0048] Fig. 7 shows the fold change (*in vivo* ability to increase Exon-2-skipped *CD33* mRNA) of two ASOs relative to control (PBS) in mouse hippocampus at two dose levels. D2-CD33 = Exon-2-skipped *CD33* mRNA, PMO-002 = SEQ ID NO:2, MOE-012 = SEQ ID NO:12.
- [0049] Fig. 8 shows the fold change (*in vivo* ability to increase Exon-2-skipped *CD33* mRNA) of two ASOs relative to control (PBS) in mouse cortex at two dose levels. D2-CD33 = Exon-2-skipped *CD33* mRNA, PMO-002 = SEQ ID NO:2, MOE-012 = SEQ ID NO:12.
- [0050] Fig. 9 shows the percent Exon-2 skipping in *CD33* mRNA in mouse cortex and hippocampus for PMO-221, PMO-224, PMO-232, PMO-233, PMO-237, PMO-238, PMO-002, and PMO-003. D2-CD33 = Exon-2-skipped *CD33* mRNA.
- [0051] Fig. 10 shows the fold change (*in vivo* ability to increase Exon-2-skipped *CD33* mRNA) of (i) PMO-224 relative to control (PBS) in mouse cortex and hippocampus at three dose levels and (ii) PMO-002 relative to control (PBS) in mouse cortex and hippocampus at one dose level. D2-CD33 = Exon-2-skipped *CD33* mRNA.
- [0052] Fig. 11 shows HPLC chromatogram and HRMS trace of PMO-424.
- [0053] Fig. 12 shows HPLC chromatogram and HRMS trace of PMO-324.
- [0054] Fig. 13 shows T_m of PMO-324, PMO-424, and PMO-224.
- [0055] Fig. 14 shows HPLC chromatogram and HRMS trace of PMO-502.
- [0056] Fig. 15 shows HPLC chromatogram and HRMS trace of PMO-402.
- [0057] Fig. 16 shows T_m of PMO-402, PMO-502, and PMO-002.
- [0058] Fig. 17 shows chromatogram of PMO-424 with N³'-trityl group (resin cleaved).
- [0059] Fig. 18 shows the fold change (*in vivo* ability to increase Exon-2-skipped *CD33* mRNA) of PMO-324 and PMO-424 relative to control (PBS) in mouse cortex and hippocampus at two dose levels. D2-CD33 = Exon-2-skipped *CD33* mRNA.
- [0060] Fig. 19 shows the fold change (*in vivo* ability to increase Exon-2-skipped *CD33* mRNA) of PMO-402 and PMO-502 relative to control (PBS) in mouse cortex and hippocampus at two dose levels. D2-CD33 = Exon-2-skipped *CD33* mRNA.
- [0061] Fig. 20 shows the melting temperature of MOE-012, MOE-277, and MOE-278.
- [0062] Fig. 21 shows the HPLC elution profile of stereopure ASOs MOE-288 to MOE-292 and stereorandom ASO MOE-252.
- [0063] Fig. 22 shows the *in vivo* activity of ASOs MOE-012 and MOE-246 to MOE-256 with 100 µg dosing.
- [0064] Fig. 23 shows the *in vivo* activity of ASOs MOE-012 and MOE-257 to MOE-261 with 100 µg dosing.
- [0065] Fig. 24 shows the *in vivo* activity of ASOs MOE-262 to MOE-267 and MOE-252 with 30 µg dosing.
- [0066] Fig. 25 shows the *in vivo* activity of ASOs MOE-277 and MOE-279 to MOE-284 with 30 µg dosing.

[0067] Fig. 26 shows the *in vivo* activity of ASOs MOE-252, MOE-288, MOE-291, and MOE-292 with 30 µg and 100 µg dosing, and MOE-289 and MOE-290 with 30 µg dosing.

[0068] Fig. 27 shows the *in vivo* activity of ASOs MOE-293 to MOE-299 with 30 µg and 100 µg dosing.

5 [0069] Fig. 28 shows the *in vivo* activity of ASOs MOE-300, MOE-301 and MOE-303 to MOE-311 with 100 µg dosing

[0070] Fig. 29 shows the *in vivo* activity of MOE-279 with 10 µg, 30 µg, 60 µg, and 100 µg dosing.

10 [0071] Fig. 30 shows the duration of the skipping effect with a single 100 µg ICV dose of MOE-277 (up to 150 days).

[0072] Fig. 31 shows the brain concentration of MOE-277 after a single 100 µg ICV dose (up to 150 days).

Definitions

15 [0073] The term “oligonucleotide” is used herein to refer to a nucleotide sequence comprising at least ten DNA or RNA nucleotides.

[0074] The term “antisense oligonucleotide,” abbreviated as “ASO,” is used herein to refer to a nucleotide sequence comprising an antisense sequence that is sufficiently complementary to a target nucleotide sequence in order to form a stable double stranded hybrid with the target nucleotide sequence. In some embodiments, the target nucleotide sequence is an RNA
20 nucleotide sequence. Unless otherwise specified, ASOs represented herein are displayed in the 5' to 3' orientation.

[0075] The term “nucleobase” is used herein to refer to a base that is a component of a nucleoside. Example nucleobases include adenine, guanine, thymine, cytosine, and uracil.

25 [0076] The term “nucleoside” is used herein to refer to a nucleobase covalently linked to a sugar. Examples of naturally occurring and non-natural nucleosides are described below.

[0077] The term “nucleotide” is used herein to refer to a nucleoside covalently linked to a phosphate group. Examples of naturally occurring nucleotides include adenosine, thymidine, uridine, cytidine, 5-methylcytidine, and guanosine. Description and examples of non-natural nucleotides are described below.

30 [0078] Within the ASO structure, the phosphate groups are commonly referred to as forming the “internucleotide linkages” of the ASO. The naturally occurring internucleotide linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. A “phosphoramidate” group comprises phosphorus having three attached oxygen atoms and one attached nitrogen atom, while a “phosphorodiamidate” group comprises phosphorus having two attached oxygen atoms and two
35 attached nitrogen atoms. A “phosphorotriamidate” group (or a phosphoric acid triamide group) comprises phosphorus having one attached oxygen atom and three attached nitrogen atoms. In the uncharged or the cationic internucleotide linkages of the morpholino-based ASOs described

herein, one nitrogen is always pendant to the linkage chain. The second nitrogen, in a phosphorodiamidate linkage, is typically the ring nitrogen in a morpholino ring structure.

[0079] The term “non-natural” is used herein to refer to molecules that contain man-made modifications relative to their naturally occurring counterparts. In some embodiments, “non-

5 “non-natural” may refer to one or more nucleotide subunits having at least one modification selected from (i) a modified internucleotide linkage, e.g., an internucleotide linkage other than the standard phosphodiester linkage found in naturally-occurring oligonucleotides, (ii) modified sugar moieties, e.g., moieties other than ribose or deoxyribose moieties found in naturally occurring oligonucleotides, (iii) modified nucleobases, e.g., bases other than those found in
10 naturally occurring oligonucleotides, or (iv) a any combination of the foregoing. In some embodiments, the ASO is chosen from ASOs that do not have a phosphorus atom in the internucleotide linkage (backbone). In some embodiments, the ASO has a phosphorodiamidate or phosphorothioate modified internucleotide linkage (backbone).

[0080] The term “morpholino” is used herein to refer to a nucleotide that contains a morpholinyl
15 ring instead of a ribose.

[0081] The term “morpholino-based ASO” is used herein to refer to an ASO with at least one nucleotide containing a morpholinyl ring instead of a ribose.

[0082] The term “stereo-controlled” is used herein to describe when a nucleotide and/or an oligonucleotide is designed or selected to have a particular stereochemistry. In some
20 embodiments, the nucleobase portion of a nucleotide or oligonucleotide, including any and all non-natural modifications, is stereo-controlled. In some embodiments, the nucleoside portion of a nucleotide or oligonucleotide, including any and all non-natural modifications, is stereo-controlled. In some embodiments, the internucleotide linkage portion of a nucleotide or oligonucleotide, including any and all non-natural modifications, is stereo-controlled. In some
25 embodiments, a nucleotide may comprise one or a combination of these stereo-controlled portions. In some embodiments, an oligonucleotide may comprise a combination of nucleotides that comprise a combination of stereo-controlled nucleotides. In some embodiments, an oligonucleotide may comprise a combination of nucleotides that are stereo-controlled and not stereo-controlled. In some embodiments, the proportion of stereo-controlled nucleotides ranges
30 from 10%-100%, such as 15%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 50%-90%, 50%-95%, 60%-100%, 60%-90%, 60%-95%, 70%-100%, 70%-90%, 70%-95%, 80-100%, 80%-90%, 80%-95%, 90-100%, 90%-95%, 90%-96%, 90%-97%, 90%-98%, 90%-99%, 95%-98%, 95%-99%, 95-100%, 50%-90%, or 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%,
35 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of nucleotides.

[0083] When applied to nucleotides, the term “stereopure” is used herein to describe when at least 90% of nucleotides in an oligonucleotide are stereo-controlled. In some embodiments, the

proportion of stereo-controlled nucleotides in a stereopure ASO ranges from 90-100%, 95-100%, 90%-95%, 90%-96%, 90%-97%, 90%-98%, 90%-99%, 95%-98%, 95%-99%, or 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of nucleotides. In some embodiments, all or a portion of nucleotides within an oligonucleotide are stereo-controlled so that they are stereopure in the same way, i.e., all or a portion of the nucleotides are stereo-controlled, and they are designed or selected to have the same stereochemistry. In some embodiments, all or a portion of nucleotides within an oligonucleotide are stereo-controlled so that they are not stereopure in the same way, i.e., all or a portion of the nucleotides are stereo-controlled, but they are designed or selected to have different stereochemistry. When applied to the internucleotide linkage portion of an oligonucleotide, the term "stereopure" is used to describe when at least 90% of the internucleotide linkages are stereo-controlled. In some embodiments, the proportion of stereo-controlled internucleotide linkages in a stereopure ASO ranges from 90-100%, 95-100%, 90%-95%, 90%-96%, 90%-97%, 90%-98%, 90%-99%, 95%-98%, 95%-99%, or 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of internucleotide linkages. In some embodiments, all or a portion of internucleotide linkages within an oligonucleotide are stereo-controlled so that they are stereopure in the same way, i.e., all or a portion of the internucleotide linkages are stereo-controlled, and they are designed or selected to have the same stereochemistry. In some embodiments, all or a portion of internucleotide linkages within an oligonucleotide are stereo-controlled so that they are not stereopure in the same way, i.e., all or a portion of the internucleotide linkages are stereo-controlled, but they are designed or selected to have different stereochemistry. In some embodiments, the internucleotide linkages are phosphorodiamidate linkages. In some embodiments, the internucleotide linkages are phosphorothioate linkages.

[0084] Stereochemistry for (Rp, Sp) and phosphate (PO) internucleotide linkages is illustrated as the following:



Stereochemistry for Rp, Sp, and PO internucleotide linkages is also illustrated as follows: S = Sp, R = Rp, O = phosphate.

[0085] For example, the stereochemistry of the internucleotide linkages of MOE-298 can be shown using either of the following illustrations:



(5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSRSSSRSSSOSSS.

- [0086] When applied to nucleotides, the term “stereorandom” is used herein to describe when the nucleotides in an oligonucleotide are not stereo-controlled. When applied to internucleotide linkages, the term “stereorandom” is used herein to describe when the internucleotide linkages in an oligonucleotide are not stereo-controlled. In some embodiments, the internucleotide linkages are phosphorodiamidate linkages. In some embodiments, the internucleotide linkages are phosphorothioate linkages.
- [0087] The term “complementary” is used herein to describe when the corresponding positions of at least two nucleotide sequences are occupied by nucleotides which can hydrogen bond with each other.
- [0088] The term “hybridize” is used herein to describe the binding of two complementary nucleotide sequences, forming one double stranded molecule. When a sufficient number of corresponding nucleotides in two sequences can hydrogen bond with each other, i.e., they are sufficiently complementary, they may form a stable hybrid. It is understood in the art that 100% complementarity is not necessary for an ASO to hybridize with a target sequence.
- [0089] The term “sufficient complementarity” is used herein to indicate a level of complementarity sufficient to permit an ASO to bind to its target sequence and form a stable hybrid. In some embodiments, the complementarity of the ASO and the target sequence is at least 99%, or 98%, or 97%, or 96%, or 95%, or 94%, or 93%, or 92%, or 91%, or 90%, or 89%, or 88%, or 87%, or 86%, or 85%, or 84%, or 83%, or 82%, or 81%, or 80%, or 79%, or 78%, or 77%, or 76%, or 75%, or 74%, or 73%, or 72%, or 71%, or 70%.
- [0090] The term “sequence similarity” is used herein to express the similarity of two ASOs. Sequence similarity is expressed as a percentage of nucleotides shared between two ASOs. It is understood that identical sequences have 100% sequence similarity.
- [0091] The terms “target region” and “target sequence” are used interchangeably herein to designate a nucleotide sequence to which an ASO will hybridize under physiological conditions. It is not necessary for the ASO and the target region to be 100% complementary, so long as there is sufficient complementarity for the ASO to hybridize to the target sequence and form a stable hybrid. The ASO may hybridize to all or a portion of the target sequence.
- [0092] The terms “treat,” “treating,” or “treatment” are used herein to refer to ameliorating a disease or disorder (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). The terms also refer to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. The terms also refer to modulating the disease or disorder, either physically (e.g., through stabilization of a discernible symptom), physiologically, (e.g., through stabilization of a physical parameter), or both.
- [0093] The terms “prevent,” “preventing,” or “prevention” are used herein to refer to inhibiting or delaying the onset of a disease or disorder.

[0094] The term “therapeutically effective amount” is used herein to refer to the amount of a therapeutic agent or composition effective in prevention or treatment of a disorder or disease. In some embodiments, this includes an amount of a therapeutic agent or composition effective in the prevention or treatment of a neurodegenerative disease.

5 [0095] The term “pharmaceutically acceptable” is used herein to refer to a molecular entity or composition that is pharmaceutically useful and not biologically or otherwise undesirable.

[0096] The term “carrier” is used herein to refer to a diluent, adjuvant, excipient, or vehicle with which the compound is administered.

10 [0097] The term “excipient” as used herein refers to any ingredient in a pharmaceutical composition other than the active ingredient.

[0098] As used herein, “skipping efficiency” of an oligonucleotide is calculated using the following formula:

$$\text{Skipping \%} = \frac{(\text{Skipped Value})}{(\text{Skipped}) + (\text{Un-skipped})} \times 100$$

and is represented on a scale of 0 to 100, wherein 100 represents 100% skipping of *CD33*

15 Exon-2. “Skipping efficiency” of an oligonucleotide as used herein is experimentally determined using one of three Standard Exon-Skipping Efficiency Assays depending on the type of antisense oligonucleotide. For antisense oligonucleotides comprising phosphorodiamidate morpholino oligomers, the Standard Exon-Skipping Efficiency Assay for PMO ASOs defined below is used; for antisense oligonucleotides comprising methoxyethyl ribose oligomers, the
20 Standard Exon-Skipping Efficiency Assay for MOE ASOs defined below is used; and for antisense oligonucleotides that do not comprise phosphorodiamidate morpholino or methoxyethyl ribose oligomers, the Standard Exon-Skipping Efficiency Assay for non-PMOs and non-MOE described below is used.

[0099] The Standard Exon-Skipping Efficiency Assay for PMO ASOs includes using U-188 MG
25 cells that were cultured and maintained using appropriate media suggested in the vendor protocols (Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum). The Assay is performed in 96 well plate format, seeding about 20,000 cells per well and treating with the PMO ASO at a concentration of 0.5 μ M using the Endo-Porter protocol. Cells are incubated at 37°C in a cell culture incubator for 48 hours before isolating the total RNA. Total RNA is isolated
30 and converted to cDNA per vendor protocol, then Taqman gene expression assays are used to quantify Exon-2 skipped *CD33* (Forward primer: CGCTGCTGCTACTGCTG (SEQ ID NO:207); Reverse Primer: TTCTAGAGTGCCAGGGATGA (SEQ ID NO:208); and probe: TGTGGGCAGACTTGACCCACAG (SEQ ID NO:209)) and un-skipped *CD33* (Forward primer: GGATGGAGAGAGGAAGTA (SEQ ID NO:210); Reverse Primer: GTGCCAGGGATGAGGATTT
35 (SEQ ID NO:211); and probe: TGCATGTGACAGACTTGACCCACA (SEQ ID NO:212)) mRNA transcripts. Human house-keeping genes such as HPRT1 (Assay ID: Hs02800695_m1;

ThermoFisher Scientific) or GAPDH1 (Hs99999905_m1; ThermoFisher Scientific) expressions are used to normalize the target transcript expressions.

[0100] The Standard Exon-Skipping Efficiency Assay for MOE ASOs includes using U-188 MG cells that are cultured and maintained using appropriate media suggested in the vendor protocols (Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum). The Assay is performed in 96 well plate format, seeding about 20,000 cells per well and treating with the MOE ASO at a concentration of 10 nM using the Lipofectamine protocol. Cells are incubated at 37°C in a cell culture incubator for 48 hours before isolating the total RNA. Total RNA is isolated and converted to cDNA per vendor protocol, then Taqman gene expression assays are used to quantify Exon-2 skipped *CD33* (Forward primer: CGCTGCTGCTACTGCTG (SEQ ID NO:207); Reverse Primer: TTCTAGAGTGCCAGGGATGA (SEQ ID NO:208); and probe: TGTGGGCAGACTTGACCCACAG (SEQ ID NO:209)) and un-skipped *CD33* (Forward primer: GGATGGAGAGAGGAAGTA (SEQ ID NO:210); Reverse Primer: GTGCCAGGGATGAGGATTT (SEQ ID NO:211); and probe: TGCATGTGACAGACTTGACCCACA (SEQ ID NO:212)) mRNA transcripts. Human house-keeping genes such as HPRT1 (Assay ID: Hs02800695_m1; ThermoFisher Scientific) or GAPDH1 (Hs99999905_m1; ThermoFisher Scientific) expressions are used to normalize the target transcript expressions.

[0101] For ASOs that are neither PMOs or MOEs, the Standard Exon-Skipping Efficiency Assay for non-PMOs and non-MOE includes using U-188 MG cells that are cultured and maintained using appropriate media suggested in the vendor protocols (Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum). The Assay is performed in 96 well plate format, seeding about 20,000 cells per well and treating with the ASO at a concentration of 10 nM using the Lipofectamine protocol. Cells are incubated at 37°C in a cell culture incubator for 48 hours before isolating the total RNA. Total RNA is isolated and converted to cDNA per vendor protocol, then Taqman gene expression assays are used to quantify Exon-2 skipped *CD33* (Forward primer: CGCTGCTGCTACTGCTG (SEQ ID NO:207); Reverse Primer: TTCTAGAGTGCCAGGGATGA (SEQ ID NO:208); and probe: TGTGGGCAGACTTGACCCACAG (SEQ ID NO:209)) and un-skipped *CD33* (Forward primer: GGATGGAGAGAGGAAGTA (SEQ ID NO:210); Reverse Primer: GTGCCAGGGATGAGGATTT (SEQ ID NO:211); and probe: TGCATGTGACAGACTTGACCCACA (SEQ ID NO:212)) mRNA transcripts. Human house-keeping genes such as HPRT1 (Assay ID: Hs02800695_m1; ThermoFisher Scientific) or GAPDH1 (Hs99999905_m1; ThermoFisher Scientific) expressions are used to normalize the target transcript expressions.

[0102] As an alternative to the Lipofectamine protocol, free uptake (without transfection reagents) may be used for the Standard Exon-Skipping Efficiency Assay.

[0103] In some embodiments, the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 25% to 99%, such as 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. In some embodiments, the antisense oligonucleotide has

a *CD33* Exon-2 skipping efficiency of 50% to 99%. In some embodiments, the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of at least 30%.

[0104] Unless otherwise defined, all other scientific and technical terms have the same meaning as commonly understood to one of ordinary skill in the art. Such scientific and technical terms are explained in the literature, for example: J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press (1989); Martin, *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co (1990); Glover, *DNA Cloning: A Practical Approach*, Volumes I and II, MRL Press, Ltd. (1985); and Ausubel, F et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates/Wiley Intersciences (2002).

[0105] Disclosed herein are novel ASOs. In some embodiments, the ASOs are directed to a target sequence in the *CD33* pre-mRNA. In some embodiments, the ASOs are directed to all or a portion of a 16- to 30-nucleotide target sequence in the *CD33* pre-mRNA, represented in SEQ ID NO:1 (5'-GGGCAGGTGA GTGGCTGTGG GGAGAGGGGT TGTCGGGCTG

GGCCGAGCTG ACCCTCGTTT CCCCACAGGG GCCCTGGCTA TGGATCCAA
TTTCTGGCTG CAAGTGCAGG AGTCAGTGAC GGTACAGGAG GGTTTGTGCG
TCCTCGTGCC CTGCACTTTC TTCCATCCCA TACCCTACTA CGACAAGAAC
TCCCCAGTTC ATGGTTACTG GTTCCGGGAA GGAGCCATTA TATCCAGGGA
CTCTCCAGTG GCCACAAACA AGCTAGATCA AGAAGTACAG GAGGAGACTC
AGGGCAGATT CCGCCTCCTT GGGGATCCCA GTAGGAACAA CTGCTCCCTG
AGCATCGTAG ACGCCAGGAG GAGGGATAAT GGTTTCATACT TCTTTCCGAT
GGAGAGAGGA AGTACCAAAT ACAGTTACAA ATCTCCCCAG CTCTCTGTGC
ATGTGACAGG TGAGGCACAG GCTTCAGAAG TGGCCGCAAG GGAAGTTCAT
GGGTACTGCA GGGCAGGGCT GGGATGGGAC CCTGGTACTG-3'). SEQ ID NO:1 includes
Exon-2 and portions of the bordering introns of the *CD33* gene. This 16- to 30-nucleotide target sequence is involved in Exon-2 skipping that also occurs when *CD33* mRNA includes the rs3865444-A SNP. When this Exon-2 skipping occurs, pre-mRNA containing the SNP is spliced so that Exon-2 is not included in the final transcript.

[0106] In some embodiments, the ASOs are 16-30 nucleotides long. In some embodiments, the nucleotides are 20-30 nucleotides long. In some embodiments, the ASOs are 25-30 nucleotides long. In some embodiments, the ASOs are 21-30 nucleotides long. In some embodiments, the ASOs are 21-25 nucleotides long. In some embodiments, the ASOs are 18-21 nucleotides long. In some embodiments, the ASOs are 18-25 nucleotides long. In some embodiments, the ASOs are 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides long.

[0107] In some embodiments, the antisense oligonucleotide comprises 16-30, such as 18-30, nucleotides. In some embodiments, the antisense oligonucleotide consists of 16-30, such as 18-30, nucleotides.

[0108] Also disclosed herein are novel ASOs complementary to all or a portion of a 10- to 16-nucleotide target sequence in the *CD33* pre-mRNA, represented in SEQ ID NO:1, which includes Exon-2 and portions of the bordering introns of the *CD33* gene. In some embodiments, the ASOs are 10-14 nucleotides long. In some embodiments, the ASOs are 10, 11, 12, 13, 14, 5 15, or 16 nucleotides long.

[0109] In some embodiments, the ASOs are directed to the 16- to 30-nt target sequence, are sufficiently complimentary to the target sequence to form a stable hybrid, and are 16-30 nucleotides in length. In some embodiments, these ASOs are sufficiently complimentary to all or a portion of the 25-nt target sequence.

10 [0110] In some embodiments, the ASOs have one of the specific sequences disclosed in Table 3 or 4. In some embodiments, the ASOs may share sequence similarity with one of the ASOs disclosed in Table 3 or 4. In some embodiments, the ASO shares at least 99%, or 98%, or 97%, or 96%, or 95%, or 94%, or 93%, or 92%, or 91%, or 90%, or 89%, or 88%, or 87%, or 86%, or 85%, or 84%, or 83%, or 82%, or 81%, or 80%, or 79%, or 78%, or 77%, or 76%, or 75%, or 15 74%, or 73%, or 72%, or 71%, or 70% sequence similarity with one of the ASOs disclosed in Table 3 or 4.

[0111] In some embodiments, at least some nucleobases of the ASOs will have thymine instead of uracil or will have uracil instead of thymine. In some embodiments, at least some nucleosides of the ASOs will have deoxyribose replaced with ribose, or will have ribose 20 replaced with deoxyribose.

[0112] In some embodiments, the ASOs comprise at least one chemically modified nucleotide. In some embodiments, the at least one chemical modification of the nucleotide is chosen from chemical modification of at least one nucleobase, chemical modification of at least one sugar moiety, chemical modification of at least one phosphate, and any combination of these 25 modifications. In some embodiments, the at least one chemical modification improves the ability of the nucleotide to resist nuclease degradation.

[0113] Non-limiting examples of chemical modifications useful in this disclosure include chemical modifications of an ASO's phosphate backbone and chemically modified (i.e., non-natural) internucleoside linkage(s). In some embodiments, the ASO is chosen from ASOs 30 having a chemically modified phosphate backbone. In some embodiments, the ASO is chosen from ASOs that do not have a phosphorus atom in the backbone. In some embodiments, the ASO has a phosphorodiamidate or phosphorothioate modified backbone. In some embodiments, the modified backbone is stereo-controlled.

[0114] Additional non-limiting examples of chemical modifications useful in this disclosure 35 include chemical modifications of at least one sugar moiety in an ASO. In some embodiments, the ASO comprises at least one chemically modified sugar moiety. In some embodiments, the chemically modified sugar moiety is chosen from sugar moieties substituted in at least one position on the sugar moiety in the ASO. In some embodiments, the ASO is chosen from ASOs

that are substituted in at least one position on the sugar chosen from the 2', 3' and 5' positions. In some embodiments, the at least one substituent on the ASOs' sugar moieties is chosen from hydroxyl; fluoro; and substituted or unsubstituted, linear or branched C₁-C₁₀ alkyl groups, substituted or unsubstituted, linear or branched C₂-C₁₀ alkenyl groups, substituted or unsubstituted, linear or branched C₂-C₁₀ alkynyl groups, substituted or unsubstituted, linear or branched C₇-C₁₇ alkaryl groups, substituted or unsubstituted, linear or branched C₃-C₁₀ allyl groups, and substituted or unsubstituted, linear or branched C₇-C₁₇ aralkyl groups, each of which groups may optionally further comprise at least one heteroatom. In some embodiments, the sugar moiety comprises at least one substituent chosen from methoxy, aminopropoxy, methoxyethoxy, dimethylaminoethoxy, and dimethylaminoethoxyethoxy. In some embodiments, the sugar moiety is chosen from pyranoses, derivatives of pyranoses, deoxypyranoses, derivatives of deoxypyranoses, riboses, derivatives of riboses, deoxyriboses, and derivatives of deoxyribose. In some embodiments, the substituted sugar moiety is chosen from methoxyethyl substitute sugar moieties, including 2'-O-methoxyethyl. In some embodiments, the sugar moiety is stereo-controlled.

[0115] In some embodiments, the sugar moiety is modified in a manner that creates a bicyclic sugar moiety. In some embodiments, the bicyclic sugar moiety is formed from a bridge modification between the 4' and 2' furanose ring atoms. In some embodiments, the bridge modification comprises at least one group that forms a bridge between the 4' and 2' furanose ring atoms. In some embodiments, at least one nucleotide in a given ASO has a bridge modification.

[0116] In some embodiments, the sugar moiety comprises fewer than 5 ring atoms, such as 4 ring atoms. In some embodiments, the sugar moiety comprises more than 5 ring atoms, such as 6 ring atoms. In some embodiments, the sugar moiety is modified to include a morpholino. Morpholino-based ASOs refer to an ASO comprising morpholino subunits supporting a nucleobase and, instead of a ribose, containing a morpholinyl ring. Non-limiting examples of internucleotide linkages for such morpholino-based ASOs include, for example, phosphoramidate or phosphorodiamidate internucleotide linkages joining the morpholinyl ring nitrogen of one morpholino subunit to the 4' exocyclic carbon of an adjacent morpholino subunit. Each morpholino subunit comprises a purine or pyrimidine nucleobase, which may bind by base-specific hydrogen bonding to a nucleobase in an oligonucleotide. In some embodiments, the morpholino-based ASO may include at least one further modification.

[0117] In some embodiments, both the sugar moiety and the internucleoside linkage between the nucleobase and the sugar moiety of at least one nucleotide unit in the ASO are replaced with non-natural groups. In some embodiments, the nucleobase units are maintained for hybridization with an appropriate nucleic acid target compound. In some embodiments, the ASO is chosen from peptide nucleic acids (PNAs). In some embodiments, the sugar-backbone of at least one oligonucleotide in the PNA is replaced with an amide-containing backbone, for

example, an aminoethylglycine backbone. In some embodiments, the nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.

[0118] In some embodiments, the ASOs may further comprise at least one nucleobase (often referred to as "base") modification or substitutions, for example, 5-substituted pyrimidines, 6-azapyrimidines, and N-2, N-6, and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil, and 5-propynylcytosine. Certain nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the disclosure. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. In some embodiments, the modified nucleobase is stereo-controlled.

[0119] It is not necessary for all positions in a given ASO to be uniformly modified, and in fact, more than one of the aforementioned modifications may be incorporated in a single nucleoside within an ASO. ASOs may contain at least one region wherein the ASO is modified to confer upon them increased resistance to nuclease degradation, increased cellular uptake, and/or an additional region for increased binding affinity for the target nucleic acid.

[0120] Due to potential three-dimensional variation of the sugar moieties, nucleobases, and internucleotide linkages, some nucleotides may share the same molecular formula but have a different spatial arrangement, i.e., some nucleotides may be stereoisomers. In some embodiments, the stereochemistry of nucleotides within a given ASO are not controlled so as to make the ASO stereorandom. In some embodiments, the nucleotides within a given ASO are stereo-controlled. In some embodiments, the nucleotides within a given ASO are stereo-controlled so as to make the ASO stereopure. In some embodiments a given ASO is a combination of stereo-controlled and stereorandom nucleotides.

[0121] In some ASOs, it is possible for some modifications to the sugar moieties, nucleobases, internucleotide linkages and/or stereo-controlled nucleotides to be arranged in regions that create a particular motif for the ASO. In some embodiments, the ASO comprises at least two regions. In some embodiments, the ASO comprises three regions: one region near the 5' end of the ASO, one region near the 3' end of the ASO, and a gap region between the two other regions. This type of arrangement is known as a gapmer motif. The length of each motif can be equal to other motifs within the ASO, or the length of each motif can be independent of the length of other motifs within the ASO. In some embodiments, one or more sugar moieties in an ASO are modified so that a block of sugar moieties in one region of the ASO are different from a block of sugar moieties in a different region of the ASO. In some embodiments, an ASO comprises modified sugar moieties arranged in a gapmer motif. In some embodiments, one or more nucleobases in an ASO are modified so that a block of nucleobases in one region of the ASO are different from a block of nucleobases in a different region of the ASO. In some embodiments, an ASO comprises modified nucleobases arranged in a gapmer motif. In some embodiments, one or more internucleotide linkages in an ASO are modified so that a block of internucleotide linkages in one region of the ASO are different from a block of internucleotide

- linkages in a different region of the ASO. In some embodiments, a given ASO comprises modified internucleotide linkages arranged in a gapmer motif. In some embodiments, one or more stereo-controlled nucleotides in an ASO are modified so that a block of stereo-controlled nucleotides in one region of the ASO are different from a block of stereo-controlled nucleotides in a different region of the ASO. In some embodiments, an ASO comprises stereo-controlled nucleotides arranged in a gapmer motif. In some embodiments, an ASO has more than one motif. In some embodiments, an ASO has more than one motif independent of each other.

Manufacturing Antisense Oligonucleotides

- [0122] The antisense molecules used in accordance with this disclosure may be made through well-known techniques of solid phase synthesis. Equipment for such synthesis is available from several sources including, for example, Applied Biosystems (Foster City, Calif.). One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066.
- [0123] Any other methods for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides, such as phosphorothioates and alkylated derivatives. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage, et al., *Tetrahedron Letters*, 22:1859-1862 (1981).
- [0124] In some embodiments, the ASOs are synthesized in a way so that all nucleotides of the ASO are stereopure.
- [0125] In some embodiments, the ASOs are synthesized *in vitro* and do not include antisense compositions of biological origin. In some embodiments, the ASOs may also be mixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures, or mixtures of compounds, as for example, liposomes, lipids, receptor targeted molecules for assisting in uptake, distribution and/or absorption. Further information about synthesis of certain ASOs according to some embodiments is included in the Examples below.

Methods of Inducing Exon-2 Skipping During pre-mRNA Splicing

- [0126] In some embodiments, the ASOs are used to induce Exon-2 skipping during processing of *CD33* pre-mRNA. In some embodiments, at least one ASO disclosed herein is used to induce Exon-2 skipping in *CD33* pre-mRNA during pre-mRNA splicing. In some embodiments, the at least one ASO is introduced into a cell, wherein the at least one ASO comprises all or a portion of SEQ ID NO:1, wherein the ASO hybridizes to a target region of the *CD33* gene, and wherein the ASO induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene. In some embodiments, the ASO administered to induce Exon-2 skipping during pre-mRNA splicing comprises one of SEQ ID NOS:2-15, 36-39, 82, 83, 96, 97, 128, 132, 135, 136, 183, 184, 190, 196, or 197.

[0127] In some embodiments, the at least one ASO is administered by itself, as a so-called “naked” ASO. In some embodiments, the at least one naked ASO is synthesized *in vitro*. In some embodiments, the at least one naked ASO is introduced into a cell to directly hybridize to a target region of the *CD33* gene to induce Exon-2 skipping during pre-mRNA splicing.

5 [0128] Certain methods of introducing “naked” ASOs or expression vectors encoding ASOs into a cell are well known in the art. In some embodiments, an ASO or expression vector encoding an ASO can be introduced by transfection using known transfection agents. In some
10 embodiments, the use of an excipient or transfection agent aids in delivery of the ASO or expression vector encoding the ASO as defined herein to a cell and/or into a cell. In some
15 embodiments, excipients or transfection agents are capable of forming complexes, nanoparticles, micelles, vesicles, and/or liposomes that deliver each ASO or expression vector encoding each ASO as defined herein, complexed or trapped in a vesicle or liposome through a cell membrane. Many of these excipients are known in the art. Suitable excipients or
20 transfection agents include LipofectAMINE™ 2000 (Invitrogen), Endo-Porter peptide, polyethylenimine (PEI; ExGen500 (MBI Fermentas)), or derivatives thereof, or similar cationic polymers, including polypropyleneimine or polyethylenimine copolymers (PECs) and derivatives, synthetic amphiphils (SAINT-18), Lipofectin™, DOTAP and/or viral capsid proteins that are capable of self-assembly into particles that can deliver each ASO as defined herein to a cell. Such excipients have been shown to efficiently deliver an oligonucleotide such as ASOs to a
25 wide variety of cultured cells. Their high transfection potential is combined with an excepted low to moderate toxicity in terms of overall cell survival. The ease of structural modification can be used to allow further modifications and the analysis of their further (*in vivo*) nucleic acid transfer characteristics and toxicity.

[0129] In some embodiments, the ASO is administered in the form of an expression vector,
25 wherein the expression vector encodes an RNA transcript comprising the sequence of the ASO. When placed under conditions conducive to expression of the encoded ASO, the expression vector can express the encoded ASO, which can hybridize to a target region of the *CD33* gene to induce Exon-2 skipping during pre-mRNA splicing. The expression vector can be a viral or non-viral vector. In some embodiments, there is provided a plasmid-based expression vector
30 comprising an expression cassette or a transcription cassette that drives expression or transcription of an ASO for redirecting splicing.

[0130] In some embodiments, a cell can be provided with an ASO for redirecting splicing by plasmid-derived ASO expression or viral expression provided by cytomegalovirus-,
adenovirus-, or adeno-associated virus-based vectors. In some embodiments, expression may
35 be driven by an RNA polymerase II promoter (Pol II) such as a U7 RNA promoter or an RNA polymerase III (Pol III) promoter, such as a U6 RNA promoter. In some embodiments, the delivery vehicle is an expression vector. In some embodiments, plasmids and artificial

chromosomes are usable for targeted homologous recombination and integration in the human genome of cells may be applied for delivery of an ASO for redirecting splicing.

Therapeutic Methods

- 5 [0131] Disclosed herein are methods of treating a subject having a neurodegenerative disease comprising administering at least one ASO disclosed herein. In some embodiments, the methods comprise administering a therapeutically effective amount of at least one ASO disclosed herein. In some embodiments, the methods comprise administering a therapeutically effective amount of at least one ASO that hybridizes to all or a portion of SEQ ID NO:1. In some
- 10 embodiments, the methods comprise administering a therapeutically effective amount of at least one ASO comprising one of SEQ ID NOS:2-10. In some embodiments, the neurodegenerative disease is characterized by a mutation in the *CD33* gene. In some embodiments, the neurodegenerative disease is characterized by an aberrant microglial phenotype. In some embodiments, the neurodegenerative disease is Alzheimer's Disease, microfibromialgia, or
- 15 multiple sclerosis.
- [0132] In some embodiments, the ASO administered to a subject having a neurodegenerative disease may be administered in a pharmaceutical composition. In some embodiments, the amount of ASO administered in a pharmaceutical composition may be dependent on the subject being treated, the subject's weight, the manner of administration, and the judgment of the
- 20 prescribing physician. For example, in some embodiments, a dosing schedule may involve the daily or semi-daily administration of the pharmaceutical composition at a perceived dosage of about 1 µg to about 1000 mg. In some embodiments, intermittent administration, such as on a monthly or yearly basis, of a dose of the pharmaceutical composition may be employed. In accordance with standard dosing regimens, in some embodiments, physicians will readily
- 25 determine optimum dosages and will be able to readily modify administration to achieve such dosages.
- [0133] A therapeutically effective amount of a compound or composition disclosed herein can be measured by the therapeutic effectiveness of the compound. In some embodiments, the dosages, however, may be varied depending upon the requirements of the patient, the severity
- 30 of the condition being treated, and the compound being used. In some embodiments, the therapeutically effective amount of a disclosed compound is sufficient to establish a maximal plasma concentration. In some embodiments, preliminary doses as, for example, determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices.
- 35 [0134] In some embodiments, toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the

therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . In some embodiments, compositions that exhibit large therapeutic indices are desirable.

[0135] In some embodiments, data obtained from the cell culture assays or animal studies can be used in formulating a range of dosage for use in humans. In some embodiments,

- 5 therapeutically effective dosages achieved in one animal model may be converted for use in another animal, including humans, using conversion factors known in the art (see, e.g., Freireich et al., *Cancer Chemother. Reports* 50(4):219-244 (1966).

[0136] The ASOs herein may be administered in a pharmaceutical composition comprising therapeutically effective amounts of an ASO together with pharmaceutically acceptable

- 10 excipients, diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. In some embodiments, such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH, and ionic strength, and additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol), and bulking substances (e.g.,
15 lactose, mannitol). In some embodiments, the material may be incorporated into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. In some embodiments, Hyaluronic acid may also be used. Such compositions may influence the physical state, stability, rate of *in vivo* release, and/or rate of *in vivo* clearance of the present ASOs and derivatives. In some embodiments, the compositions may be prepared in
20 liquid form, or may be in dried powder, such as lyophilized form.

Administration

[0137] In some embodiments, a pharmaceutical composition comprising an ASO and a pharmaceutically acceptable carrier or excipient may be prepared for administration according
25 to techniques well known in the pharmaceutical industry. In some embodiments, such techniques include combining the ASO with the carrier and/or excipient(s) into association in a unit dosage form.

- [0138] In some embodiments, compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined
30 amount of a compound of the present disclosure as powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. In some embodiments, such formulations may be prepared by any suitable method which includes the step of bringing into association at least one embodiment of the present disclosure as the active compound and at least one carrier or excipient (which may constitute one or more
35 accessory ingredients). In some embodiments, the at least one carrier is acceptable in the sense of being compatible with the other ingredients of the formulation and is not deleterious to the recipient. In some embodiments, the carrier may be a solid or a liquid, or both, and may be formulated with at least one compound described herein as the active compound in a unit-dose

formulation, for example, a tablet, which may contain from about 0.05% to about 95% by weight of the at least one active compound. In some embodiments, other pharmacologically active substances may also be present including other compounds. In some embodiments, the formulations of the present disclosure may be prepared by any of the well-known techniques of pharmacy consisting essentially of admixing the components.

[0139] For solid compositions, in some embodiments, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. In some embodiments, liquid pharmacologically administrable compositions can, for example, be prepared by, for example, dissolving or dispersing, at least one active compound of the present disclosure as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. In general, in some embodiments, suitable formulations may be prepared by uniformly and intimately admixing the at least one active compound of the present disclosure with a liquid or finely divided solid carrier, or both, and then, if desired, shaping the product. For example, in some embodiments, a tablet may be prepared by compressing or molding a powder or granules of at least one embodiment of the present disclosure, which may be optionally combined with one or more accessory ingredients. In some embodiments, compressed tablets may be prepared by compressing, in a suitable machine, at least one embodiment of the present disclosure in a free-flowing form, such as a powder or granules, which may be optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). In some embodiments, molded tablets may be made by molding, in a suitable machine, where the powdered form of at least one embodiment of the present disclosure is moistened with an inert liquid diluent.

[0140] In some embodiments, formulations suitable for buccal (sub-lingual) administration include lozenges comprising at least one embodiment of the present disclosure in a flavored base, for example, sucrose and acacia or tragacanth, and pastilles comprising the at least one compound in an inert base such as gelatin and glycerin or sucrose and acacia.

[0141] In some embodiments, formulations suitable for parenteral administration comprise sterile aqueous preparations of at least one embodiment of the present disclosure, which are approximately isotonic with the blood of the intended recipient. In some embodiments, these preparations are administered intravenously, although administration may also be affected by subcutaneous, intramuscular, intraperitoneal, intracerebroventricular, or intradermal injection. In some embodiments, these preparations are administered via osmotic pump. In some embodiments, such preparations may conveniently be prepared by admixing at least one embodiment described herein with water and rendering the resulting solution sterile and isotonic with the blood. In some embodiments, injectable compositions according to the present disclosure may contain from about 0.1 to about 5% w/w of the active compound.

[0142] In some embodiments, formulations suitable for rectal administration are presented as unit-dose suppositories. In some embodiments, these may be prepared by admixing at least one embodiment as described herein with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

- 5 [0143] In some embodiments, formulations suitable for topical application to the skin may take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. In some embodiments, carriers and excipients which may be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combinations of two or more thereof. In some embodiments, the ASO is generally present at a concentration of from about 0.1% to about 15% w/w of the composition, for
10 example, from about 0.5 to about 2%.

EXAMPLES

[0144] The following Examples serve to more fully describe the invention. They are meant for illustrative purposes and are not meant to limit the invention in any way.

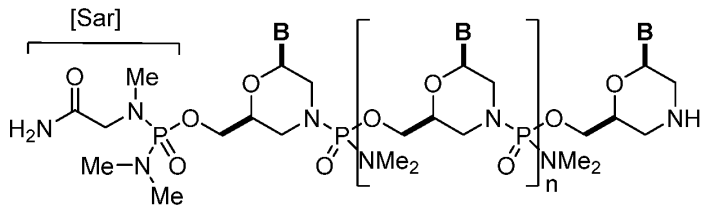
15 **Abbreviations**

- [0145] ASO: antisense oligonucleotide
[0146] DNA: deoxyribonucleic acid
[0147] cDNA: complementary deoxyribonucleic acid
[0148] RNA: ribonucleic acid
20 [0149] mRNA: messenger ribonucleic acid
[0150] PMO: phosphorodiamidate morpholino oligomer
[0151] MOE: methoxyethyl
[0152] LOAD: late onset Alzheimer's Disease
[0153] SNP: single nucleotide polymorphism
25 [0154] PNA: peptide nucleic acid
[0155] DOTAP: 1,2 dioleoyl 3 trimethylammonio propane
[0156] PEI: polyethylenimine
[0157] PEC: polyethylenimine copolymers
[0158] HRMS: high resolution mass spectrometry
30 [0159] MW: molecular weight
[0160] SP: stereopure
[0161] UPLC: ultra performance liquid chromatography
[0162] MS: mass spectrometry
[0163] MTBE: Methyl tert-butyl ether
35 [0164] DCM: dichloromethane
[0165] TFA: trifluoroacetic acid
[0166] RT: room temperature

- [0167] H: hour
 [0168] Min: minute
 [0169] EtOAc: ethyl acetate
 [0170] HPRT1: hypoxanthine phosphoribosyltransferase 1
 5 [0171] GAPDH1: glyceraldehyde 3 phosphate dehydrogenase 1
 [0172] NTC: non-targeting control
 [0173] WP: well plate
 [0174] Bz – benzoyl
 [0175] CE – 2-cyanoethyl
 10 [0176] Trt – trityl
 [0177] iPr- isopropyl
 [0178] Sar – Sarcosine
 [0179] ESI-TOF-MS – electrospray ionization – time-of-flight mass spectrometry
- 15 **Example 1: Reducing or Interfering with Full Length *CD33***
 [0180] SNP rs3865444 was reported to be associated with an increased skipping of Exon-2 of *CD33* and with reduced levels of full length *CD33* on the surface of monocytes. The allele was found to be associated with decreased levels of full length *CD33* in human cerebrospinal fluid (CSF) and plasma when measured using Somascan technology (Fig.1). In a study by the
 20 Alzheimer's Disease Neuroimaging Initiative (ADNI), the allele was found to be associated with decreased ventricle volume and increased midtemporal volume, which are both consistent with protection against Alzheimer's Disease (Fig 2). Moreover, in the longitudinal analysis, the allele was associated with improved slope for Alzheimer's Disease Assessment Scale (ADAS) 11, mini-mental state examination (MMSE), Rey Auditory Verbal Learning Test (RAVLT) immediate,
 25 Trial Making Test-B (TRABSCOR), Functional Activities Questionnaire (FAQ), ¹⁸F-fluorodeoxyglucose-positron emission tomography (FDG PET), ventricle volume, fusiform gyrus, and midtemporal volume (Fig 3), indicating protection against the disease.
 [0181] On the other hand, rs201074739 is a 4-base pair deletion in exon3 of the *CD33* gene. This causes a frameshift in the open reading frame and a premature translation termination. The
 30 indel was associated with decreased levels of full length *CD33* in human CSF and plasma when measured using SomaScan technology (Fig. 4). However, this indel has not been associated with a reduced risk of the disease so far. Moreover, it was associated with increased ventricle volume and a worse functional activities questionnaire (FAQ) score, suggesting a deleterious effect (Fig. 2).
 35 [0182] Accordingly, successfully inducing Exon-2 skipping of *CD33* may have therapeutic benefits.

Example 2: General ASO Formulas

[0183] PMO oligonucleotides were designed for screening. The designed oligonucleotides listed in Tables 1 and 3 below were made by GeneTools LLC (www.gene-tools.com). Table 1 lists the top PMO oligonucleotides with their deconvoluted MS data. Table 3 includes the top PMO oligonucleotides in Table 1, as well as other PMO oligonucleotides. All PMO oligonucleotides listed in Tables 1 and 3 below contain a phosphorodiamidate-attached sarcosine linker (Sar) at the 5' end. All PMO oligonucleotides in Tables 1 and 3 below were synthesized with unmodified cytosine PMO nucleotide. All PMO oligonucleotides listed in Tables 1 and 3 below have stereorandom internucleotide linkages, and thus are called stereorandom PMO oligonucleotides. The general formula of the PMO oligonucleotides listed in Tables 1 and 3 below is:

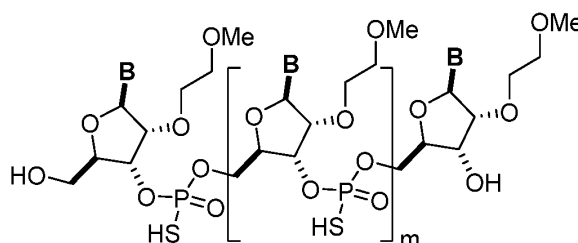


General formula for stereorandom PMO oligonucleotides

Table 1

ASO #	Seq.	SEQ ID NO	MW (with Sarcosine linker)	MS observed
PMO-002	5' -CCTCACCTGTCACATGCACAGAGAG-3'	2	8398.06	8403.0
PMO-003	5' -CCTGTCACATGCACAGAGAGCTGGG-3'	3	8494.11	8498.4
PMO-004	5' -ATTTGGTACTTCCTCTCTCCATCCG-3'	4	8311.96	8316.4
PMO-005	5' -TCTCCATCCGAAAGAAGTATGAACC-3'	5	8421.09	8423.7
PMO-006	5' -TGGGATGGAAGAAAGTGCAGGGCAC-3'	6	8647.24	8649.7
PMO-007	5' -CAGCCAGAAATTTGGATCCATAGCC-3'	7	8437.09	8444.7
PMO-008	5' -CCCTGTGGGGAAACGAGGGTCAGCT-3'	8	8550.14	8552.5

[0184] MOE oligonucleotides were designed for screening. The designed oligonucleotides listed in Tables 2 and 4 below were made by either Integrated DNA Technologies (www.idtdna.com) or GeneDesign (Ajinomoto Bio Pharma, <https://ajibio-pharma.com/>). Table 2 lists the top MOE sequences with their deconvoluted MS data. All MOE oligonucleotide listed in Tables 2 and 4 below contain a hydroxyl at the 5' end. All MOE oligonucleotides listed in Tables 2 and 4 below contain 2'-O-MOE-modified ribonucleotides with phosphorothioate backbone except when noted. All MOE oligonucleotides listed in Tables 2 and 4 below were synthesized with 5-methylcytosine 2'-O-MOE ribonucleotide. All MOE oligonucleotides listed in Tables 2 and 4 below have stereorandom internucleotide linkages, and thus are called stereorandom MOE oligonucleotides. The general formula of the MOE oligonucleotides listed in Tables 2 and 4 depicted as free form is:



General formula for MOE oligonucleotide sequences

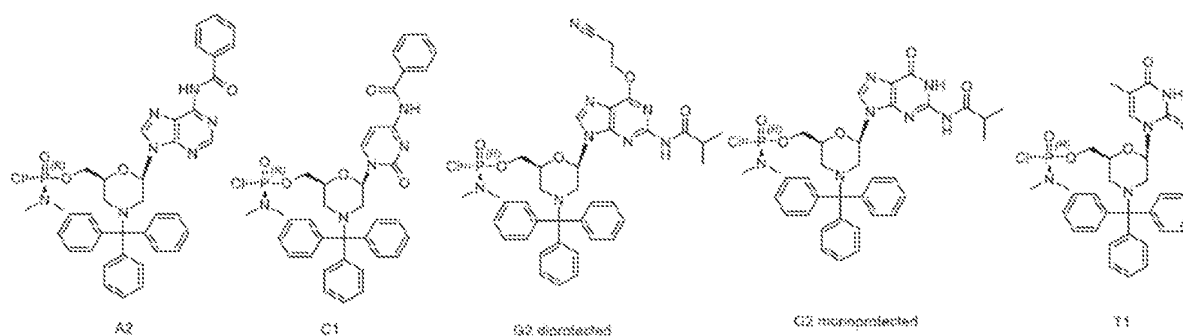
Table 2

ASO #	Seq. (5' to 3')	SEQ. ID NO:	MW as free form	MS observed
MOE-009	CACATGCACAGAGAGCTGGG	9	8031.7	8033.80
MOE-010	GAGAGCTGGGGAGATTTGTA	10	8077.1	8077.31
MOE-011	TCTCCATCCGAAAGAAGTAT	11	7940.9	7942.85
MOE-012	ATCCGAAAGAAGTATGAACC	12	7985.2	7986.62
MOE-013	ATGCTCAGGGAGCAGTTGTT	13	8016.1	8016.17
MOE-014	GAGTCTCCTCCTGTACTTCT	14	7894.4	7895.30
MOE-015	CGCACAAACCCTCCTGTACC	15	7893.0	7893.84

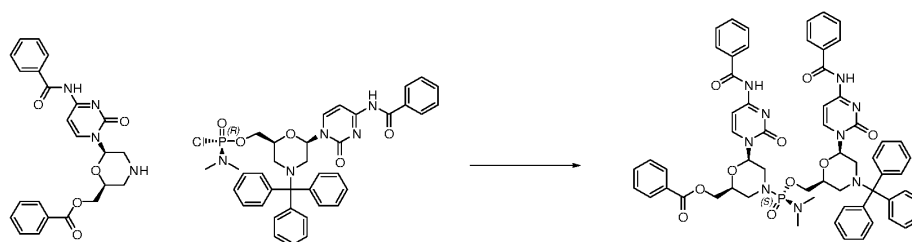
Example 3: Synthesis of PMO-302 (stereopure internucleotide linkages (Sp))

Synthesis of stereopure PMO-302 oligonucleotide with unfunctionalized 5'-OH (CCTCACCTGTCACATGCACAGAGAG (SEQ ID NO: 2)).

[0185] Monomers used in the synthesis of PMO-302 are as follows: (reported in WO2017024264A2):



5 Synthesis of PMO-302 with 5'-OH and stereopure internucleotide linkages: 2-mer synthesis:

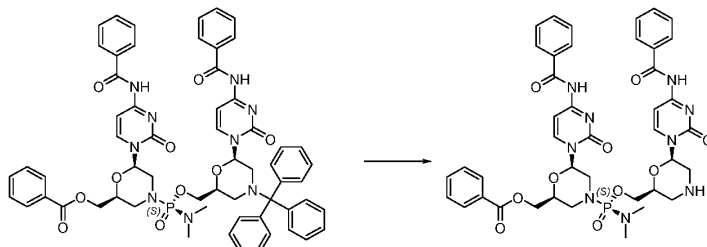


[0186] Unless otherwise noted all liquid ingredients were added via an appropriate size syringe. All reactions were conducted under N₂ atmosphere. Filtrations and workup were done open to the air. Filtrations were conducted on a glass sintered funnel.

[0187] A flask with the amine ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)morpholin-2-yl)methyl benzoate (130 mg) was equipped with a stir bar and rubber septum. The atmosphere was exchanged with nitrogen and sparged. After 5 minutes, added 1,3-dimethyl-2-imidazolidinone (2.2mL) followed by 1,2,2,6,6-pentamethylpiperidine (164 μ L) via syringe at rt and the mixture was allowed to form a solution. Solid ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (219 mg) was added in one portion and the flask was sealed with the rubber septum. Stirring was continued for 3h and the reaction monitored by UPLC MS. Upon completion, while stirring, MTBE (11.7mL) was added over 1 minutes. Precipitate was formed towards the end of the addition. n-Heptane (10mL) was added. The oily mixture was allowed to settle for 10 minutes. While the heavy oil was settled, the cloudy supernatant was transferred by decantation to a 30mL vial and centrifuged. This formed an additional oily residue on the bottom. The solvent was removed by decantation and the two oily residues were combined by dissolving into 1mL of DCM and purified by flash chromatography with 0-5% MeOH in DCM. The fractions containing desired

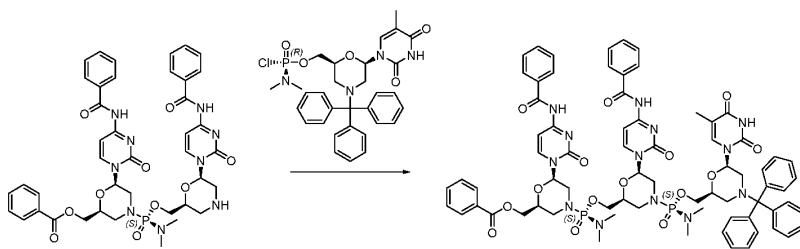
product were dried under vacuum to obtain ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (270 mg) as a white foam. MS (ESI) *m/z*: [M+H]⁺ Calcd for C₆₀H₅₈N₉O₁₀P: 1096.40; Found: 1096.53.

5 Deprotection of 2-mer:



- [0188] To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (350 mg) was added DCM (3.5mL). Added ethanol (186 μ L) via syringe at rt. Added TFA (160 μ L) at rt dropwise over 30 seconds. Stirred for 30 min and monitored by UPLC-MS. Upon completion, added MTBE (14mL) over 1 minute with a syringe. The suspension was stirred for 10 min and then sonicated. Filtered over a sintered filter funnel and rinsed with MTBE 10mL (2x5mL). The solids were dried, transferred to a new flask and then dissolved by addition of DCM (3.5mL). 1,2,2,6,6-pentamethylpiperidine (292 μ L) was added via syringe. After 10 min at rt added MTBE (15.8mL) over 1 minute. White solids were formed. After 10 minutes the slurry was sonicated, filtered and rinsed with MTBE (e.g. 2x10mL). Dried for 20 minutes under air flow and 1 hour under vacuum to obtain ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (243 mg). MS (ESI) *m/z*: [M+H]⁺ Calcd for C₄₁H₄₄N₉O₁₀P: 854.29; Found: 854.65.

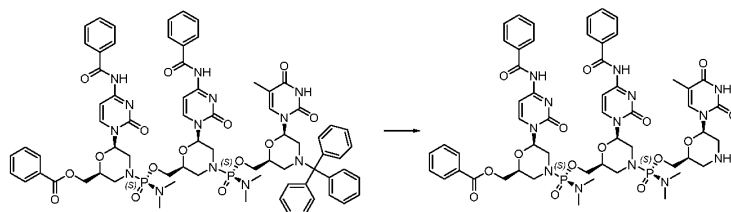
3-mer synthesis:



- [0189] To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (215 mg) were added 1,3-Dimethyl-2-imidazolidinone (2mL) and 1,2,2,6,6-pentamethylpiperidine (138 μ L) under nitrogen. After 2-min added ((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-

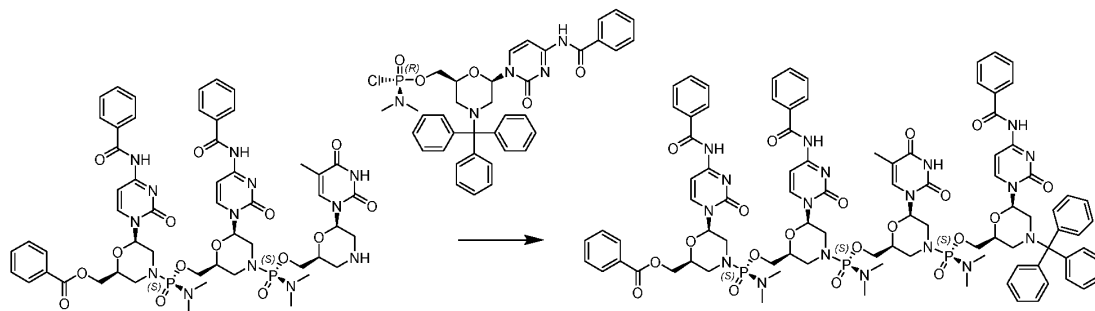
tritylmorpholin-2-yl)methyl-(*R*)-dimethylphosphoramidochloridate (169 mg) and the reaction was stirred for 3h at rt. Upon completion, added ethyl acetate (2.6mL) and then MTBE (14mL). The resulting white precipitate was filtered, rinsed with MTBE (2 x 5mL) and dried under vacuum to obtain ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(dimethylamino))((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methoxy)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (350 mg). MS (ESI) m/z : $[M+H]^+$ Calcd for $C_{72}H_{77}N_{13}O_{15}P_2$: 1426.51; Found: 1427.74.

Deprotection of 3-mer:



[0190] To a flask was added ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(dimethylamino))((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methoxy)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (350 mg) and DCM (4.2mL). Added ethanol (143 μ L) and then slowly at rt added TFA (95 μ L). The reaction mixture was stirred for 2 hours at rt. Upon completion, added MTBE (15mL). The solids were filtered and rinsed with MTBE (10mL). The solids were dried and then transferred to a flask and dissolved by addition of DCM (2.7mL). Added 1,2,2,6,6-pentamethylpiperidine (224 μ L) and rt and the reaction mixture was stirred at rt for 10 minutes. Added MTBE (15mL) and the resulting slurry was stirred for 10 minutes and sonicated. Filtered and rinsed with MTBE (10mL). Obtained trimer as free base (297 mg). MS (ESI) m/z : $[M+H]^+$ Calcd for $C_{53}H_{63}N_{13}O_{15}P_2$: 1184.40; Found: 1185.

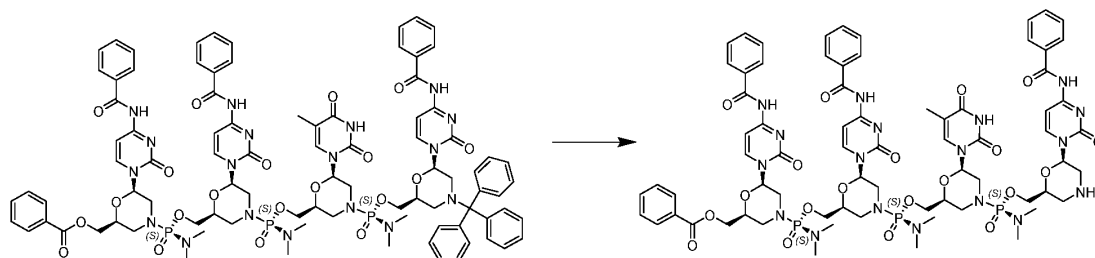
4-mer synthesis:



[0191] To a flask was added ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(dimethylamino))((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)phosphoryl)morpholin-

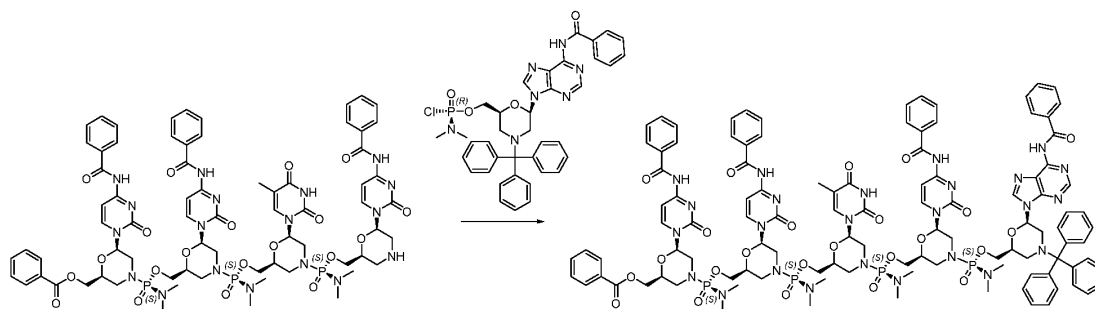
2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (292 mg) and the flask was purged with nitrogen. Added 1,3-Dimethyl-2-imidazolidinone (2.9mL) and then 1,2,2,6,6-pentamethylpiperidine (135 μ L). ((2*S*,6*R*)-6-(4-Benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (207 mg) was added in one
 5 portion and the reaction was stirred at rt for at least 1h monitoring for completion by HPLC-MS. Ethyl acetate (2.9mL) was charged followed by MTBE (14mL). The slurry was stirred for 15 minutes and filtered, washed with MTBE 2x5mL. The resulting solids were dried under vacuum for 10 minutes and then collected to a new flask, dried under vacuum to afford 430 mg of 4-mer. MS (ESI) m/z : $[M+H]^+$ Calcd for $C_{90}H_{99}N_{18}O_{20}P_3$: 1846.65; Found: 1847.

10 Deprotection of 4-mer:



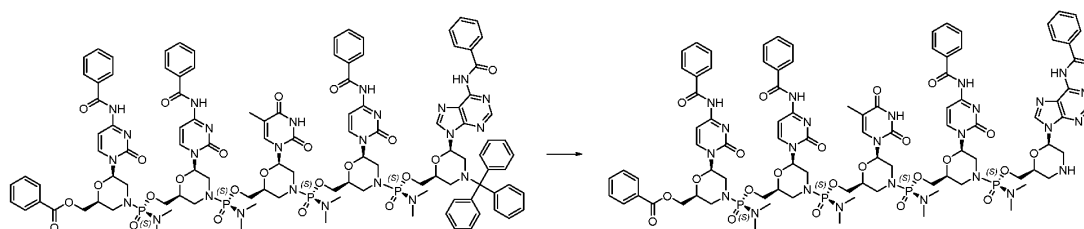
[0192] To a flask was added ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (430 mg).
 15 Added DCM (4.3mL) and ethanol (272 μ L). After a solution was formed, added TFA (135 μ L). The reaction mixture was stirred for 2.5h when it was deemed completed by HPLC analysis.
 20 Added ethyl acetate (3.0mL) and MTBE (10.8mL) over 1 minute. Solid precipitate were formed during MTBE addition. Upon completed MTBE addition, the solids were stirred for 10 minutes and sonicated three times. Filtered and rinsed with MTBE 2x5mL. The solids were dried and then dissolved in DCM (4.3mL) and treated with 1,2,2,6,6-pentamethylpiperidine (319 μ L). After 5 min, the desired product was precipitated by addition of ethyl acetate (3.0mL) and MTBE
 25 (10.8mL) over 1 minute. The solids were filtered, rinsed with MTBE and dried under vacuum overnight to afford ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (330 mg). MS (ESI)
 30 m/z : $[M+H]^+$ Calcd for $C_{71}H_{85}N_{18}O_{20}P_3$: 1603.54; Found: 1605.

5-mer synthesis:



[0193] To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl (R)-dimethylphosphoramidochloridate (178 mg) at rt. The reaction mixture was stirred at rt for 5h and then added ethyl acetate (6.6 mL) and MTBE (13.2mL). The white precipitate was filtered and dried. The solid was dissolved in DCM 2mL and purified by automated silica gel chromatography on a 25g cartridge with 0-20% MeOH in DCM. Afforded 345 mg of desired product 5-mer. MS (ESI) *m/z*: Calcd for C₁₀₉H₁₂₁N₂₅O₂₄P₄: [(M+2H)/2]⁺ 1145.4; Found: 1145.6.

15 Deprotection of 5-mer:



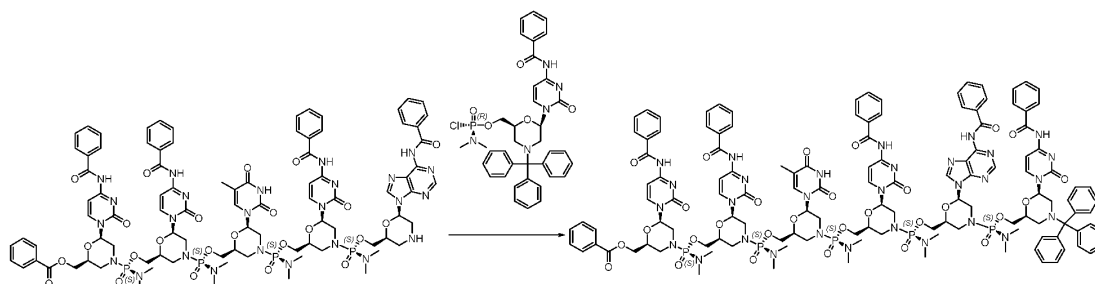
[0194] To a flask was added ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(6-benzamido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl (R)-dimethylphosphoramidochloridate (178 mg) at rt. The reaction mixture was stirred at rt for 5h and then added ethyl acetate (6.6 mL) and MTBE (13.2mL). The white precipitate was filtered and dried. The solid was dissolved in DCM 2mL and purified by automated silica gel chromatography on a 25g cartridge with 0-20% MeOH in DCM. Afforded 345 mg of desired product 5-mer. MS (ESI) *m/z*: Calcd for C₁₀₉H₁₂₁N₂₅O₂₄P₄: [(M+2H)/2]⁺ 1145.4; Found: 1145.6.

20 tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (328 mg). Added DCM (3.1mL) and then ethanol (167 μL). Added TFA (66.2 μL) at rt and stirred at rt for 3h. Added additional 3 drops (ca 15 μL) of TFA. The reaction was monitored by HPLC-MS and upon completion (disappearance of starting material peak), added ethyl acetate (11.8mL) followed by stirring for 5 min. Filtered and rinsed with EtOAc (2mL) and MTBE (5mL). Additional solids were

formed in the mother liquor which were also harvested by second filtration. The combined solids were placed into a reaction flask. Added DCM (2.3mL) and 1,2,2,6,6-pentamethylpiperidine (209 μ L). Stirred for 15 min then added EtOAc (2.6mL) and MTBE (10.5 mL). The resulting solids were filtered and rinsed by MTBE 2 x 3mL then dried under vacuum, collected to afford

5 280 mg deprotected 5-mer. MS (ESI) m/z: Calcd for $C_{90}H_{107}N_{25}O_{24}P_4$: $[(M+2H)/2]^+$ 1023.8; Found: 1024.12.

6-mer synthesis:



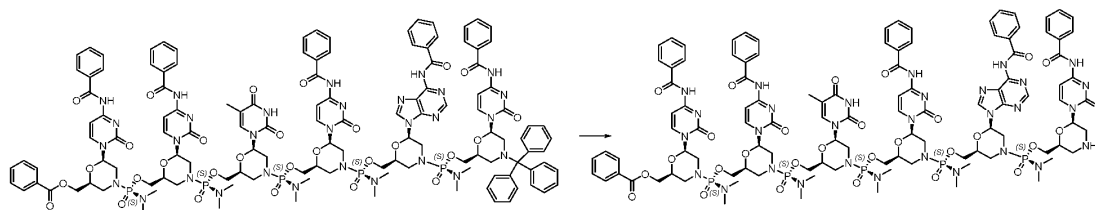
[0195] To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(6-benzamido-9H-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (265 mg) was added

10 1,3-Dimethyl-2-imidazolidinone (2.7mL). Added 1,2,2,6,6-pentamethylpiperidine (71.0 μ L). Added ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (108 mg) at rt as solid. After 3 hours at rt, upon completion as judged by HPLC analysis, added EtOAc (5.3mL) and MTBE (10.6mL) over 2-3 min each.

15 20 Filtered and rinsed with MTBE 2x3mL. After drying with air flow for 2-3 min the solids turned to sticky mass. The solids transferred to same flask with 10mL DCM and concentrated under vacuum. Isolated ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(6-benzamido-9H-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (354 mg). MS (ESI)

25 30 m/z: Calcd for $C_{127}H_{143}N_{30}O_{29}P_5$: $[M+2H/2]^+$ 1354.97; Found: 1354.73.

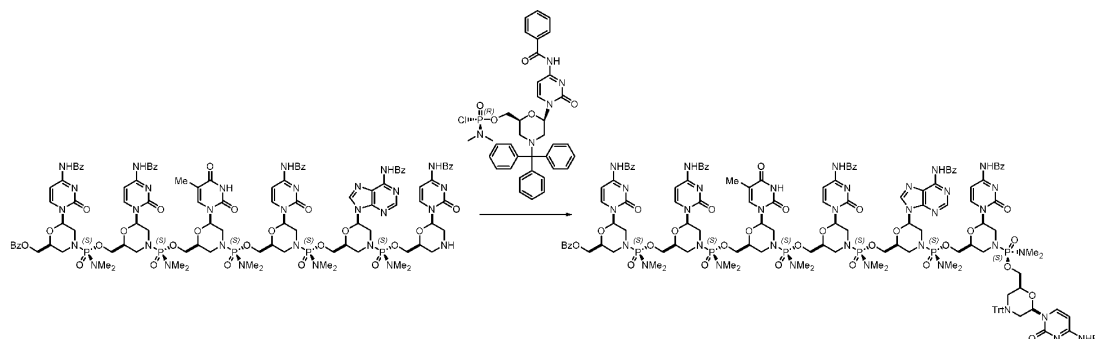
Deprotection of 6-mer:



[0196] To a flask with the dried evaporated solids from previous step (6-mer) was added DCM (3.2mL) and ethanol (155 μ L). After the solids dissolved completely, added TFA (71.7 μ L). The mixture was stirred for 2h and the reaction was not completed according to HPLC analysis.

- 5 Added additional 50 μ L TFA and continued to stir for additional 6h. Added EtOAc (2.9mL) then MTBE (11mL). The resulting solids were filtered and rinsed with 4:1 MTBE/EtOAc (12mL). Isolated ((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(6-benzamido-9H-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (295 mg). MS (ESI)
- 10
- 15 m/z: Calcd for $C_{108}H_{129}N_{30}O_{29}P_5$: [(M+2H)/2]⁺ 1233.92; Found: 1233.68.

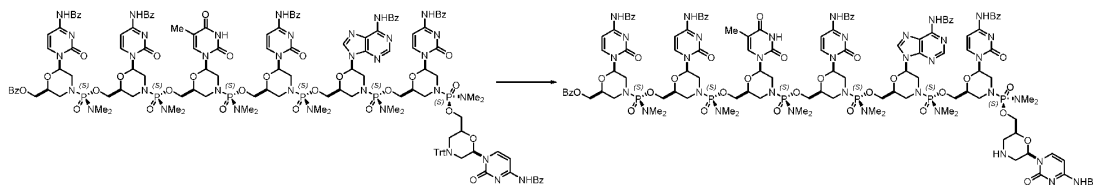
Synthesis of 7-mer:



- 20 To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(6-benzamido-9H-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (295 mg) was added
- 25 1,3-Dimethyl-2-imidazolidinone (2.9mL) and then 1,2,2,6,6-pentamethylpiperidine (65.6 μ L) at rt. ((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-

dimethylphosphoramidochloridate was added at rt as a solid (100 mg). The reaction was stirred for 3h at rt. Upon completion by HPLC analysis, added EtOAc (5.9mL) and MTBE (11.8 mL) over 2-3 min each. The solids were filtered and rinsed with MTBE 2x5mL. After drying with air flow for 2-3 min the solids were transferred to a flask and dried under vacuum for 1h to afford 7-mer (430 mg). MS (ESI) m/z: Calcd for C₁₄₅H₁₆₅N₃₅O₃₄P₆: [(M+2H)/2]⁺ 1564.85; Found: 1564.77.

Deprotection of 7-mer:



[0198] To a flask with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(6-benzamido-9*H*-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (374 mg) was added DCM (3.0mL) and ethanol (140 μ L). Added TFA (138 μ L) dropwise at rt over 30 sec. After 30 minutes, added EtOAc (7.5mL) and added MTBE (7.5mL). Filtered and rinsed with MTBE 2x3mL. The solids were dried in the filter funnel under air flow and then transferred to a flask. Dissolved in DCM (3.9mL) and EtOH (140 μ L), and added 1,2,2,6,6-pentamethylpiperidine (109 μ L). After 10 minutes, the solution was treated with added EtOAc (7.5mL) and MTBE (7.5mL). The resulting solids were filtered and rinsed with MTBE 2x3mL. The solids were dried in funnel and then transferred to a flask, dried under vacuum to obtain total ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-((*S*)-(((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(6-benzamido-9*H*-purin-9-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methoxy)(dimethylamino)phosphoryl)morpholin-2-yl)methyl benzoate (345 mg). MS (ESI) m/z: Calcd for C₁₂₆H₁₅₁N₃₅O₃₄P₆: [(M+2H)/2]⁺ 1443.48; Found: 1444.

[0199] From 8-mer to 25-mer, general procedures were used for coupling, deprotection and free basing:

[0200] **General procedure A for coupling:** To a flask with dried PMO oligonucleotide (free base PMO oligonucleotide) (1 wt, 1 equiv.) was added 1,3-dimethyl-2-imidazolidinone (6-10 volumes compared to free base PMO oligonucleotide) and then 1,2,2,6,6-pentamethylpiperidine (3-5 equiv.). The mixture was stirred and sonicated until all solids dissolved. Activated monomer (*R*)-dimethylphosphoramidochloridate (1.3-2.5 equiv.) was added as a solid in a single portion under N₂ atmosphere. The reaction mixture was stirred for minimum of 3h (18-24h at stages 15-25mer) and monitored for completion by UPLC MS (>99.5% target by UV or no detectable starting material mass). Additional (*R*)-dimethylphosphoramidochloridate may be added if target conversion criteria is not reached. Upon completion, the reaction mixture was charged with EtOAc (10-40 vols) and MTBE (10-40 volumes as compared to free base PMO oligonucleotide) to form a white precipitate. The solids were, filtered on a sintered funnel, rinsed with EtOAc/MTBE 1:1, dried under vacuum and collected to afford "trityl-protected PMO oligonucleotide" for the next step. General yield 90-100%.

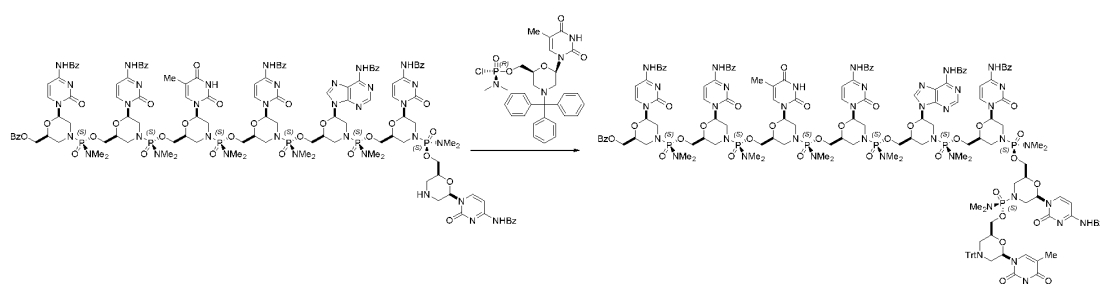
[0201] **General procedure B for trityl deprotection and free basing:**

[0202] **Trityl deblock solution** was prepared as follows: To a flask were added DCM (8 mL), 2,2,2-trifluoroethanol (2 mL), 4-cyanopyridine (100 mg), ethanol (100 µL) and trifluoroacetic acid (105 mg) in that order. The solution was mixed until all components are dissolved and then used in deprotection as is.

[0203] **Step 1 - trityl deprotection:** To a flask with "trityl-protected PMO oligonucleotide" (1 wt, 1 equiv.) was added trityl deblock solution (8 volumes compared to trityl-protected PMO oligonucleotide mass). The reaction mixture was stirred for 5-30 minutes and monitored by UPLC MS. Upon completion (>99.5% target), added EtOAc (10-40 vols) and MTBE (10-40 volumes) to form a white precipitate. The solids were filtered on a sintered funnel, rinsed with EtOAc/MTBE 1:1, dried under vacuum and collected to afford "TFA salt PMO oligonucleotide" for the next step.

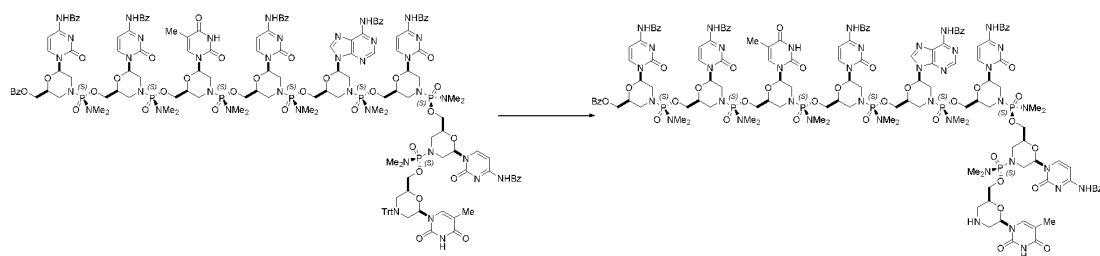
[0204] **Step 2 – free basing:** To a flask with "TFA salt PMO oligonucleotide" (1 wt, 1 equiv.) was added DCM (7-10 vols compared to TFA salt PMO oligonucleotide mass) and EtOH (0.3-0.5 vol). The solution was treated with 1,2,2,6,6-pentamethylpiperidine (5 equiv.). The reaction mixture was stirred for 5-10 minutes and then treated with EtOAc (10-40 vols) and MTBE (10-40 volumes) to form a white precipitate. The solids were rinsed with EtOAc/MTBE 1:1, dried under vacuum and collected for the next step.

Coupling to 8-mer:



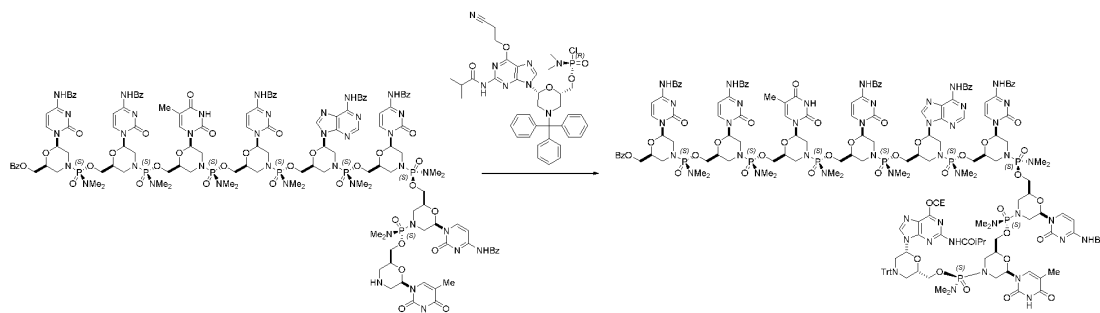
- [0205] Using General Procedure A: reaction of 7-mer (340 mg) with ((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (86 mg) afforded 8-mer (403 mg). MS (ESI) m/z : Calcd for $C_{157}H_{184}N_{39}O_{39}P_7$: $[M+2H/2]^+$ 1730.09; Found: 1730.

Deprotection of 8-mer:



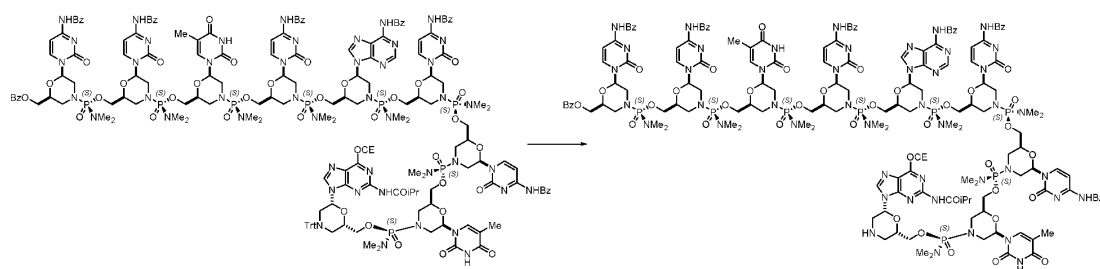
- [0206] Using General Procedure B: reaction of trityl protected 8-mer (380 mg) afforded free base 8-mer (353 mg). MS (ESI) m/z : Calcd for $C_{138}H_{170}N_{39}O_{39}P_7$: $[M+2H/2]^+$ 1608.53; Found: 1609.

Coupling to 9-mer:



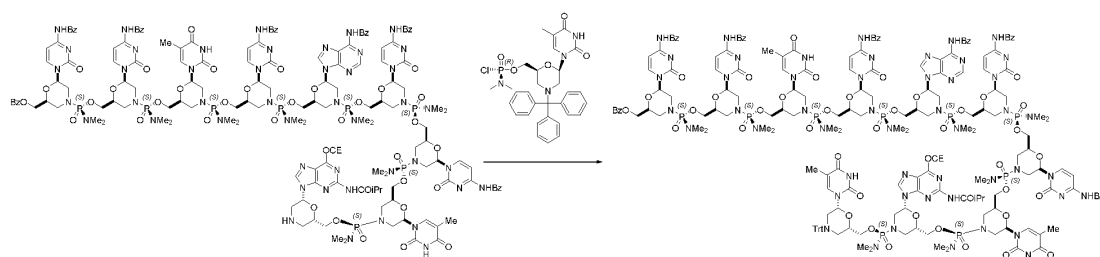
- [0207] Using General Procedure A: reaction of 8-mer (370 mg) with ((2*S*,6*R*)-6-(6-(2-cyanoethoxy)-2-isobutyramido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (105 mg) afforded 9-mer (453 mg).

Deprotection of 9-mer:



[0208] Using General Procedure B: reaction of trityl protected 9-mer (453 mg) afforded free base 9-mer (411 mg).

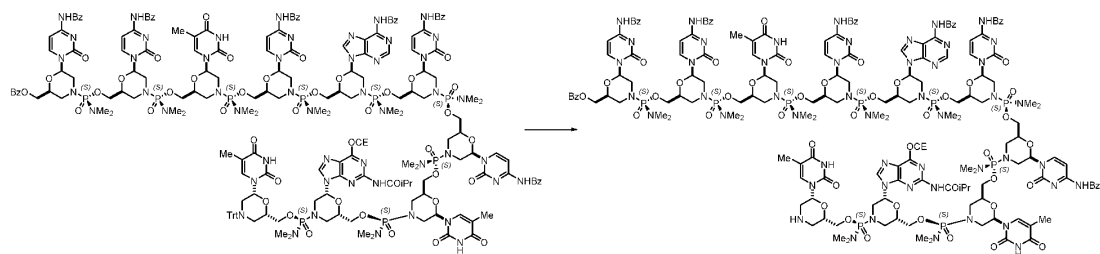
Coupling to 10-mer:



5

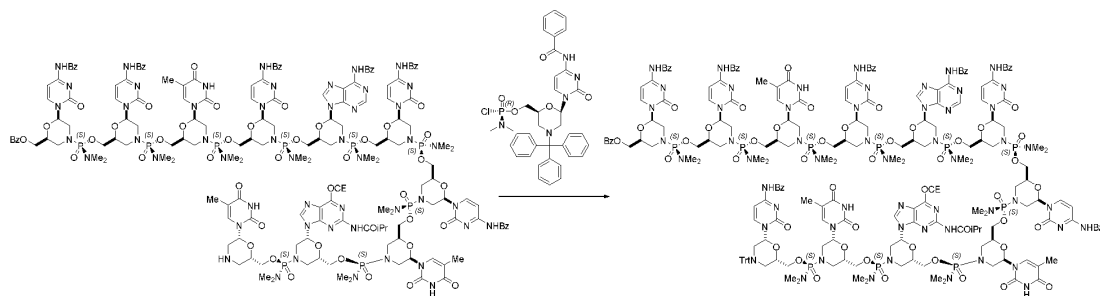
[0209] Using General Procedure A: reaction of 9-mer (405 mg) with ((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (80 mg) afforded 10-mer (469 mg). MS (ESI) m/z : Calcd for $C_{188}H_{230}N_{51}O_{49}P_9$: $[M+3H/3]^+$ 1422.8; Found: 1423.3.

10 Deprotection of 10-mer:



[0210] Using General Procedure B: reaction of trityl protected 10-mer (450 mg) afforded free base 10-mer (435 mg).

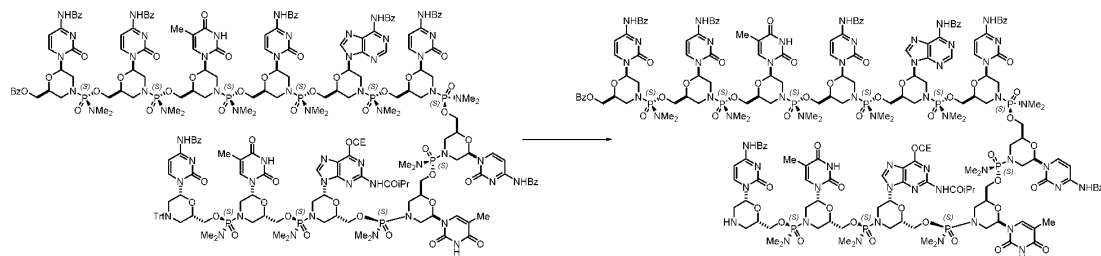
Coupling to 11-mer:



15

[0211] Using General Procedure A: reaction of 10-mer (435 mg) with ((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (98 mg) afforded 11-mer (512 mg).

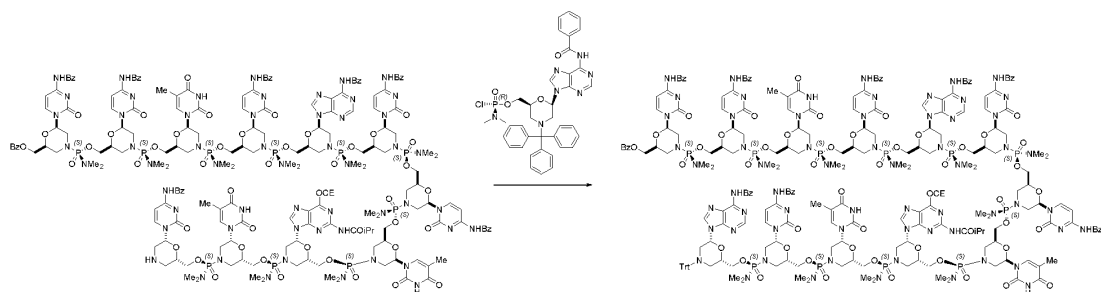
Deprotection of 11-mer:



5

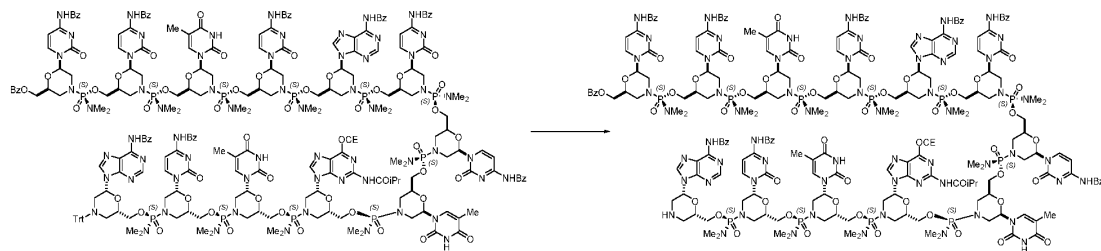
[0212] Using General Procedure B: reaction of trityl protected 11-mer (500 mg) afforded free base 11-mer (481 mg).

Coupling to 12-mer:



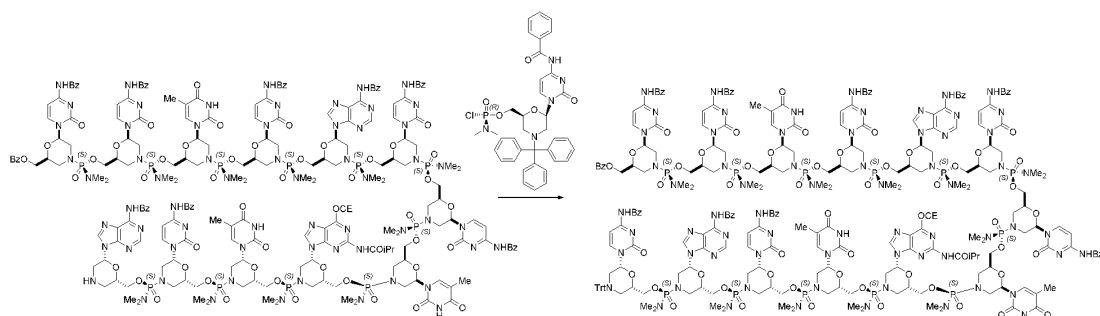
10 [0213] Using General Procedure A: reaction of 11-mer (481 mg) with ((2*S*,6*R*)-6-(6-benzamido-9*H*-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (102 mg) afforded 12-mer (525 mg).

Deprotection of 12-mer:



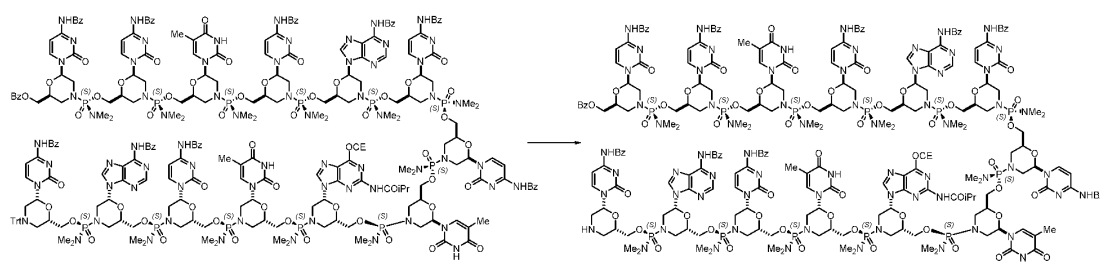
15 [0214] Using General Procedure B: reaction of trityl protected 12-mer (525 mg) afforded free base 12-mer (490 mg).

Coupling to 13-mer:



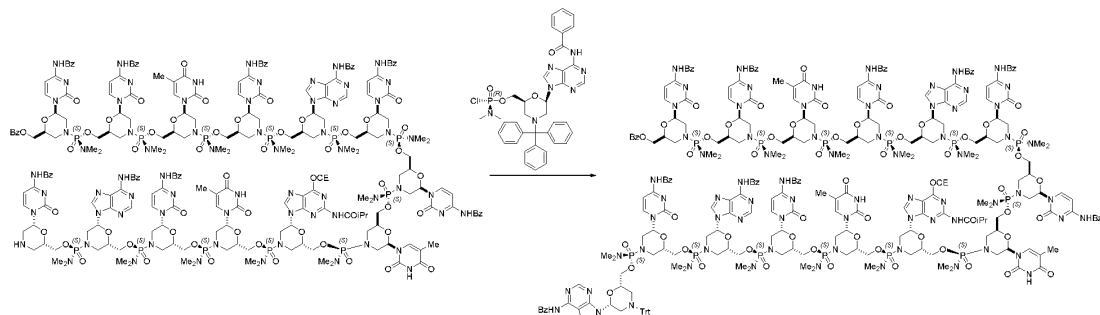
[0215] Using General Procedure A: reaction of 12-mer (484 mg) with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (86 mg) afforded 13-mer (550 mg).

5 Deprotection of 13-mer:



[0216] Using General Procedure B: reaction of trityl protected 13-mer (550 mg) afforded free base 13-mer (550 mg).

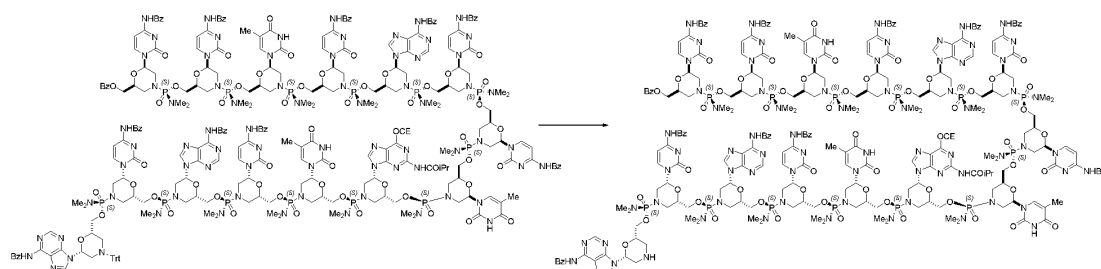
Coupling to 14-mer:



10

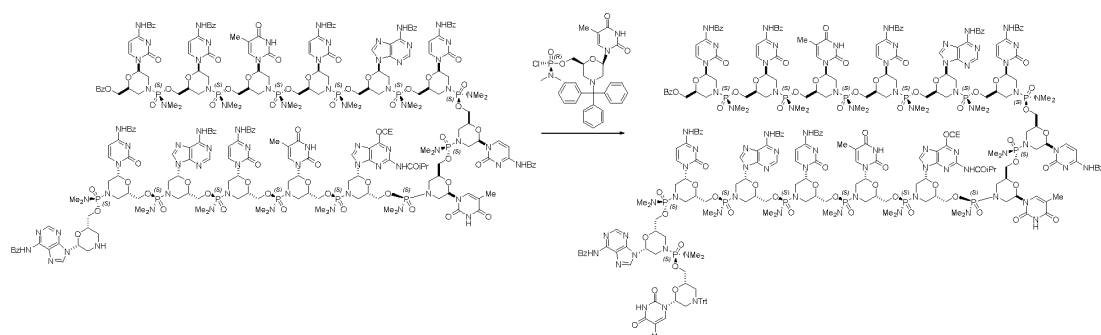
[0217] Using General Procedure A: reaction of 13-mer (550 mg) with ((2*S*,6*R*)-6-(6-benzamido-9*H*-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (94 mg) afforded 14-mer (621 mg).

Deprotection of 14-mer:



Using General Procedure B: reaction of trityl protected 14-mer (621 mg) afforded free base 14-mer (596 mg).

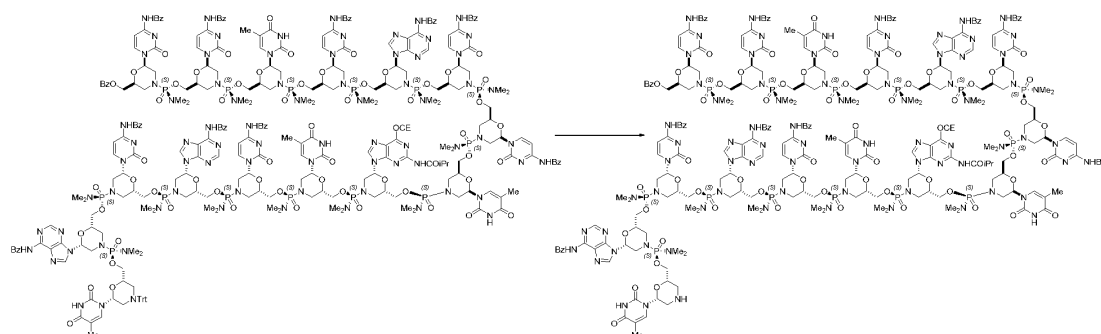
Coupling to 15-mer:



5

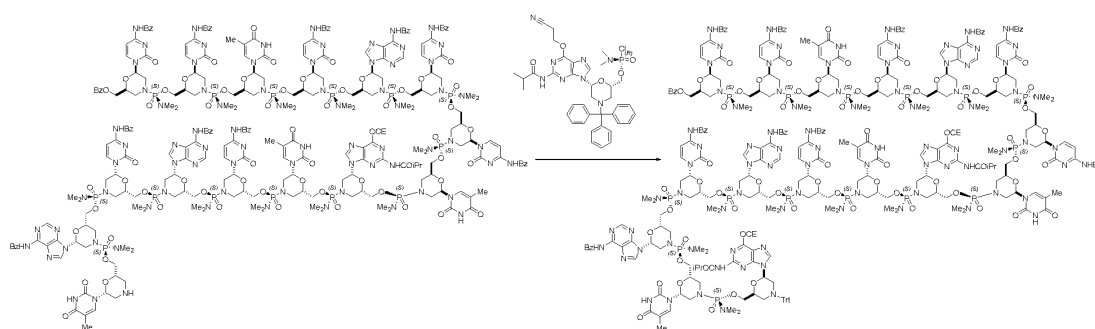
[0218] Using General Procedure A: reaction of 14-mer (596 mg) with ((2*S*,6*R*)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (84 mg) afforded 15-mer (655 mg). MS (ESI) m/z : Calcd for $C_{274}H_{337}N_{79}O_{72}P_{14}$: $[M+4H/4]^+$ 1581.28; Found: 1582.

Deprotection of 15-mer:



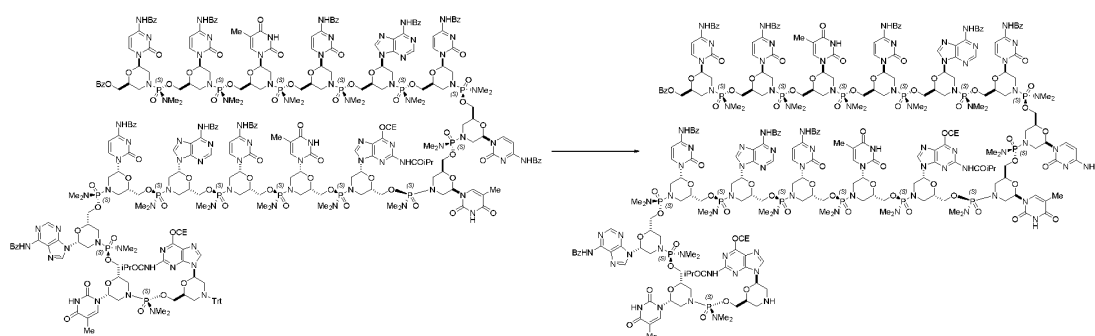
[0219] Using General Procedure B: reaction of trityl protected 15-mer (650 mg) afforded free base 15-mer (613 mg). MS (ESI) m/z : Calcd for $C_{255}H_{323}N_{79}O_{72}P_{14}$: $[M+4H/4]^+$ 1520.76; Found: 1521.

Coupling to 16-mer:



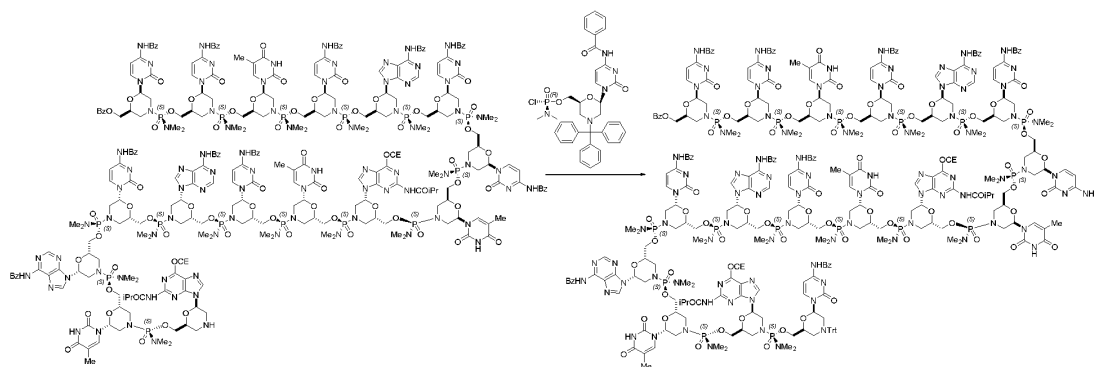
[0220] Using General Procedure A: reaction of 15-mer (613 mg) with ((2*S*,6*R*)-6-(6-(2-cyanoethoxy)-2-isobutyramido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (103 mg) afforded 16-mer (680 mg).

5 Deprotection of 16-mer:



[0221] Using General Procedure B: reaction of trityl protected 16-mer (680 mg) afforded free base 16-mer (623 mg).

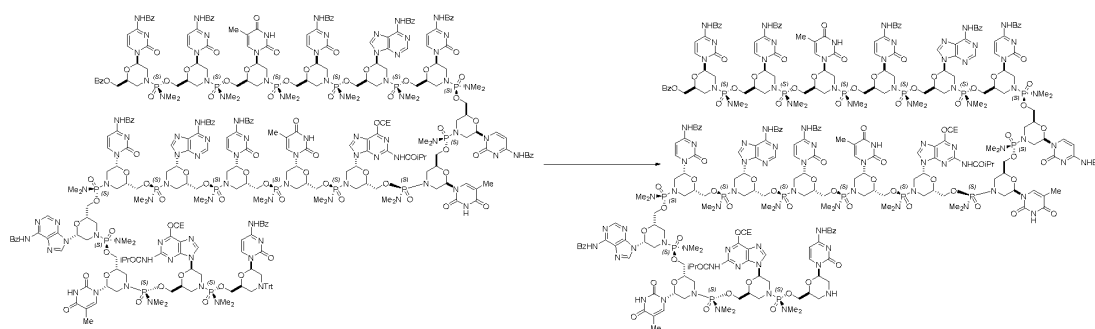
Coupling to 17-mer:



10

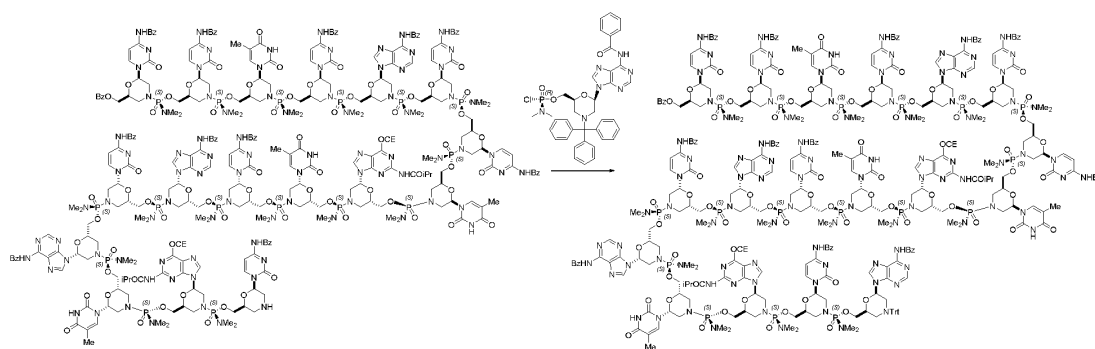
[0222] Using General Procedure A: reaction of 16-mer (623 mg) with ((2*S*,6*R*)-6-(4-benzamido-2-oxypyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (93 mg) afforded 17-mer (690 mg).

Deprotection of 17-mer:



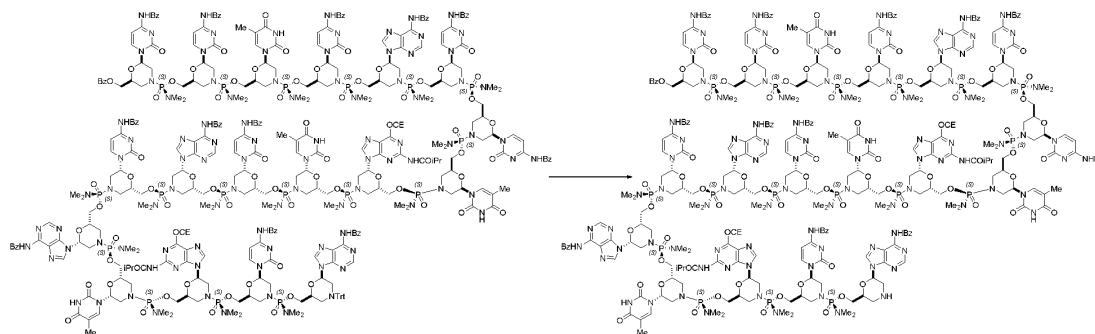
[0223] Using General Procedure B: reaction of trityl protected 17-mer (690 mg) afforded free base 17-mer (670 mg).

Coupling to 18-mer:



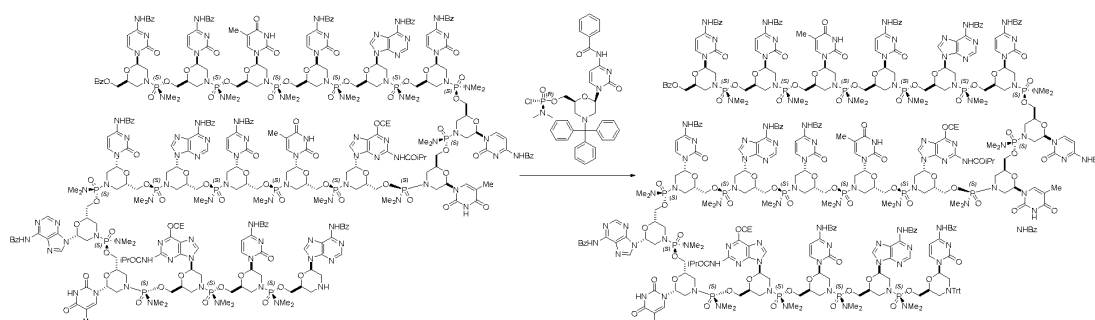
[0224] Using General Procedure A: reaction of 17-mer (673 mg) with ((2*S*,6*R*)-6-(6-benzamido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (R)-dimethylphosphoramidochloridate (98 mg) afforded 18-mer (740 mg).

Deprotection of 18-mer:



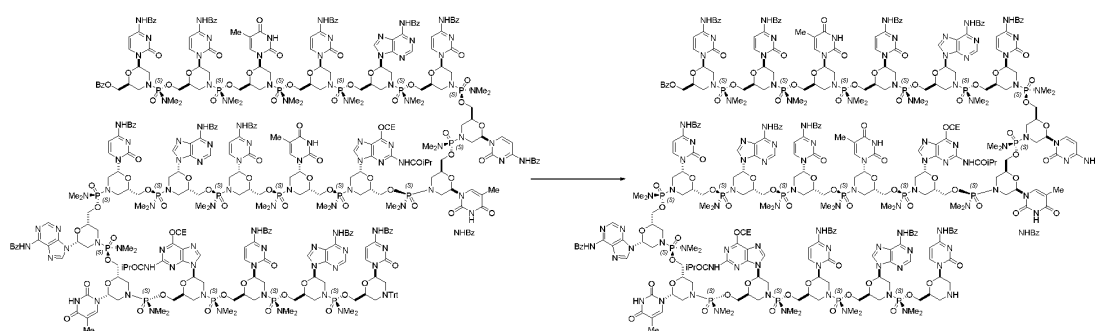
[0225] Using General Procedure B: reaction of trityl protected 18-mer (739 mg) afforded free base 18-mer (675 mg).

Coupling to 19-mer:



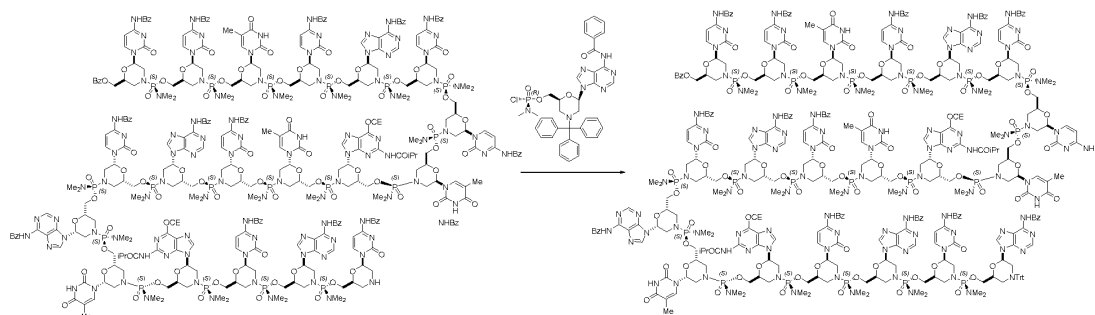
[0226] Using General Procedure A: reaction of 18-mer (675 mg) with ((2*S*,6*R*)-6-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (127 mg) afforded 19-mer (735 mg).

5 Deprotection of 19-mer:



[0227] Using General Procedure B: reaction of trityl protected 19-mer (735 mg) afforded free base 19-mer (732 mg).

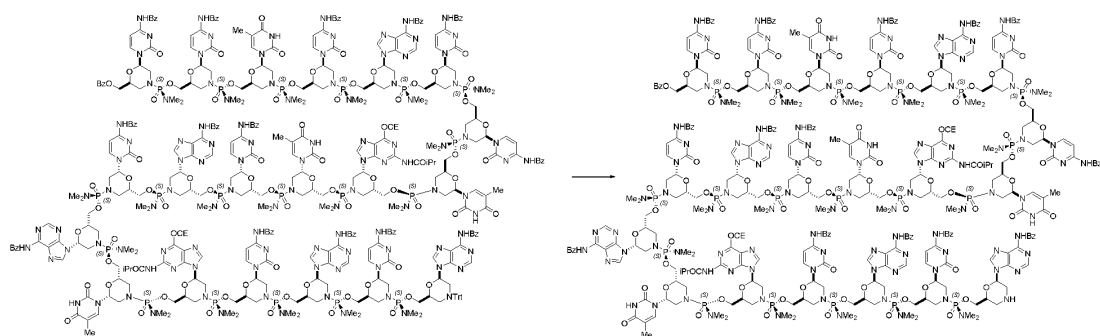
Coupling to 20-mer:



10

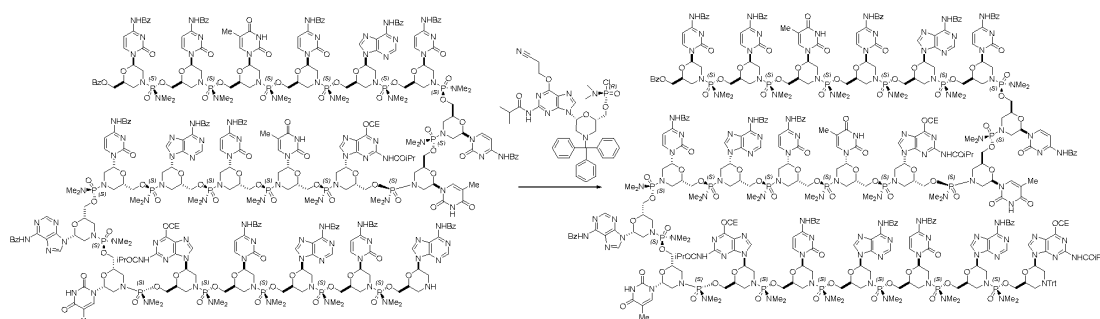
[0228] Using General Procedure A: reaction of 19-mer (732 mg) with ((2*S*,6*R*)-6-(6-benzamido-9*H*-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (135 mg) afforded 20-mer (790 mg). MS (ESI) *m/z*: Calcd for $C_{367}H_{452}N_{111}O_{95}P_{19}$: $[M+5H/5]^+$ 1706; Found: 1707.

15 Deprotection of 20-mer:



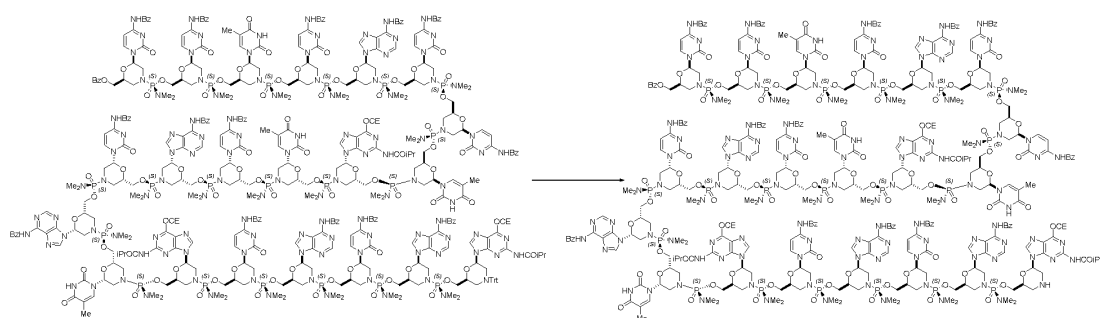
[0229] Using General Procedure B: reaction of trityl protected 20-mer (790 mg) afforded free base 20-mer (743 mg). MS (ESI) m/z : Calcd for $C_{348}H_{438}N_{111}O_{95}P_{19}$: $[M+5H/5]^+$ 1657.6; Found: 1658.

5 Coupling to 21-mer:



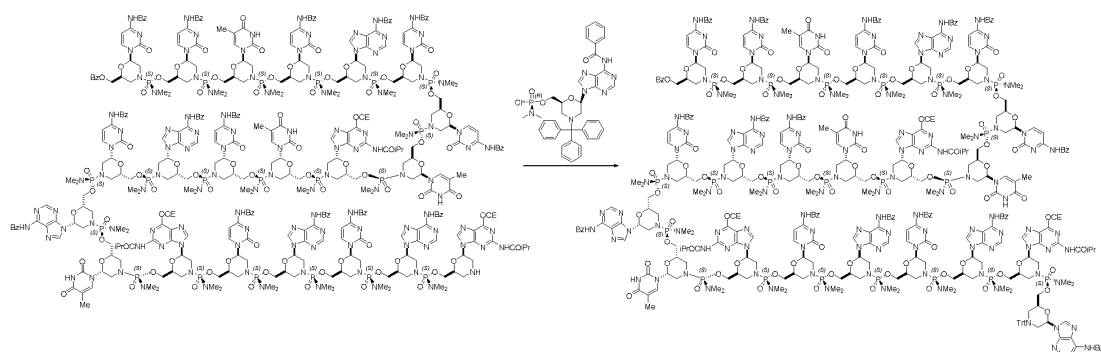
[0230] Using General Procedure A: reaction of 20-mer (743 mg) with ((2*S*,6*R*)-6-(6-(2-cyanoethoxy)-2-isobutyramido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (129 mg) afforded 21-mer (795 mg).

10 Deprotection of 21-mer:



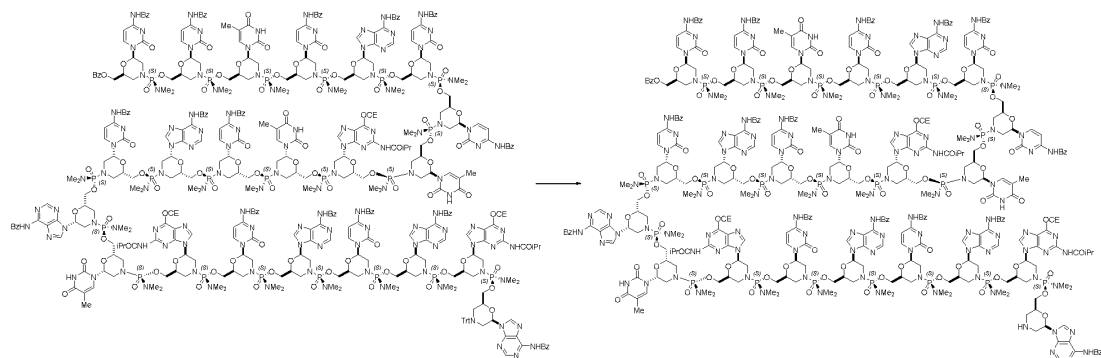
[0231] Using General Procedure B: reaction of trityl protected 21-mer (800 mg) afforded free base 21-mer (756 mg).

Coupling to 22-mer:



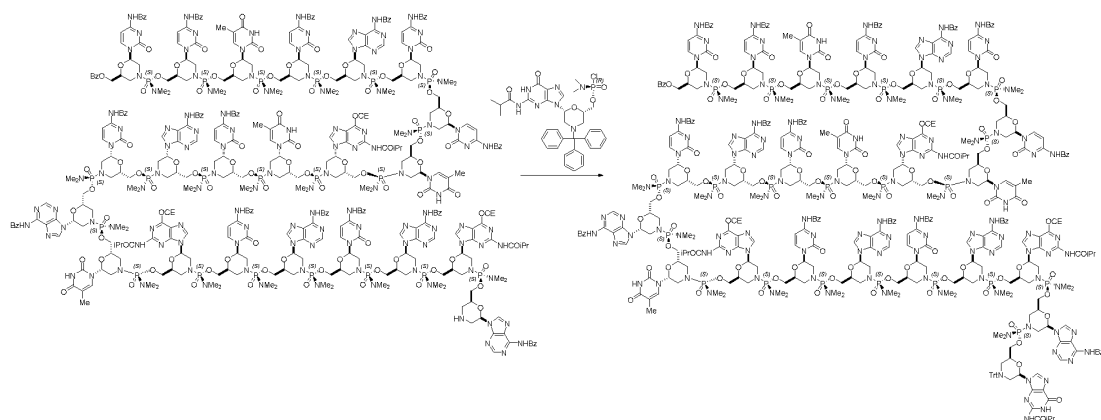
[0232] Using General Procedure A: reaction of 21-mer (753mg) with ((2*S*,6*R*)-6-(6-benzamido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (137 mg) afforded 22-mer (806 mg).

5 Deprotection of 22-mer:



[0233] Using General Procedure B: reaction of trityl protected 22-mer (806 mg) afforded free base 22-mer (785 mg).

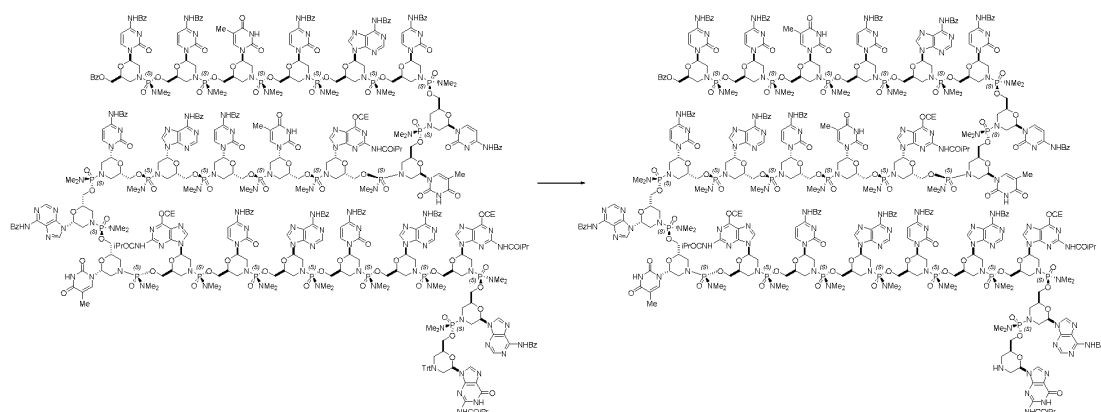
Coupling to 23-mer:



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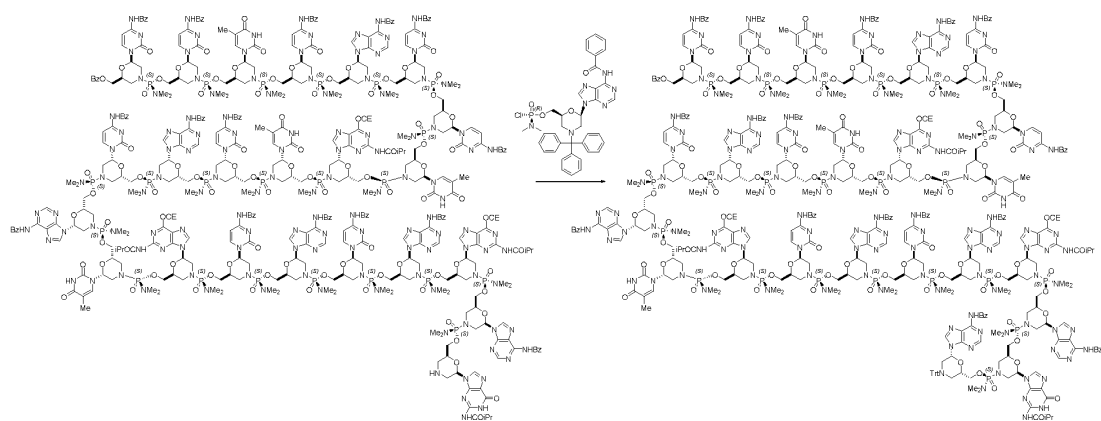
[0234] Using General Procedure A: reaction of 22-mer (780 mg) with ((2*S*,6*R*)-6-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (161 mg) afforded 23-mer (837 mg).

Deprotection of 23-mer:



[0235] Using General Procedure B: reaction of trityl protected 23-mer (837 mg) afforded free base 23-mer (830 mg).

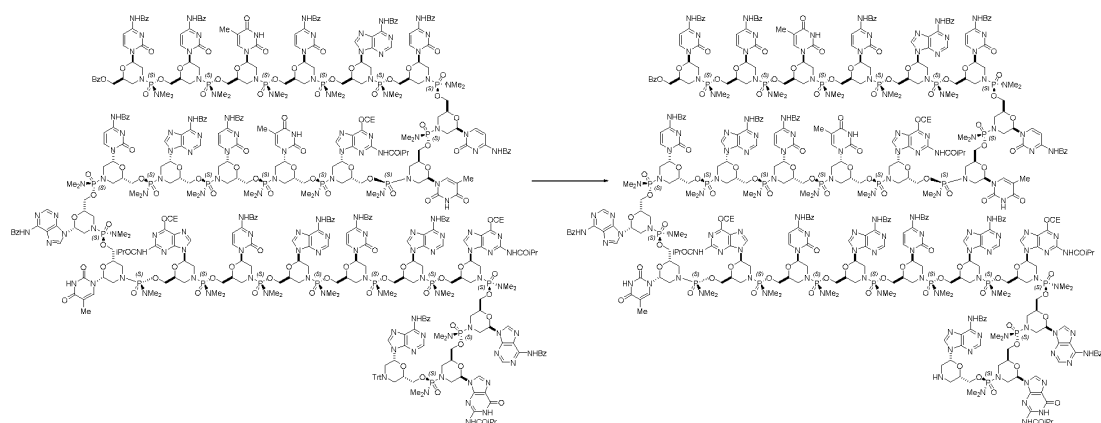
Coupling to 24-mer:



5

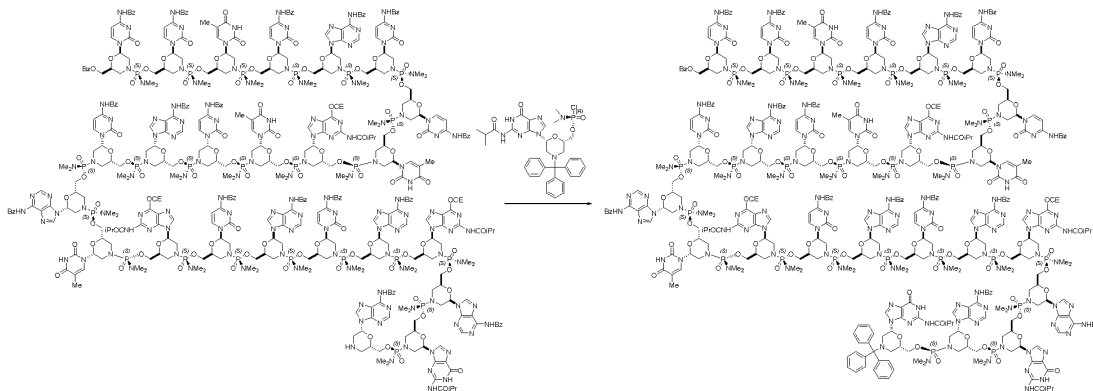
[0236] Using General Procedure A: reaction of 23-mer (830 mg) with ((2*S*,6*R*)-6-(6-benzamido-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (177 mg) afforded 24-mer (800 mg).

Deprotection of 24-mer:

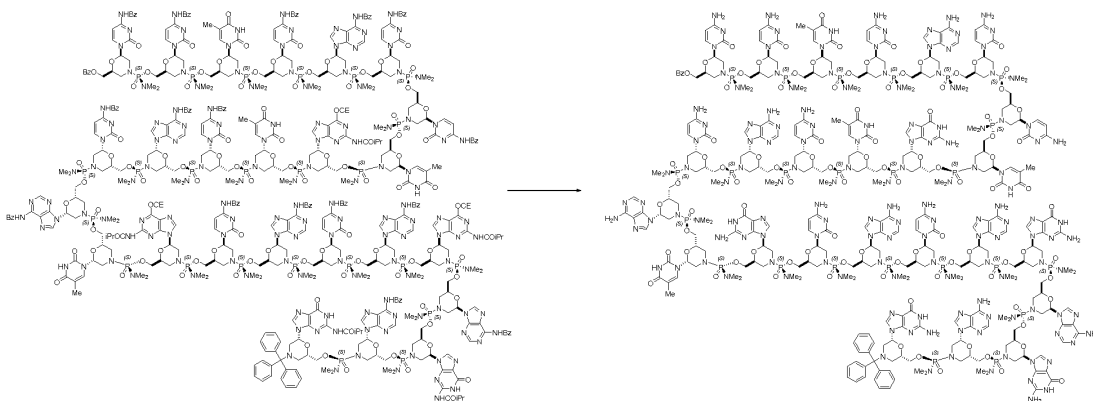


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[0237] Using General Procedure B: reaction of trityl protected 24-mer (800 mg) afforded free base 24-mer (793 mg).

Coupling to 25-mer:

- [0238] Using General Procedure A: reaction of 24-mer (793 mg) with ((2*S*,6*R*)-6-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-tritylmorpholin-2-yl)methyl (*R*)-dimethylphosphoramidochloridate (150 mg) afforded 25-mer (818 mg). MS (ESI) m/z : Calcd for $C_{456}H_{571}N_{147}O_{118}P_{24}$: $[M+7H/7]^+$ 1535.53; Found: 1535.56.

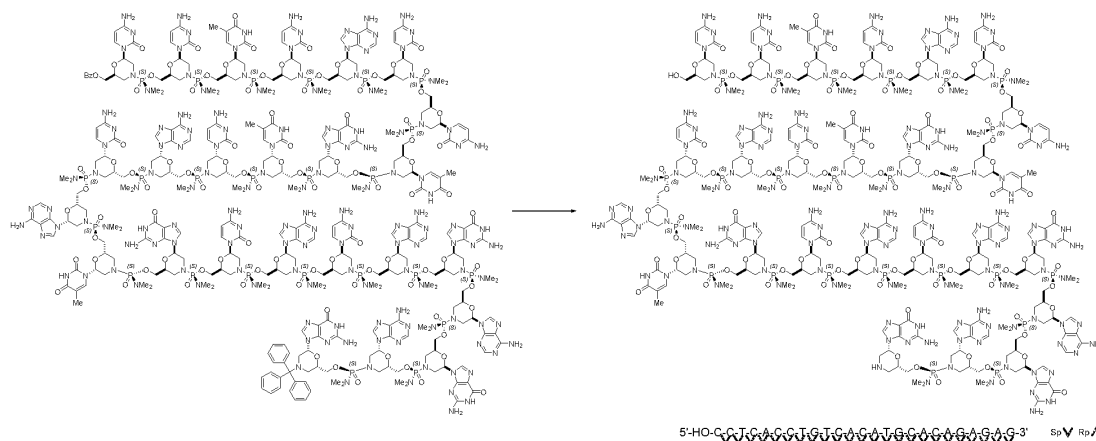
Base deprotection of 25-mer:

- [0239] To a 100mL flask with 25-mer (710 mg) were added methanol (19.5 mL) and 28% aqueous ammonium hydroxide (19.5 mL) under nitrogen atmosphere. The reaction mixture was stirred at 50 °C for 2 days affording a clear solution. The solution was then evaporated under vacuum to a volume of ca 20mL at 35 °C. The slightly cloudy mixture was filtered through a plastic fritted funnel and rinsed with ca 5-10mL of water for a total adjusted volume of 25mL. The resulting solution was used for purification by reverse phase HPLC using the conditions below. The desired peak fractions were evaporated to afford a total of 148mg deprotected Trityl-on 25-mer as a white solid. MS (ESI) m/z : Calcd for $C_{308}H_{464}N_{144}O_{96}P_{24}$: $[M+1H]^+$ 8462.97; Found: 8463.00 (deconvoluted HRMS spectrum).

Purification conditions for trityl-protected PMO:

Column:	Waters Xbridge prep C18 5um OBD 19x100mm (Part Number: 186002978)						
Instrument:	Waters Prep HPLC						
Mobile phase A:	10 mM NH4HCO3, pH 10 (with NH4OH)						
Mobile phase B:	15% of Mobile Phase A and 85% acetonitrile						
Column Temperature:	Unregulated						
Gradient:							
	TIME (min)	A%	B%				
	0	80	20		Initial		
	12	65	35		Elution Gradient		
	13	0	100				
	14	0	100				
	15	80	20		Reset Conditions		
Flow Rate (mL/min):	40						
Wavelength:	DAD + 260nm + timed collection						

Trityl deprotection of 25-mer:



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[0240] To a vial with trityl protected 25-mer (3.7 mg) was added 0.1 M phosphoric acid (250 μ L). The vial was agitated at rt for 4h when the reaction was deemed completed (two consecutive checks by UPLC MS shows starting material peak was converted to a earlier eluting peak). Added 0.1M ammonium hydroxide (250 μ L) and filtered through a syringe filter of 0.2 μ M. The filter was rinsed with 0.4mL of water and collected into a vial. The sample was purified by reverse phase HPLC using the method in the below table. The desired fractions were combined and evaporated under vacuum, then lyophilized to afford the desired product 1.2 mg of 25-mer PMO (PMO-302). MS (ESI) m/z: Calcd for C₂₈₉H₄₅₀N₁₄₄O₉₆P₂₄: [M+1H]⁺ 8220.86; Found: 8220.87 (deconvoluted HRMS spectrum).

15

Purification conditions for fully-deprotected PMO:

Column:	Waters Xbridge prep C18 5um OBD 19x100mm (Part Number: 186002978)						
Instrument:	Waters Prep HPLC						
Mobile phase A:	Water + 0.1% Ammonium Hydroxide						
Mobile phase B:	Acetonitrile + 0.1% Ammonium Hydroxide						
Column Temperature:	Unregulated						
Gradient:							
	TIME (min)	A%	B%				
	0	95	5		Initial		
	1.1	95	5		Elution Gradient		
	11.1	80	20				
	12.1	5	95				
	13.1	5	95				
	13.2	95	5		Reset Conditions		
	15	95	5				
Flow Rate (mL/min):	40						
Wavelength:	DAD + 260nm + timed collection						

Example 4: Evaluation of splice modulation properties of CD33 Exon-2 targeting

oligonucleotides:

[0241] Different technologies can be used to assess the activity/properties of *CD33* targeting oligonucleotides using various human, mouse, and non-human primate cell lines.

In vitro assay methods:

- [0242] U-188 MG (human glioblastoma cell lines) and human iCell Microglia cells were used for screening of *CD33* Exon-2 skipping ASOs (*CD33* Oligonucleotides). U-118 MG cell lines were purchased from ATCC. iCell Microglia cells were purchased from Fujifilm (Cellular Dynamics). Both cellular models were cultured and maintained using appropriate media suggested in the vendor protocols. Screening was performed in 96 WP formats, seeding about 20,000 cells per well and treating with specified concentrations of modified ASOs using Endo-Porter or Lipofectamine reagents. Cells were further incubated at 37°C in a cell culture incubator for 48 hours before isolating the total RNA. Various experiments were carried out in biological duplicates. Total RNA was isolated and converted to cDNA as per vendor protocol, then Taqman gene expression assays were used to quantify Exon-2 skipped and un-skipped *CD33* mRNA transcripts. Human house-keeping genes such as HPRT1 or GAPDH1 expressions were used to normalize the target transcript expressions.

Evaluation of PMO-ASO sequences

- [0243] PMO ASOs were tested for their efficacy in inducing skipping of Exon-2 in a *CD33* gene transcript in U-118 MG glioblastoma cells *in vitro*. PMO ASOs were designed to cover *CD33* Exon-2 and its surrounding introns (SEQ ID NO:1) in 25 nucleotide sections that moved down

SEQ ID NO:1 5' to 3' five nucleotides at a time. Oligonucleotides were tested using two concentrations (0.5 μ M and 0.167 μ M) and delivered using Endo-Porter reagents. Cells harvested and RNA isolated at 48 hours of post treatment. Total RNA was converted to cDNA as per vendor protocol and Taqman gene expression assays were used to quantify Exon-2 skipped and un-skipped *CD33* mRNA transcripts. Human house-keeping gene HPRT1 expression was used as a loading control for each experiment. Each oligonucleotide was tested twice at each concentration.

[0244] The skipping efficiency of the oligonucleotides was calculated using the following formula.

$$\text{Skipping \%} = \frac{\text{(Skipped Value)}}{\text{(Skipped)} + \text{(Un-skipped)}} \times 100$$

[0245] Skipping efficiency is represented on a scale of 0 to 100, wherein 100 represents 100% skipping of *CD33* Exon-2 skipping. Each experiment used a negative control oligonucleotide, NTC (Non-Targeting Control), which does not target *CD33*.

Results are shown in Table 3.

Table 3

ASO NO:	Sequence (5' to 3')	SEQ. ID NO:	Conc. (μ M)	Exon-2 Skipping %	
PBS	Not Applicable	PBS	0	15.69	22.01
PBS	Not Applicable	PBS	0	18.13	13.26
PMO-016	CAGTACCAGGGTCCCATCCCAGCCC	16	0.5	20.58	25.01
PMO-016	CAGTACCAGGGTCCCATCCCAGCCC	16	0.167	17.69	20.34
PMO-017	CCAGGGTCCCATCCCAGCCCTGCCC	17	0.5	17.00	21.53
PMO-017	CCAGGGTCCCATCCCAGCCCTGCCC	17	0.167	19.12	19.13
PMO-018	GTCCCATCCCAGCCCTGCCCTGCAG	18	0.5	19.50	19.62
PMO-018	GTCCCATCCCAGCCCTGCCCTGCAG	18	0.167	18.34	20.68
PMO-019	ATCCCAGCCCTGCCCTGCAGTACCC	19	0.5	18.31	17.77
PMO-019	ATCCCAGCCCTGCCCTGCAGTACCC	19	0.167	17.82	18.09
PMO-020	AGCCCTGCCCTGCAGTACCCATGAA	20	0.5	ND	17.85
PMO-020	AGCCCTGCCCTGCAGTACCCATGAA	20	0.167	19.28	17.35
PMO-021	TGCCCTGCAGTACCCATGAACTTCC	21	0.5	17.12	14.33
PMO-021	TGCCCTGCAGTACCCATGAACTTCC	21	0.167	19.94	15.79
PMO-022	TGCAGTACCCATGAACTTCCCTTGC	22	0.5	10.76	9.32
PMO-022	TGCAGTACCCATGAACTTCCCTTGC	22	0.167	17.90	17.11

PMO-023	TACCCATGAACTTCCCTTGCGGCCA	23	0.5	ND	10.73
PMO-023	TACCCATGAACTTCCCTTGCGGCCA	23	0.167	16.04	12.77
PMO-024	ATGAACTTCCCTTGCGGCCACTTCT	24	0.5	14.47	12.34
PMO-024	ATGAACTTCCCTTGCGGCCACTTCT	24	0.167	16.55	16.86
PMO-025	CTTCCCTTGCGGCCACTTCTGAAGC	25	0.5	11.82	11.09
PMO-025	CTTCCCTTGCGGCCACTTCTGAAGC	25	0.167	16.10	16.30
PMO-026	CTTGCGGCCACTTCTGAAGCCTGTG	26	0.5	11.01	10.46
PMO-026	CTTGCGGCCACTTCTGAAGCCTGTG	26	0.167	12.54	14.08
PMO-027	GGCCACTTCTGAAGCCTGTGCCTCA	27	0.5	23.17	24.10
PMO-027	GGCCACTTCTGAAGCCTGTGCCTCA	27	0.167	17.64	18.26
PMO-028	CTTCTGAAGCCTGTGCCTCACCTGT	28	0.5	29.39	27.55
PMO-028	CTTCTGAAGCCTGTGCCTCACCTGT	28	0.167	19.87	17.91
PMO-029	GAAGCCTGTGCCTCACCTGTCACAT	29	0.5	28.57	24.78
PMO-029	GAAGCCTGTGCCTCACCTGTCACAT	29	0.167	20.96	20.73
PMO-030	CTGTGCCTCACCTGTCACATGCACA	30	0.5	25.85	27.90
PMO-030	CTGTGCCTCACCTGTCACATGCACA	30	0.167	19.42	22.08
PMO-002	CCTCACCTGTCACATGCACAGAGAG	2	0.5	38.65	37.45
PMO-002	CCTCACCTGTCACATGCACAGAGAG	2	0.167	26.85	26.74
PMO-003	CCTGTCACATGCACAGAGAGCTGGG	3	0.5	34.57	48.48
PMO-003	CCTGTCACATGCACAGAGAGCTGGG	3	0.167	22.33	29.52
PMO-031	CACATGCACAGAGAGCTGGGGAGAT	31	0.5	18.84	23.01
PMO-031	CACATGCACAGAGAGCTGGGGAGAT	31	0.167	17.14	15.10
PMO-032	GCACAGAGAGCTGGGGAGATTTGTA	32	0.5	17.35	16.34
PMO-032	GCACAGAGAGCTGGGGAGATTTGTA	32	0.167	17.78	14.79
PMO-033	GAGAGCTGGGGAGATTTGTAAGTGT	33	0.5	23.37	18.65
PMO-033	GAGAGCTGGGGAGATTTGTAAGTGT	33	0.167	22.58	21.78
PMO-034	CTGGGGAGATTTGTAAGTGTATTTG	34	0.5	ND	18.38
PMO-034	CTGGGGAGATTTGTAAGTGTATTTG	34	0.167	17.67	16.19
PMO-035	GAGATTTGTAAGTGTATTTGGTACT	35	0.5	27.36	26.10
PMO-035	GAGATTTGTAAGTGTATTTGGTACT	35	0.167	ND	23.64
PMO-036	TTGTAAGTGTATTTGGTACTTCCTC	36	0.5	40.31	31.62
PMO-036	TTGTAAGTGTATTTGGTACTTCCTC	36	0.167	25.77	26.98
PMO-037	ACTGTATTTGGTACTTCCTCTCTCC	37	0.5	39.08	44.31
PMO-037	ACTGTATTTGGTACTTCCTCTCTCC	37	0.167	32.28	32.79
PMO-004	ATTTGGTACTTCCTCTCTCCATCCG	4	0.5	38.03	43.18
PMO-004	ATTTGGTACTTCCTCTCTCCATCCG	4	0.167	35.68	27.95
PMO-038	GTACTTCCTCTCTCCATCCGAAAGA	38	0.5	32.51	37.10

PMO-038	GTACTTCCTCTCTCCATCCGAAAGA	38	0.167	25.18	23.40
PMO-039	TCCTCTCTCCATCCGAAAGAAGTAT	39	0.5	43.59	35.11
PMO-039	TCCTCTCTCCATCCGAAAGAAGTAT	39	0.167	28.26	31.00
PMO-005	TCTCCATCCGAAAGAAGTATGAACC	5	0.5	35.76	37.72
PMO-005	TCTCCATCCGAAAGAAGTATGAACC	5	0.167	28.69	28.28
PMO-040	ATCCGAAAGAAGTATGAACCATTAT	40	0.5	35.90	29.79
PMO-040	ATCCGAAAGAAGTATGAACCATTAT	40	0.167	26.41	24.05
PMO-041	AAAGAAGTATGAACCATTATCCCTC	41	0.5	26.90	27.85
PMO-041	AAAGAAGTATGAACCATTATCCCTC	41	0.167	25.80	27.27
PMO-042	AGTATGAACCATTATCCCTCCTCCT	42	0.5	21.24	19.46
PMO-042	AGTATGAACCATTATCCCTCCTCCT	42	0.167	19.56	21.66
PMO-043	GAACCATTATCCCTCCTCCTGGCGT	43	0.5	30.13	27.10
PMO-043	GAACCATTATCCCTCCTCCTGGCGT	43	0.167	24.70	25.35
PMO-044	ATTATCCCTCCTCCTGGCGTCTACG	44	0.5	26.86	24.63
PMO-044	ATTATCCCTCCTCCTGGCGTCTACG	44	0.167	20.68	26.29
PMO-045	CCCTCCTCCTGGCGTCTACGATGCT	45	0.5	23.56	22.61
PMO-045	CCCTCCTCCTGGCGTCTACGATGCT	45	0.167	19.23	23.34
PMO-046	CTCCTGGCGTCTACGATGCTCAGGG	46	0.5	24.16	28.53
PMO-046	CTCCTGGCGTCTACGATGCTCAGGG	46	0.167	26.02	25.22
PMO-047	GGCGTCTACGATGCTCAGGGAGCAG	47	0.5	23.39	26.54
PMO-047	GGCGTCTACGATGCTCAGGGAGCAG	47	0.167	24.89	23.35
PMO-048	CTACGATGCTCAGGGAGCAGTTGTT	48	0.5	23.09	26.70
PMO-048	CTACGATGCTCAGGGAGCAGTTGTT	48	0.167	24.86	20.24
PMO-049	ATGCTCAGGGAGCAGTTGTTCTAC	49	0.5	23.21	23.31
PMO-049	ATGCTCAGGGAGCAGTTGTTCTAC	49	0.167	20.58	ND
PMO-050	CAGGGAGCAGTTGTTCTACTGGGA	50	0.5	26.92	21.31
PMO-050	CAGGGAGCAGTTGTTCTACTGGGA	50	0.167	17.69	20.22
PMO-051	AGCAGTTGTTCTACTGGGATCCCC	51	0.5	20.55	21.10
PMO-051	AGCAGTTGTTCTACTGGGATCCCC	51	0.167	21.30	18.81
PMO-052	TTGTTCTACTGGGATCCCCAAGGA	52	0.5	14.29	17.26
PMO-052	TTGTTCTACTGGGATCCCCAAGGA	52	0.167	26.38	19.68
PMO-053	CCTACTGGGATCCCCAAGGAGGCGG	53	0.5	18.37	15.87
PMO-053	CCTACTGGGATCCCCAAGGAGGCGG	53	0.167	13.70	21.48
PMO-054	TGGGATCCCCAAGGAGGCGGAATCT	54	0.5	18.17	26.18
PMO-054	TGGGATCCCCAAGGAGGCGGAATCT	54	0.167	19.79	19.06
PMO-055	TCCCCAAGGAGGCGGAATCTGCCCT	55	0.5	19.77	18.45
PMO-055	TCCCCAAGGAGGCGGAATCTGCCCT	55	0.167	21.96	13.22

PMO-056	AAGGAGGCGGAATCTGCCCTGAGTC	56	0.5	18.42	28.31
PMO-056	AAGGAGGCGGAATCTGCCCTGAGTC	56	0.167	20.39	18.38
PMO-057	GGCGGAATCTGCCCTGAGTCTCCTC	57	0.5	16.83	14.78
PMO-057	GGCGGAATCTGCCCTGAGTCTCCTC	57	0.167	21.10	19.33
PMO-058	AATCTGCCCTGAGTCTCCTCCTGTA	58	0.5	14.23	13.92
PMO-058	AATCTGCCCTGAGTCTCCTCCTGTA	58	0.167	15.61	20.25
PMO-059	GCCCTGAGTCTCCTCCTGTACTTCT	59	0.5	15.60	17.24
PMO-059	GCCCTGAGTCTCCTCCTGTACTTCT	59	0.167	16.31	17.25
PMO-060	GAGTCTCCTCCTGTACTTCTTGATC	60	0.5	17.48	11.05
PMO-060	GAGTCTCCTCCTGTACTTCTTGATC	60	0.167	17.37	17.20
PMO-061	TCCTCCTGTACTTCTTGATCTAGCT	61	0.5	16.82	19.32
PMO-061	TCCTCCTGTACTTCTTGATCTAGCT	61	0.167	16.57	16.78
PMO-062	CTGTACTTCTTGATCTAGCTTGTTT	62	0.5	12.61	15.76
PMO-062	CTGTACTTCTTGATCTAGCTTGTTT	62	0.167	15.85	16.60
PMO-063	CTTCTTGATCTAGCTTGTTTGTGGC	63	0.5	7.95	7.13
PMO-063	CTTCTTGATCTAGCTTGTTTGTGGC	63	0.167	14.81	16.45
PMO-064	TGATCTAGCTTGTTTGTGGCCACTG	64	0.5	11.67	15.59
PMO-064	TGATCTAGCTTGTTTGTGGCCACTG	64	0.167	15.34	16.81
PMO-065	TAGCTTGTTTGTGGCCACTGGAGAG	65	0.5	9.86	12.58
PMO-065	TAGCTTGTTTGTGGCCACTGGAGAG	65	0.167	14.97	14.60
PMO-066	TGTTTGTGGCCACTGGAGAGTCCCT	66	0.5	16.61	13.93
PMO-066	TGTTTGTGGCCACTGGAGAGTCCCT	66	0.167	15.52	14.44
PMO-067	GTGGCCACTGGAGAGTCCCTGGATA	67	0.5	14.40	12.62
PMO-067	GTGGCCACTGGAGAGTCCCTGGATA	67	0.167	14.38	16.49
PMO-068	CACTGGAGAGTCCCTGGATATAATG	68	0.5	16.74	19.00
PMO-068	CACTGGAGAGTCCCTGGATATAATG	68	0.167	16.44	16.67
PMO-069	GAGAGTCCCTGGATATAATGGCTCC	69	0.5	17.04	17.70
PMO-069	GAGAGTCCCTGGATATAATGGCTCC	69	0.167	19.01	19.95
PMO-070	TCCCTGGATATAATGGCTCCTTCCC	70	0.5	17.32	16.87
PMO-070	TCCCTGGATATAATGGCTCCTTCCC	70	0.167	16.41	14.39
PMO-071	GGATATAATGGCTCCTTCCCGGAAC	71	0.5	15.59	18.00
PMO-071	GGATATAATGGCTCCTTCCCGGAAC	71	0.167	18.67	28.86
PMO-072	TAATGGCTCCTTCCCGGAACCAGTA	72	0.5	17.61	18.80
PMO-072	TAATGGCTCCTTCCCGGAACCAGTA	72	0.167	16.00	22.11
PMO-073	GCTCCTTCCCGGAACCAGTAACCAT	73	0.5	15.92	16.69
PMO-073	GCTCCTTCCCGGAACCAGTAACCAT	73	0.167	15.96	15.51
PMO-074	TTCCCGGAACCAGTAACCATGAACT	74	0.5	14.79	19.48

PMO-074	TTCCCGGAACCAGTAACCATGAACT	74	0.167	16.78	15.98
PMO-075	GGAACCAGTAACCATGAACTGGGGA	75	0.5	15.96	14.76
PMO-075	GGAACCAGTAACCATGAACTGGGGA	75	0.167	20.20	20.33
PMO-076	CAGTAACCATGAACTGGGGAGTTCT	76	0.5	15.12	15.51
PMO-076	CAGTAACCATGAACTGGGGAGTTCT	76	0.167	15.78	17.18
PMO-077	ACCATGAACTGGGGAGTTCTTGTCG	77	0.5	10.79	15.88
PMO-077	ACCATGAACTGGGGAGTTCTTGTCG	77	0.167	17.29	4.46
PMO-078	GAACTGGGGAGTTCTTGTCGTAGTA	78	0.5	18.50	11.38
PMO-078	GAACTGGGGAGTTCTTGTCGTAGTA	78	0.167	13.09	16.04
PMO-079	GGGGAGTTCTTGTCGTAGTAGGGTA	79	0.5	11.62	12.30
PMO-079	GGGGAGTTCTTGTCGTAGTAGGGTA	79	0.167	15.89	15.01
PMO-080	GTTCTTGTCGTAGTAGGGTATGGGA	80	0.5	16.44	16.32
PMO-080	GTTCTTGTCGTAGTAGGGTATGGGA	80	0.167	19.96	20.31
PMO-081	TGTCGTAGTAGGGTATGGGATGGAA	81	0.5	20.16	15.18
PMO-081	TGTCGTAGTAGGGTATGGGATGGAA	81	0.167	17.13	16.78
PMO-082	TAGTAGGGTATGGGATGGAAGAAAG	82	0.5	37.51	32.78
PMO-082	TAGTAGGGTATGGGATGGAAGAAAG	82	0.167	23.94	24.65
PMO-083	GGGTATGGGATGGAAGAAAGTGCAG	83	0.5	29.55	31.59
PMO-083	GGGTATGGGATGGAAGAAAGTGCAG	83	0.167	26.54	25.26
PMO-006	TGGGATGGAAGAAAGTGCAGGGCAC	6	0.5	33.94	28.96
PMO-006	TGGGATGGAAGAAAGTGCAGGGCAC	6	0.167	22.10	21.87
PMO-084	TGGAAGAAAGTGCAGGGCACGAGGA	84	0.5	21.23	22.41
PMO-084	TGGAAGAAAGTGCAGGGCACGAGGA	84	0.167	20.11	20.47
PMO-085	GAAAGTGCAGGGCACGAGGACGCAC	85	0.5	19.04	18.64
PMO-085	GAAAGTGCAGGGCACGAGGACGCAC	85	0.167	18.98	17.74
PMO-086	TGCAGGGCACGAGGACGCACAAACC	86	0.5	17.81	16.75
PMO-086	TGCAGGGCACGAGGACGCACAAACC	86	0.167	18.70	17.72
PMO-087	GGCACGAGGACGCACAAACCCTCCT	87	0.5	15.65	17.94
PMO-087	GGCACGAGGACGCACAAACCCTCCT	87	0.167	17.82	17.12
PMO-088	GAGGACGCACAAACCCTCCTGTACC	88	0.5	22.94	18.52
PMO-088	GAGGACGCACAAACCCTCCTGTACC	88	0.167	19.69	16.19
PMO-089	CGCACAAACCCTCCTGTACCGTCAC	89	0.5	19.29	26.13
PMO-089	CGCACAAACCCTCCTGTACCGTCAC	89	0.167	23.21	24.50
PMO-090	AAACCCTCCTGTACCGTCACTGACT	90	0.5	22.94	23.01
PMO-090	AAACCCTCCTGTACCGTCACTGACT	90	0.167	19.34	21.74
PMO-091	CTCCTGTACCGTCACTGACTCCTGC	91	0.5	18.54	21.34
PMO-091	CTCCTGTACCGTCACTGACTCCTGC	91	0.167	22.92	21.23

PMO-092	GTACCGTCACTGACTCCTGCACTTG	92	0.5	27.95	25.22
PMO-092	GTACCGTCACTGACTCCTGCACTTG	92	0.167	20.83	21.67
PMO-093	GTCAGTCACTCCTGCACTTGACAGCC	93	0.5	30.17	27.61
PMO-093	GTCAGTCACTCCTGCACTTGACAGCC	93	0.167	16.83	20.18
PMO-094	TGACTCCTGCACTTGACAGCCAGAAA	94	0.5	22.59	24.97
PMO-094	TGACTCCTGCACTTGACAGCCAGAAA	94	0.167	19.41	19.40
PMO-095	CCTGCACTTGACAGCCAGAAATTTGG	95	0.5	24.04	24.47
PMO-095	CCTGCACTTGACAGCCAGAAATTTGG	95	0.167	20.75	25.75
PMO-096	ACTTGACAGCCAGAAATTTGGATCCA	96	0.5	27.13	31.68
PMO-096	ACTTGACAGCCAGAAATTTGGATCCA	96	0.167	20.64	23.17
PMO-007	CAGCCAGAAATTTGGATCCATAGCC	7	0.5	33.54	24.73
PMO-007	CAGCCAGAAATTTGGATCCATAGCC	7	0.167	22.72	20.38
PMO-097	AGAAATTTGGATCCATAGCCAGGGC	97	0.5	27.54	30.55
PMO-097	AGAAATTTGGATCCATAGCCAGGGC	97	0.167	24.97	26.23
PMO-098	TTTGGATCCATAGCCAGGGCCCCTG	98	0.5	24.69	19.34
PMO-098	TTTGGATCCATAGCCAGGGCCCCTG	98	0.167	20.23	13.18
PMO-099	ATCCATAGCCAGGGCCCCTGTGGGG	99	0.5	21.37	16.72
PMO-099	ATCCATAGCCAGGGCCCCTGTGGGG	99	0.167	17.63	17.91
PMO-100	TAGCCAGGGCCCCTGTGGGGAAACG	100	0.5	18.47	16.44
PMO-100	TAGCCAGGGCCCCTGTGGGGAAACG	100	0.167	17.48	14.77
PMO-101	AGGGCCCCTGTGGGGAAACGAGGGT	101	0.5	23.22	20.19
PMO-101	AGGGCCCCTGTGGGGAAACGAGGGT	101	0.167	18.70	15.95
PMO-008	CCCTGTGGGGAAACGAGGGTCAGCT	8	0.5	25.81	22.09
PMO-008	CCCTGTGGGGAAACGAGGGTCAGCT	8	0.167	18.18	15.45
PMO-102	TGGGGAAACGAGGGTCAGCTCGGCC	102	0.5	25.88	26.13
PMO-102	TGGGGAAACGAGGGTCAGCTCGGCC	102	0.167	21.60	16.73
PMO-103	AAACGAGGGTCAGCTCGGCCAGCC	103	0.5	29.05	21.15
PMO-103	AAACGAGGGTCAGCTCGGCCAGCC	103	0.167	19.36	14.31
PMO-104	AGGGTCAGCTCGGCCAGCCGACA	104	0.5	21.67	17.87
PMO-104	AGGGTCAGCTCGGCCAGCCGACA	104	0.167	15.37	14.73
PMO-105	CAGCTCGGCCAGCCGACAACCCC	105	0.5	14.61	16.34
PMO-105	CAGCTCGGCCAGCCGACAACCCC	105	0.167	16.89	14.93
PMO-106	CGGCCAGCCGACAACCCCTCTCC	106	0.5	17.13	14.47
PMO-106	CGGCCAGCCGACAACCCCTCTCC	106	0.167	15.72	14.80
PMO-107	CAGCCGACAACCCCTCTCCCCACA	107	0.5	16.70	13.34
PMO-107	CAGCCGACAACCCCTCTCCCCACA	107	0.167	15.72	12.60
PMO-108	CGACAACCCCTCTCCCCACAGCCAC	108	0.5	14.21	12.88

PMO-108	CGACAACCCCTCTCCCCACAGCCAC	108	0.167	16.03	12.42
PMO-109	ACCCCTCTCCCCACAGCCACTCACC	109	0.5	18.03	11.73
PMO-109	ACCCCTCTCCCCACAGCCACTCACC	109	0.167	14.53	11.90
PMO-110	TCTCCCCACAGCCACTCACCTGCCC	110	0.5	14.10	12.30
PMO-110	TCTCCCCACAGCCACTCACCTGCCC	110	0.167	15.90	12.20

Evaluation of MOE-ASO Sequences

[0246] MOE ASOs were tested for their efficacy in inducing skipping of Exon-2 in a *CD33* gene transcript in U-118 MG glioblastoma cells *in vitro*. MOE ASOs were designed to cover *CD33* Exon-2 and its surrounding introns (SEQ ID NO:1) in 20 nucleotide sections that moved down SEQ ID NO:1 5' to 3' five nucleotides at a time. Oligonucleotides were tested using different concentrations (10 nM and 3.3 nM) and delivered using the Lipofectamine protocol. Cells were harvested and RNA was isolated at 48 hours of post treatment. Total RNA was converted to cDNA as per vendor protocol and Taqman gene expression assays were used to quantify Exon-2 skipped and un-skipped *CD33* mRNA transcripts. Human house-keeping gene HPRT1 expression was used as a loading control for each experiment. Each oligonucleotide was tested twice at each concentration.

[0247] The skipping efficiency of the oligonucleotides was calculated using the following formula.

$$\text{Skipping \%} = \frac{(\text{Skipped Value})}{(\text{Skipped}) + (\text{Un-skipped})} \times 100$$

[0248] Skipping efficiency is represented on a scale of 0 to 100, wherein 100 represents 100% skipping of *CD33* Exon-2 skipping. Each experiment used a negative control oligonucleotide, NTC (Non-Targeting Control), which does not target *CD33*. Results are reported in Table 4 below.

Table 4

ASO NO:	Sequence (5' to 3')	SEQ. ID NO:	Conc.(nM)	Exon-2 Skipping %	
PBS	Not Applicable	PBS	0	18.47	13.36
PBS	Not Applicable	PBS	0	17.74	12.61
MOE-111	CAGTACCAGGGTCCCATCCC	111	10	40.31	24.93
MOE-111	CAGTACCAGGGTCCCATCCC	111	3.3	29.81	24.10
MOE-112	CCAGGGTCCCATCCCAGCCC	112	10	31.01	22.56
MOE-112	CCAGGGTCCCATCCCAGCCC	112	3.3	30.80	21.53
MOE-113	GTCCCATCCCAGCCCTGCCC	113	10	22.45	9.58

MOE-113	GTCCCATCCCAGCCCTGCCC	113	3.3	22.13	11.13
MOE-114	ATCCCAGCCCTGCCCTGCAG	114	10	14.91	8.40
MOE-114	ATCCCAGCCCTGCCCTGCAG	114	3.3	17.69	11.98
MOE-115	AGCCCTGCCCTGCAGTACCC	115	10	18.87	12.24
MOE-115	AGCCCTGCCCTGCAGTACCC	115	3.3	18.83	13.28
MOE-116	TGCCCTGCAGTACCCATGAA	116	10	ND	6.70
MOE-116	TGCCCTGCAGTACCCATGAA	116	3.3	9.33	5.16
MOE-117	TGCAGTACCCATGAACTTCC	117	10	6.59	4.56
MOE-117	TGCAGTACCCATGAACTTCC	117	3.3	6.01	3.33
MOE-118	TACCCATGAACTTCCCTTGC	118	10	4.61	2.80
MOE-118	TACCCATGAACTTCCCTTGC	118	3.3	3.41	2.33
MOE-119	ATGAACTTCCCTTGCGGCCA	119	10	13.83	6.59
MOE-119	ATGAACTTCCCTTGCGGCCA	119	3.3	10.67	7.25
MOE-120	CTTCCCTTGCGGCCACTTCT	120	10	11.64	6.49
MOE-120	CTTCCCTTGCGGCCACTTCT	120	3.3	12.08	7.49
MOE-121	CTTGCGGCCACTTCTGAAGC	121	10	26.01	16.70
MOE-121	CTTGCGGCCACTTCTGAAGC	121	3.3	17.39	11.23
MOE-122	GGCCACTTCTGAAGCCTGTG	122	10	3.21	2.84
MOE-122	GGCCACTTCTGAAGCCTGTG	122	3.3	4.52	3.12
MOE-123	CTTCTGAAGCCTGTGCCTCA	123	10	63.47	55.19
MOE-123	CTTCTGAAGCCTGTGCCTCA	123	3.3	59.19	46.69
MOE-124	GAAGCCTGTGCCTCACCTGT	124	10	ND	49.44
MOE-124	GAAGCCTGTGCCTCACCTGT	124	3.3	60.09	43.86
MOE-125	CTGTGCCTCACCTGTCACAT	125	10	54.24	41.03
MOE-125	CTGTGCCTCACCTGTCACAT	125	3.3	37.81	26.33
MOE-126	CCTCACCTGTCACATGCACA	126	10	74.18	59.06
MOE-126	CCTCACCTGTCACATGCACA	126	3.3	69.41	60.05
MOE-127	CCTGTCACATGCACAGAGAG	127	10	71.43	49.59
MOE-127	CCTGTCACATGCACAGAGAG	127	3.3	44.56	37.84
MOE-009	CACATGCACAGAGAGCTGGG	9	10	87.63	71.68
MOE-009	CACATGCACAGAGAGCTGGG	9	3.3	78.49	63.41
MOE-128	GCACAGAGAGCTGGGGAGAT	128	10	87.43	77.61
MOE-128	GCACAGAGAGCTGGGGAGAT	128	3.3	82.29	73.73
MOE-010	GAGAGCTGGGGAGATTTGTA	10	10	85.60	72.24
MOE-010	GAGAGCTGGGGAGATTTGTA	10	3.3	81.85	69.90
MOE-129	CTGGGGAGATTTGTAAGTGT	129	10	74.19	63.26
MOE-129	CTGGGGAGATTTGTAAGTGT	129	3.3	65.32	56.15

MOE-130	GAGATTTGTAAGTGTATTTG	130	10	62.12	44.93
MOE-130	GAGATTTGTAAGTGTATTTG	130	3.3	60.10	38.70
MOE-131	TTGTAAGTGTATTTGGTACT	131	10	66.00	67.32
MOE-131	TTGTAAGTGTATTTGGTACT	131	3.3	61.73	68.50
MOE-132	ACTGTATTTGGTACTTCCTC	132	10	70.65	73.37
MOE-132	ACTGTATTTGGTACTTCCTC	132	3.3	68.40	66.93
MOE-133	ATTTGGTACTTCCTCTCTCC	133	10	75.12	75.05
MOE-133	ATTTGGTACTTCCTCTCTCC	133	3.3	67.22	67.63
MOE-134	GTACTTCCTCTCTCCATCCG	134	10	67.31	68.05
MOE-134	GTACTTCCTCTCTCCATCCG	134	3.3	58.11	63.24
MOE-135	TCCTCTCTCCATCCGAAAGA	135	10	72.52	78.37
MOE-135	TCCTCTCTCCATCCGAAAGA	135	3.3	65.56	71.32
MOE-011	TCTCCATCCGAAAGAAGTAT	11	10	77.95	79.80
MOE-011	TCTCCATCCGAAAGAAGTAT	11	3.3	71.38	73.17
MOE-012	ATCCGAAAGAAGTATGAACC	12	10	79.43	81.71
MOE-012	ATCCGAAAGAAGTATGAACC	12	3.3	71.98	73.17
MOE-136	AAAGAAGTATGAACCATTAT	136	10	65.32	64.65
MOE-136	AAAGAAGTATGAACCATTAT	136	3.3	63.72	64.55
MOE-137	AGTATGAACCATTATCCCTC	137	10	55.07	55.88
MOE-137	AGTATGAACCATTATCCCTC	137	3.3	60.39	60.78
MOE-138	GAACCATTATCCCTCCTCCT	138	10	45.84	49.89
MOE-138	GAACCATTATCCCTCCTCCT	138	3.3	42.18	41.02
MOE-139	ATTATCCCTCCTCCTGGCGT	139	10	46.81	47.81
MOE-139	ATTATCCCTCCTCCTGGCGT	139	3.3	38.49	42.29
MOE-140	CCCTCCTCCTGGCGTCTACG	140	10	48.67	51.53
MOE-140	CCCTCCTCCTGGCGTCTACG	140	3.3	45.10	45.81
MOE-141	CTCCTGGCGTCTACGATGCT	141	10	53.96	58.13
MOE-141	CTCCTGGCGTCTACGATGCT	141	3.3	48.52	53.06
MOE-142	GGCGTCTACGATGCTCAGGG	142	10	57.10	63.60
MOE-142	GGCGTCTACGATGCTCAGGG	142	3.3	44.22	49.17
MOE-143	CTACGATGCTCAGGGAGCAG	143	10	16.30	19.21
MOE-143	CTACGATGCTCAGGGAGCAG	143	3.3	17.28	18.92
MOE-013	ATGCTCAGGGAGCAGTTGTT	13	10	69.53	67.97
MOE-013	ATGCTCAGGGAGCAGTTGTT	13	3.3	60.30	62.96
MOE-144	CAGGGAGCAGTTGTTCTAC	144	10	32.12	30.95
MOE-144	CAGGGAGCAGTTGTTCTAC	144	3.3	29.78	30.24
MOE-145	AGCAGTTGTTCTACTGGGA	145	10	54.95	57.38

MOE-145	AGCAGTTGTTCCCTACTGGGA	145	3.3	48.94	51.28
MOE-146	TTGTTCCCTACTGGGATCCCC	146	10	30.91	34.58
MOE-146	TTGTTCCCTACTGGGATCCCC	146	3.3	27.88	30.50
MOE-147	CCTACTGGGATCCCCAAGGA	147	10	23.32	22.90
MOE-147	CCTACTGGGATCCCCAAGGA	147	3.3	20.41	23.17
MOE-148	TGGGATCCCCAAGGAGGCGG	148	10	28.55	30.05
MOE-148	TGGGATCCCCAAGGAGGCGG	148	3.3	23.57	25.39
MOE-149	TCCCCAAGGAGGCGGAATCT	149	10	35.42	39.05
MOE-149	TCCCCAAGGAGGCGGAATCT	149	3.3	32.06	31.12
MOE-150	AAGGAGGCGGAATCTGCCCT	150	10	26.82	27.76
MOE-150	AAGGAGGCGGAATCTGCCCT	150	3.3	23.00	26.50
MOE-151	GGCGGAATCTGCCCTGAGTC	151	10	24.81	30.28
MOE-151	GGCGGAATCTGCCCTGAGTC	151	3.3	21.85	21.58
MOE-152	AATCTGCCCTGAGTCTCCTC	152	10	20.14	20.99
MOE-152	AATCTGCCCTGAGTCTCCTC	152	3.3	21.41	22.14
MOE-153	GCCCTGAGTCTCCTCCTGTA	153	10	39.75	41.02
MOE-153	GCCCTGAGTCTCCTCCTGTA	153	3.3	24.02	26.60
MOE-014	GAGTCTCCTCCTGTACTTCT	14	10	75.96	78.90
MOE-014	GAGTCTCCTCCTGTACTTCT	14	3.3	49.08	53.79
MOE-154	TCCTCCTGTACTTCTTGATC	154	10	60.29	62.38
MOE-154	TCCTCCTGTACTTCTTGATC	154	3.3	33.27	34.20
MOE-155	CTGTACTTCTTGATCTAGCT	155	10	37.05	32.79
MOE-155	CTGTACTTCTTGATCTAGCT	155	3.3	22.07	21.02
MOE-156	CTTCTTGATCTAGCTTGTTT	156	10	28.56	24.36
MOE-156	CTTCTTGATCTAGCTTGTTT	156	3.3	21.49	19.64
MOE-157	TGATCTAGCTTGTTTGTGGC	157	10	23.31	20.74
MOE-157	TGATCTAGCTTGTTTGTGGC	157	3.3	19.90	22.50
MOE-158	TAGCTTGTTTGTGGCCACTG	158	10	23.59	24.40
MOE-158	TAGCTTGTTTGTGGCCACTG	158	3.3	20.35	24.34
MOE-159	TGTTTGTGGCCACTGGAGAG	159	10	23.61	27.17
MOE-159	TGTTTGTGGCCACTGGAGAG	159	3.3	20.37	24.19
MOE-160	GTGGCCACTGGAGAGTCCCT	160	10	25.04	26.30
MOE-160	GTGGCCACTGGAGAGTCCCT	160	3.3	27.86	21.83
MOE-161	CACTGGAGAGTCCCTGGATA	161	10	21.19	19.37
MOE-161	CACTGGAGAGTCCCTGGATA	161	3.3	21.64	21.42
MOE-162	GAGAGTCCCTGGATATAATG	162	10	30.10	30.87
MOE-162	GAGAGTCCCTGGATATAATG	162	3.3	27.48	25.80

MOE-163	TCCCTGGATATAATGGCTCC	163	10	16.89	19.97
MOE-163	TCCCTGGATATAATGGCTCC	163	3.3	20.87	20.85
MOE-164	GGATATAATGGCTCCTTCCC	164	10	20.18	18.88
MOE-164	GGATATAATGGCTCCTTCCC	164	3.3	19.10	26.05
MOE-165	TAATGGCTCCTTCCCGGAAC	165	10	15.35	15.55
MOE-165	TAATGGCTCCTTCCCGGAAC	165	3.3	18.20	23.35
MOE-166	GCTCCTTCCCGGAACCAGTA	166	10	24.43	23.98
MOE-166	GCTCCTTCCCGGAACCAGTA	166	3.3	23.04	28.04
MOE-167	TTCCCGGAACCAGTAACCAT	167	10	17.30	18.36
MOE-167	TTCCCGGAACCAGTAACCAT	167	3.3	19.68	20.56
MOE-168	GGAACCAGTAACCATGAACT	168	10	28.07	33.82
MOE-168	GGAACCAGTAACCATGAACT	168	3.3	29.39	27.36
MOE-169	CAGTAACCATGAACTGGGGA	169	10	25.12	35.14
MOE-169	CAGTAACCATGAACTGGGGA	169	3.3	18.07	16.65
MOE-170	ACCATGAACTGGGGAGTTCT	170	10	36.13	40.54
MOE-170	ACCATGAACTGGGGAGTTCT	170	3.3	24.50	27.23
MOE-171	GAACTGGGGAGTTCTTGTCG	171	10	36.74	40.62
MOE-171	GAACTGGGGAGTTCTTGTCG	171	3.3	25.99	25.29
MOE-172	GGGGAGTTCTTGTCGTAGTA	172	10	33.33	34.65
MOE-172	GGGGAGTTCTTGTCGTAGTA	172	3.3	28.76	29.44
MOE-173	GTTCTTGTCGTAGTAGGGTA	173	10	28.88	28.86
MOE-173	GTTCTTGTCGTAGTAGGGTA	173	3.3	23.21	25.74
MOE-174	TGTCGTAGTAGGGTATGGGA	174	10	25.89	27.21
MOE-174	TGTCGTAGTAGGGTATGGGA	174	3.3	22.43	22.39
MOE-175	TAGTAGGGTATGGGATGGAA	175	10	25.20	23.10
MOE-175	TAGTAGGGTATGGGATGGAA	175	3.3	22.85	24.57
MOE-176	GGGTATGGGATGGAAGAAAG	176	10	22.14	20.68
MOE-176	GGGTATGGGATGGAAGAAAG	176	3.3	28.49	23.76
MOE-177	TGGGATGGAAGAAAGTGCAG	177	10	31.21	31.46
MOE-177	TGGGATGGAAGAAAGTGCAG	177	3.3	23.98	27.84
MOE-178	TGGAAGAAAGTGCAGGGCAC	178	10	27.29	28.99
MOE-178	TGGAAGAAAGTGCAGGGCAC	178	3.3	19.99	27.48
MOE-179	GAAAGTGCAGGGCACGAGGA	179	10	12.36	18.07
MOE-179	GAAAGTGCAGGGCACGAGGA	179	3.3	18.80	18.04
MOE-180	TGCAGGGCACGAGGACGCAC	180	10	26.85	31.33
MOE-180	TGCAGGGCACGAGGACGCAC	180	3.3	25.68	24.68
MOE-181	GGCACGAGGACGCACAAACC	181	10	26.06	25.11

MOE-181	GGCACGAGGACGCACAAACC	181	3.3	30.02	30.36
MOE-182	GAGGACGCACAAACCCTCCT	182	10	34.00	32.47
MOE-182	GAGGACGCACAAACCCTCCT	182	3.3	33.30	32.57
MOE-015	CGCACAAACCCTCCTGTACC	15	10	65.75	69.90
MOE-015	CGCACAAACCCTCCTGTACC	15	3.3	54.43	56.86
MOE-183	AAACCCTCCTGTACCGTCAC	183	10	72.72	72.60
MOE-183	AAACCCTCCTGTACCGTCAC	183	3.3	56.83	58.76
MOE-184	CTCCTGTACCGTCACTGACT	184	10	74.45	77.20
MOE-184	CTCCTGTACCGTCACTGACT	184	3.3	47.20	51.71
MOE-185	GTACCGTCACTGACTCCTGC	185	10	55.41	57.23
MOE-185	GTACCGTCACTGACTCCTGC	185	3.3	42.08	40.45
MOE-186	GTCACGTCACTCCTGCACTTG	186	10	41.60	47.16
MOE-186	GTCACGTCACTCCTGCACTTG	186	3.3	32.79	39.43
MOE-187	TGACTCCTGCACTTGCAAGCC	187	10	57.89	63.61
MOE-187	TGACTCCTGCACTTGCAAGCC	187	3.3	41.11	42.17
MOE-188	CCTGCACTTGCAAGCCAGAAA	188	10	54.71	57.79
MOE-188	CCTGCACTTGCAAGCCAGAAA	188	3.3	36.56	37.48
MOE-189	ACTTGCAAGCCAGAAATTTGG	189	10	24.18	28.93
MOE-189	ACTTGCAAGCCAGAAATTTGG	189	3.3	20.14	21.92
MOE-190	CAGCCAGAAATTTGGATCCA	190	10	69.64	73.25
MOE-190	CAGCCAGAAATTTGGATCCA	190	3.3	59.35	66.00
MOE-191	AGAAATTTGGATCCATAGCC	191	10	50.39	58.23
MOE-191	AGAAATTTGGATCCATAGCC	191	3.3	42.00	44.93
MOE-192	TTTGGATCCATAGCCAGGGC	192	10	24.03	23.58
MOE-192	TTTGGATCCATAGCCAGGGC	192	3.3	21.98	22.59
MOE-193	ATCCATAGCCAGGGCCCCTG	193	10	38.04	40.05
MOE-193	ATCCATAGCCAGGGCCCCTG	193	3.3	26.12	26.68
MOE-194	TAGCCAGGGCCCCTGTGGGG	194	10	25.24	25.25
MOE-194	TAGCCAGGGCCCCTGTGGGG	194	3.3	23.36	23.26
MOE-195	AGGGCCCCTGTGGGGAAACG	195	10	23.50	19.99
MOE-195	AGGGCCCCTGTGGGGAAACG	195	3.3	20.64	16.35
MOE-196	CCCTGTGGGGAAACGAGGGT	196	10	50.10	51.30
MOE-196	CCCTGTGGGGAAACGAGGGT	196	3.3	30.51	29.87
MOE-197	TGGGGAAACGAGGGTCAGCT	197	10	48.33	45.21
MOE-197	TGGGGAAACGAGGGTCAGCT	197	3.3	35.56	29.45
MOE-198	AAACGAGGGTCAGCTCGGCC	198	10	47.94	49.11
MOE-198	AAACGAGGGTCAGCTCGGCC	198	3.3	31.84	29.60

MOE-199	AGGGTCAGCTCGGCCCAGCC	199	10	32.57	37.93
MOE-199	AGGGTCAGCTCGGCCCAGCC	199	3.3	22.79	23.61
MOE-200	CAGCTCGGCCCAGCCCGACA	200	10	19.08	19.10
MOE-200	CAGCTCGGCCCAGCCCGACA	200	3.3	18.51	20.80
MOE-201	CGGCCCAGCCCGACAACCCC	201	10	13.05	11.72
MOE-201	CGGCCCAGCCCGACAACCCC	201	3.3	15.20	16.19
MOE-202	CAGCCCGACAACCCCTCTCC	202	10	15.84	15.39
MOE-202	CAGCCCGACAACCCCTCTCC	202	3.3	16.45	14.82
MOE-203	CGACAACCCCTCTCCCCACA	203	10	21.09	19.86
MOE-203	CGACAACCCCTCTCCCCACA	203	3.3	20.06	16.31
MOE-204	ACCCCTCTCCCCACAGCCAC	204	10	18.35	17.90
MOE-204	ACCCCTCTCCCCACAGCCAC	204	3.3	17.42	21.23
MOE-205	TCTCCCCACAGCCACTCACC	205	10	16.97	17.30
MOE-205	TCTCCCCACAGCCACTCACC	205	3.3	17.67	18.40
MOE-206	CCACAGCCACTCACCTGCCC	206	10	26.79	27.65
MOE-206	CCACAGCCACTCACCTGCCC	206	3.3	25.58	24.45

Identification of *CD33* Regions with Increased Exon-2 Skipping Activity Using ASOs

[0249] PMO and MOE ASOs were designed to cover *CD33* Exon-2 and its surrounding introns (SEQ ID NO:1) in 20-25 nucleotide sections that moved down SEQ ID NO:1 5' to 3' five

- 5 nucleotides at a time. Exon-2 skipping activity was generated in Tables 3 and 4 for these PMO and MOE ASOs, respectively. Regions that exhibited increased Exon-2 skipping activity were identified where two or more consecutive PMO or MOE ASOs that are complementary to a section of SEQ ID NO:1 showed increased Exon-2 skipping activity. Those regions were:

- a. Region 1: (SEQ ID NO:213)
10 (5'-TCTCCCCAGCTCTCTGTGCATGTGACAGGTGAGGCACA-3') (see, e.g., PMO-002 and PMO-003)
- b. Region 2: (SEQ ID NO:214)
(5'-TAATGGTTCATACTTCTTTTCGGATGGAGAGAGGAAGTACCAAATACAGTTACAAA TCT-3') (see, e.g., PMO-036, PMO-037, PMO-004, PMO-038, PMO-039, and PMO-005)
- 15 c. Region 3: (SEQ ID NO:215)
(5'-CCTCGTGCCCTGCACTTTCTTCCATCCCATACCCTACTACGAC-3') (see, e.g., PMO-082, PMO-083, and PMO-006)
- d. Region 4: (SEQ ID NO:216)
20 (5'-AGGGGCCCTGGCTATGGATCCAAATTTCTGGCTGCAAGTGCAG-3') (see, e.g., PMO-096, PMO-007, and PMO-097)

- e. Region 5: (SEQ ID NO:217)
(5'-ACAGTTACAAATCTCCCCAGCTCTCTGTGCATGTGACAGGTGAGG-3') (see, e.g., MOE-009, MOE-128, and MOE-010)
- f. Region 6: (SEQ ID NO:218)
5 (5'-GGTTCATACTTCTTTTCGGATGGAGAGAGGAAGTACCAAAT-3') (see, e.g., MOE-135, MOE-011, and MOE-012)
- g. Region 7: (SEQ ID NO:219)
(5'-GCAGGAGTCAGTGACGGTACAGGAGGGTTTGTGCG-3') (see, e.g., MOE-015, MOE-183, and MOE-184)
- h. Region 8: (SEQ ID NO:220)
10 (5'-GGCCGAGCTGACCCTCGTTTCCCCACAGGGGCCC-3') (see, e.g., MOE-196 and MOE-197).

Evaluation of PMO-ASO Sequences at Multiple Concentrations

[0250] Oligonucleotides were tested for their efficacy in inducing skipping of Exon-2 in a *CD33* gene transcript in U-118 MG glioblastoma cells *in vitro*. Oligonucleotides were tested using
15 different concentrations (0.156, 0.313, 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 μ M) and delivered using the Endo-Porter protocol. Cells were harvested and RNA was isolated at 48 hours of post treatment.

[0251] The skipping efficiency of the oligonucleotides was calculated using the following
20 formula.

$$\text{Skipping \%} \approx \frac{(\text{Skipped Value})}{(\text{Skipped}) + (\text{Un-skipped})} \times 100$$

[0252] Skipping efficiency is represented on a scale of 0 to 100, wherein 100 represents 100% skipping of *CD33* Exon-2 skipping. Each experiment used a negative control oligonucleotide, NTC (Non-Targeting Control), which does not target *CD33*. Skipping efficiency (%*CD33*-D2
25 Transcript Level (Normalized)) are shown in Fig. 5.

Evaluation of MOE-ASO Sequences at Multiple Concentrations

[0253] Oligonucleotides were tested for their efficacy in inducing skipping of Exon-2 in a *CD33* gene transcript in U-118 MG glioblastoma cells *in vitro*. Oligonucleotides were tested using
30 different concentrations (0.082, 0.205, 0.512, 1.28, 3.2, 8.0, 20.0, and 50.0 nM) and delivered using the Lipofectamine protocol. Cells were harvested and RNA was isolated at 48 hours of post treatment.

[0254] The skipping efficiency of the oligonucleotides was calculated using the following formula.

$$\text{Skipping \%} = \frac{(\text{Skipped Value})}{(\text{Skipped}) + (\text{Un-skipped})} \times 100$$

[0255] Skipping efficiency is represented on a scale of 0 to 100, wherein 100 represents 100% skipping of *CD33* Exon-2 skipping. Each experiment used a negative control oligonucleotide, NT (Non-Targeting Control), which does not target *CD33*. Skipping efficiency (%*CD33*-D2 Transcript Level (Normalized)) are shown in Fig. 6.

Example 5: Evaluation of *in vivo* activity of PMO-002 (SEQ ID NO:2) and MOE-012 (SEQ ID NO:12)

[0256] Different technologies can be used to assess the activity/properties of *CD33* targeting oligonucleotides using various human, mouse, and non-human primate cell lines.

***In vivo* assay methods:**

[0257] Humanized *CD33* mouse models were used to study *CD33* Exon-2 skipping ASOs. CRISPR/Cas9 mediated gene editing was used to replace murine *CD33* with human genomic *CD33*, including the signal peptide. Murine 3' and 5' untranslated regions were retained. For *in vivo* experiments, mixed gender cohorts of human *CD33* mouse lines on a C57BL/6 background were used, mice were 12-24 weeks old at the time of dosing.

[0258] PMO-002 (SEQ ID NO:2) and MOE-012 (SEQ ID NO:12) were administered via intracerebroventricular injection at 30 µg or 100 µg into the right lateral ventricle in a 3 µL bolus on day 1. Mice were necropsied 1 week after the injection. At necropsy, mice were transcardially perfused with PBS under avertin anesthesia. Brains were rapidly removed from the skull and the cortex and hippocampus were dissected from the injected hemisphere for exon skipping evaluation. For RNA isolation, frozen tissue was added with 9X volume of Trizol and homogenized for 3 minutes. 500 µL of the Trizol lysate was transferred to a 1 mL deep well plate. 100 µL of chloroform was added to each sample, shaken vigorously, and centrifuged at 4000xg for 5 minutes. The supernatant (250 µL) was transferred to the binding plate from SV96 total RNA extraction kit (Promega) and RNA was extracted per the same protocol. Total RNA was isolated and converted to cDNA per SV96 protocol (Promega), then Taqman gene expression assays were used to quantify Exon-2 skipped *CD33* mRNA transcripts. Mouse house-keeping genes such as HPRT1 or GAPDH1 expressions were used to normalize the target transcript expressions. The fold change of Exon-2 skipped *CD33* mRNA in murine hippocampus is displayed in Fig. 7 (n=4, technical duplicates shown in figure). The fold change of Exon-2 skipped *CD33* mRNA in murine cortex is displayed in Fig. 8 (n=4, technical duplicates shown in figure). In both the hippocampus and cortex, each ASO increased the amount of Exon-2 skipped *CD33* mRNA *in vivo* for both doses relative to PBS control.

Example 6: Additional Exemplary PMO-ASOs

[0259] PMO oligonucleotides were designed for screening. The designed oligonucleotides were made by GeneTools LLC (website:www.gene-tools.com) by solid-phase method. Table 5 below lists synthesized PMO oligonucleotides with their deconvoluted MS data. These PMO oligonucleotides are complementary to a section of SEQ ID NO:1 showing increased Exon-2 skipping activity. In particular, PMO-221 through PMO 240, PMO-324, PMO-424, PMO-402 and PMO-502 are complementary to Region 1; and PMO-241 through PMO-244 are complementary to Region 2. All PMO oligonucleotides listed in Table 5 below contain a phosphorodiamidate-attached sarcosine (Sar) linker at the 5' end. All PMO oligonucleotides listed in Table 5 below were synthesized with unmodified cytosine PMO nucleotide. All PMO oligonucleotides listed in Table 5 below have stereorandom internucleotide linkages, and thus are called stereorandom PMO oligonucleotides. The structure of PMO-224 is as follows:

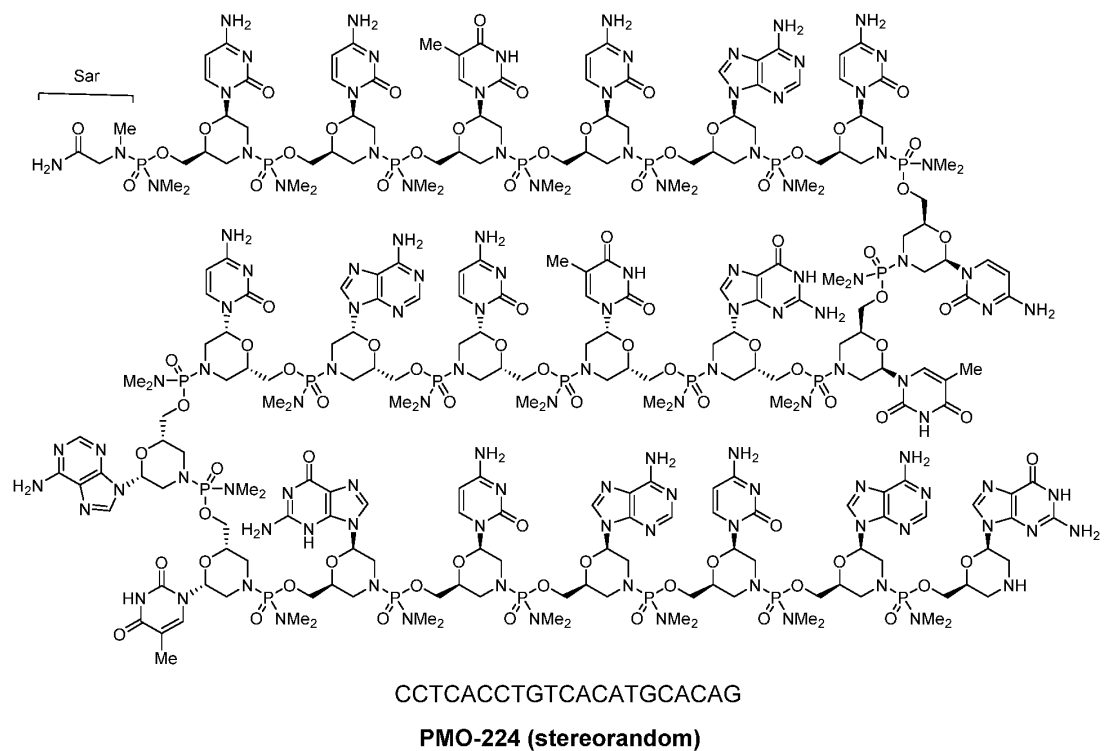


Table 5

ASO #	Seq.	SEQ. ID NO:	MW (with Sarcosine linker)	MS observed
PMO-221	5' -CCTCACCTGTCACATGCACAGAG-3'	221	7703.47	7709.4

PMO-222	5' -TCACCTGTCACATGCACAGAGAG-3'	222	7767.52	7773.6
PMO-223	5' -CTCACCTGTCACATGCACAGAGA-3'	223	7727.49	7733.6
PMO-224	5' -CCTCACCTGTCACATGCACAG-3'	224	7008.88	7013.9
PMO-225	5' -ACCTGTCACATGCACAGAGAG-3'	225	7121.98	7127.1
PMO-226	5' -TCACCTGTCACATGCACAGAG-3'	226	7072.93	7077.9
PMO-227	5' - TCACCTGTCACATGCACAGAGAGCT-3'	227	8413.07	8418.5
PMO-228	5' - CCTGTGCCTCACCTGTCACATGCAC-3'	228	8315.97	8320.0
PMO-229	5' - GTGCCTCACCTGTCACATGCACAGA-3'	229	8389.04	8393.6
PMO-230	5' - TGCCTCACCTGTCACATGCACAGAG-3'	230	8389.04	8393.7
PMO-231	5' - CTCACCTGTCACATGCACAGAGAGC-3'	231	8398.06	8386.1
PMO-232	5' - CACCTGTCACATGCACAGAGAGCTG-3'	232	8438.08	8441.8
PMO-233	5' - ACCTGTCACATGCACAGAGAGCTGG-3'	233	8478.11	8483.7
PMO-234	5' - CTGTCACATGCACAGAGAGCTGGGG-3'	234	8534.14	8539.6
PMO-235	5' -CCTGTCACATGCACAGAGAGCTG-3'	235	7783.52	7789.6
PMO-236	5' -TGTCACATGCACAGAGAGCTGGG-3'	236	7863.58	7868.6
PMO-237	5' -CTGTCACATGCACAGAGAGCTGG-3'	237	7823.55	7828.0

PMO-238	5' -TGTCACATGCACAGAGAGCTGG-3'	238	7508.28	7513.6
PMO-239	5' -TCACATGCACAGAGAGCTGGG-3'	239	7178	7183.4
PMO-240	5' -TGTCACATGCACAGAGAGCTG-3'	240	7152.99	7157.2
PMO-241	5' - CTGTATTTGGTACTTCCTCTCTCCA-3'	241	8326.97	8332.8
PMO-242	5' - TGTATTTGGTACTTCCTCTCTCCAT-3'	242	8341.98	8347.2
PMO-243	5' - GTATTTGGTACTTCCTCTCTCCATC-3'	243	8326.97	8332.8
PMO-244	5' - TATTTGGTACTTCCTCTCTCCATCC-3'	244	8286.94	8292.3

Example 7: Evaluation of *in vivo* activity of PMO-002, PMO-003, PMO-224, PMO-232, PMO-233, PMO-237, and PMO-238

- [0260] To examine the *in vivo* effect of five PMO sequences in Tables 1 and 5, a study in
- 5 hCD33 mice was performed with an ICV administered 30 µg dose in a manner identical to
- Example 5 with the exception of the injection volume being 2.5 µL. Skipping effect was
- assessed after 7-days. The data represented as Exon-2 *CD33* skipping % is shown in Fig. 9.
- [0261] The skipping effect of PMO-224 was assessed in a separate *in vivo* study with 30 µg,
- 100 µg and 300 µg doses and injection volume of 10 µL. PMO-002 was also assessed with a
- 10 100 µg dose. The data represented as fold-change relative to PBS control is shown in Fig. 10.

Example 8: Synthesis of PMO oligonucleotides with stereopure internucleotide linkages and 5'-sarcosine linkers

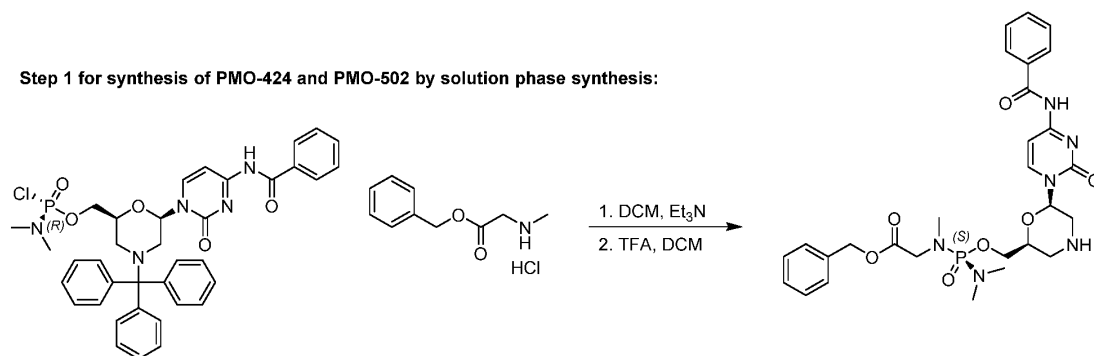
- 15 **Table 6.** Stereopure PMO oligonucleotides

ASO name	Sequence
PMO-324 (all <i>Rp</i> internucleotide linkages)	5' -CCTCACCTGTCACATGCACAG-3'
PMO-424 (all <i>Sp</i> internucleotide linkages)	5'-CCTCACCTGTCACATGCACAG-3'
PMO-402 (all <i>Rp</i> internucleotide linkages)	5'-CCTCACCTGTCACATGCACAGAGAG-3'
PMO-502 (all <i>Sp</i> internucleotide linkages)	5'-CCTCACCTGTCACATGCACAGAGAG-3'

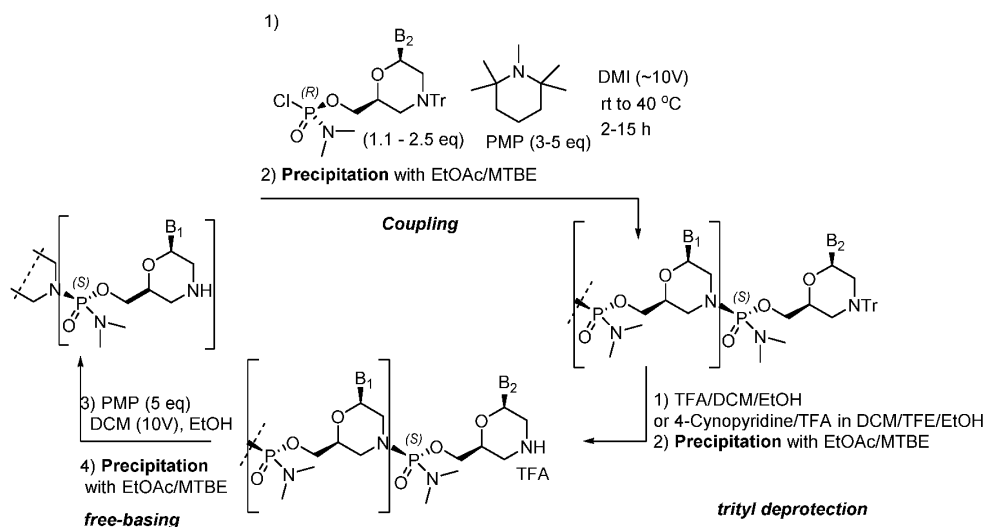
Solution phase synthesis of stereopure PMO oligonucleotides:

- [0262] Solution phase synthesis of 5'-sarcosine capped stereopure oligonucleotides in Table 6 was conducted using similar methods to those methods described in Example 3 (using general Procedures A and B) with the exception of Step 1 which started with coupling sarcosine benzyl ester to a stereopure cytosine dimethylphosphoramidochloridate. Briefly, the synthesis includes iterative steps of deprotection/free basing/coupling as depicted here for all Sp internucleotide linkages):

Step 1 for synthesis of PMO-424 and PMO-502 by solution phase synthesis:



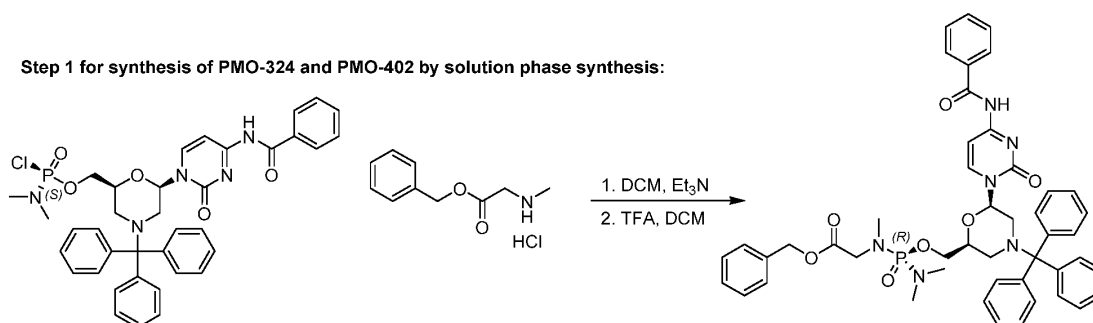
General scheme for elongation process



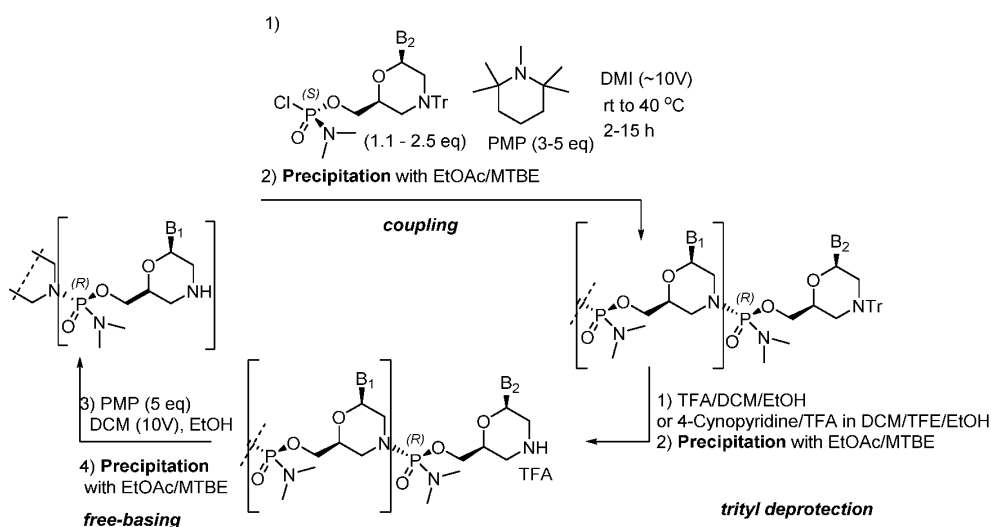
10 **General scheme for synthesis of PMO-424 and PMO-502 by solution phase.**

Briefly, the synthesis includes iterative steps of deprotection/free basing/coupling as depicted here for all Rp internucleotide linkages):

Step 1 for synthesis of PMO-324 and PMO-402 by solution phase synthesis:



General scheme for elongation process



General scheme for synthesis of PMO-324 and PMO-402 by solution phase.

At the conclusion of each individual step, precipitation of the oligonucleotide was achieved by addition of non-polar solvent such as MTBE and/or EtOAc. In elongation steps up to sixmer, purification of the 3'-N-trityl protected oligonucleotide was conducted by silica gel chromatography using DCM/MeOH as eluent.

[0263] Upon reaching the desired oligonucleotide length (21-mer for PMO-324, PMO-424, and 25-mer for PMO-402 and PMO-502), the 3'-N-trityl protected sequence was subjected to base deprotection as follows.

Base deprotection for solution phase synthesis:

[0264] The 3'-N-trityl protected PMO oligonucleotide residue from the final coupling step (1 wt.) was dissolved in MeOH (8 vols) and then 7N NH₃ in MeOH (20 vols) was added. The reaction mixture was heated to 50-55°C for at least 48 hours. The solution was filtered to remove any solids, and rinsed with 1:1 MeOH/7N NH₃ in MeOH. Purification by preparative-scale chromatography using a reverse phase gradient as outlined in Table 7 afforded the 3'-N-trityl protected PMO after solvent evaporation.

Table 7. Analytical and purification conditions for stereopure 3'-N-trityl protected PMO oligonucleotides

Analytical	
Column	XBridge, Premier BEH C18 300 Å 2.5 µm 150 x 2.1 mm (Part number : 186009894)
Flow rate	0.8 mL/min
Column temperature	room temperature
Buffer A	10 mM ammonium bicarbonate in water
Buffer B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 99% B (6 min)
Preparative	
Column	Phenomenex Luna C18 5 µm, 300 x 4.6 mm (Part number : 00H-4252-E0)
Flow rate	1.5 mL/min
Column temperature	40 °C
Buffer A	Water + 0.1% Et ₃ N
Buffer B	MeCN + 0.1% Et ₃ N
Gradient	5 to 99% B (23 min)

- 5 [0265] **Final trityl deprotection:** To the base-deprotected PMO oligonucleotide from HPLC purification was added 0.1N phosphoric acid (at least 20 equivalents) and the reaction was monitored by HPLC. Upon completion of trityl deprotection assessed by two consecutive HPLC runs, the reaction mixture was basified by addition of ammonium hydroxide (at least 40 equivalents). The solution was filtered and the final PMO oligonucleotide was purified by HPLC
- 10 under the conditions in Table 8.

Table 8. Analytical and purification conditions for fully deprotected stereopure PMO oligonucleotides

Analytical	
Column	XBridge, Premier BEH C18 300 Å 2.5 µm 150 x 2.1 mm (Part number : 186009894)
Flow rate	0.8 mL/min
Column temperature	room temperature
Buffer A	10 mM ammonium bicarbonate in water
Buffer B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 99% B (6 min)
Preparative	
Column	Waters, XBridge Prep C18 OBD 5 µm, 19 x 100 mm (Part number : 186002978)
Flow rate	25.5 mL/min
Column temperature	room temperature
Buffer A	10 mM ammonium bicarbonate in water
Buffer B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 28% B (28 min)

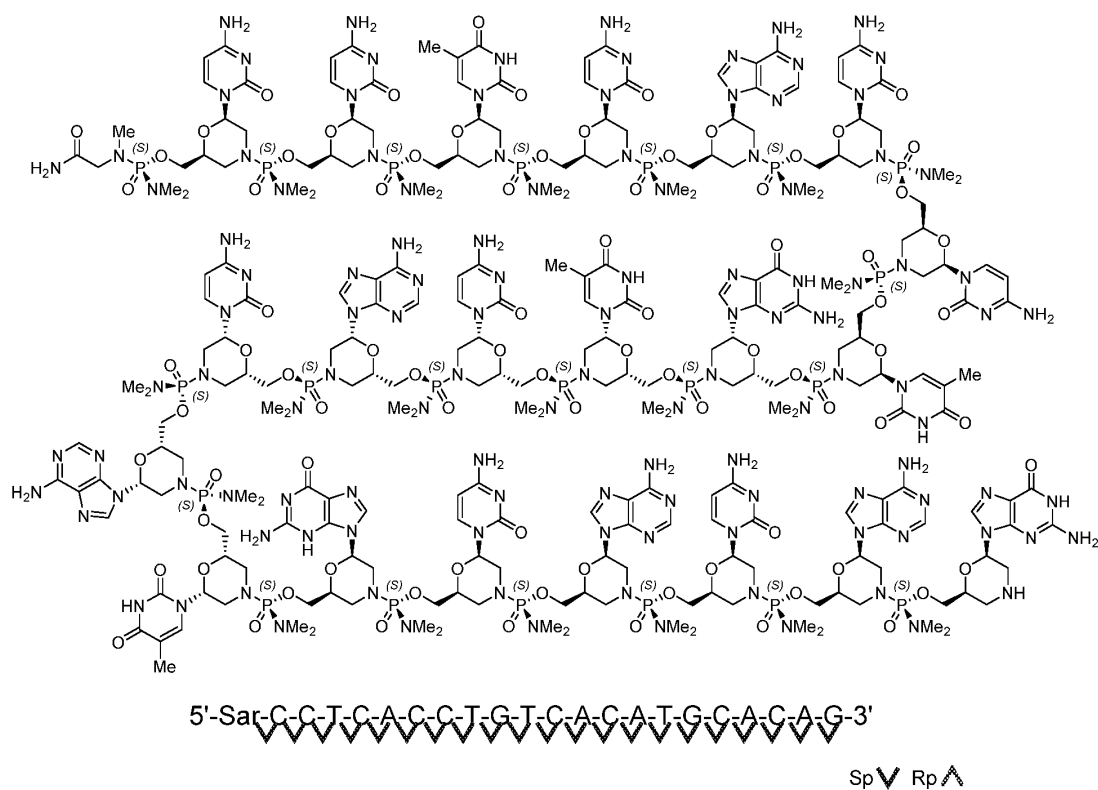
Example 9: Analytical data for stereopure PMO oligonucleotides

The Melting temperature (T_m) of PMO oligonucleotides:

- 5 T_m measurement device: Shimadzu UV-2700 UV-Vis Spectrophotometer
- [0266] ASO samples were prepared by dissolving ~0.6-0.8 mg of solid to ~3.2 µg/mL using nuclease free water. Reverse complementary RNA (obtained from IDT Technologies Inc.) was dissolved to 400 µM in nuclease free water. 10 µL aliquots of each stock solutions were diluted to 1 mL using nuclease free water to determine their concentrations by UV-Vis
- 10 Spectrophotometer. Test Samples (500 µL) were prepared containing 4.0 µM PMO with 4.0 µM reverse complimentary RNA in buffer (100 mM NaCl, 10 mM Na Phosphate pH 7.0 with 0.1 mM EDTA). Test samples were incubated in a 1 mL cuvette and heated from 15 °C to 105 °C at 0.5 °C/min. UV absorbance increase due to strand melting was monitored at 260 nm. Prior to the experiment, the samples were melted and reannealed by heating from 25 °C to 95 °C at 5
- 15 °C/min and cooling to starting temperatures to ensure complete annealing. Shimadzu T_m Analysis software was used to calculate the T_m (curve inflection point: 50% melting) using the derivative function.

Analytical data for stereopure PMO oligonucleotides.

[0267] PMO-424:



P^{31} NMR (D_2O , 162 MHz) δ 21.5, 18.7, 18.6, 18.5, 18.4, 18.3, 18.3, 18.1, 18.0, 17.9.

ESI-TOF-MS Calcd.: 7009.02 for $C_{246}H_{390}N_{119}O_{84}P_{21}$;

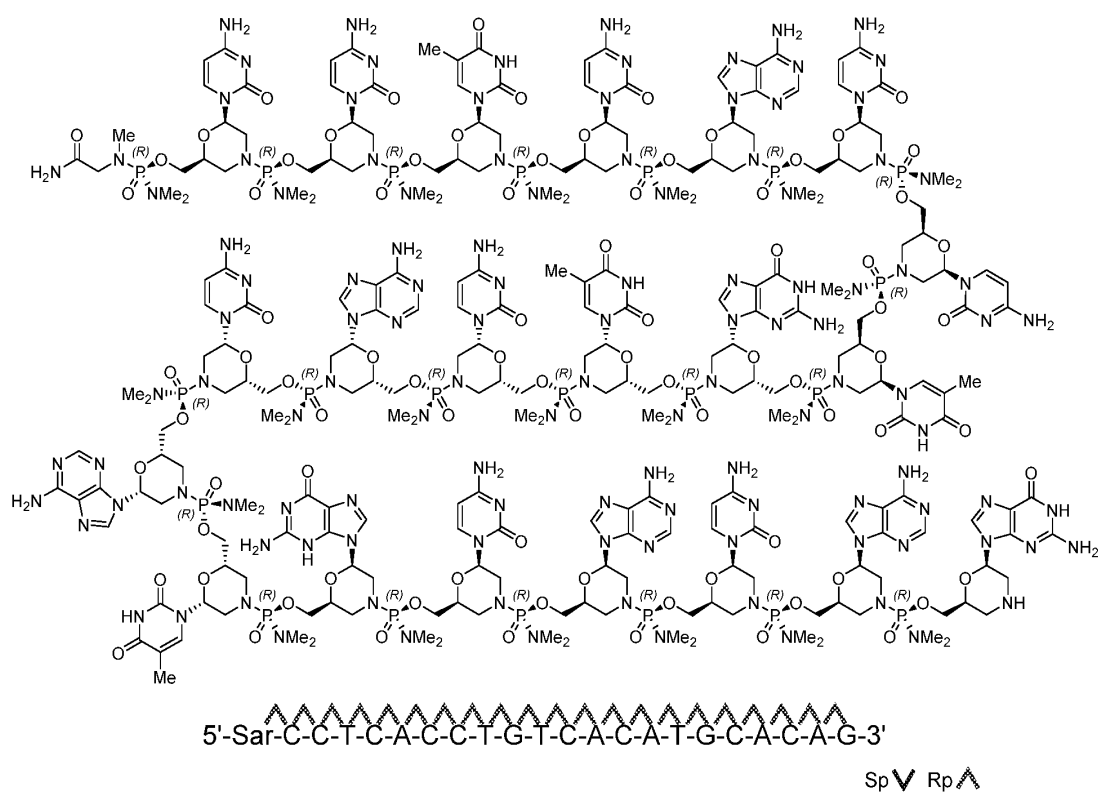
Found: 7008.51.

5

T_m = 80.1 °C (T_m of stereorandom = 75.0 °C). See Fig. 13.

See Fig. 11 for HPLC and HRMS data.

[0268]PMO-324:



ESI-TOF-MS Calcd.: 7009.02 for $C_{246}H_{390}N_{119}O_{84}P_{21}$;

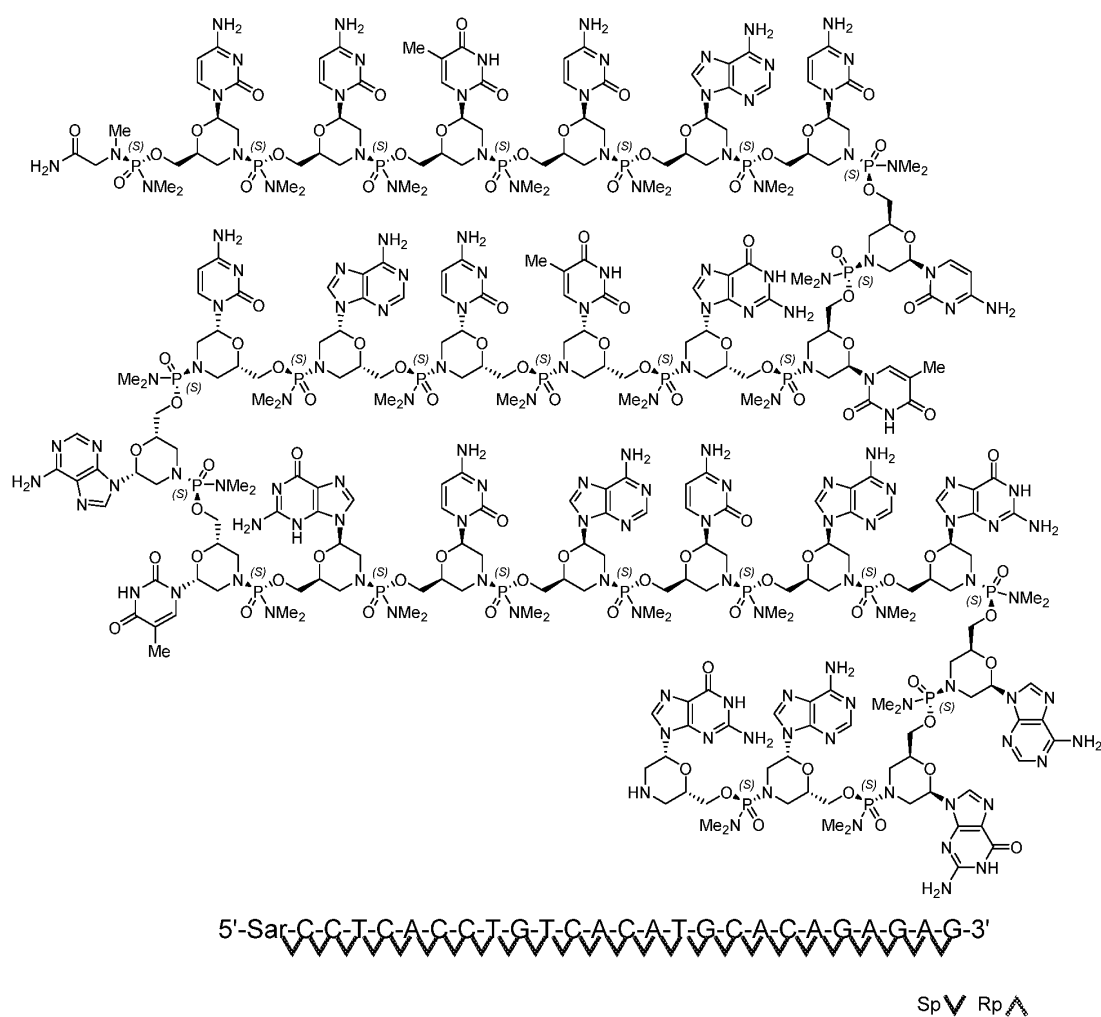
Found: 7008.50.

$T_m = 66.5^\circ\text{C}$ (T_m of stereorandom = 75.0°C). See Fig. 13.

5

See Fig. 12 for HPLC and HRMS data.

[0269] PMO-502:



ESI-TOF-MS Calcd.: 8398.20 for $C_{294}H_{462}N_{147}O_{98}P_{25}$

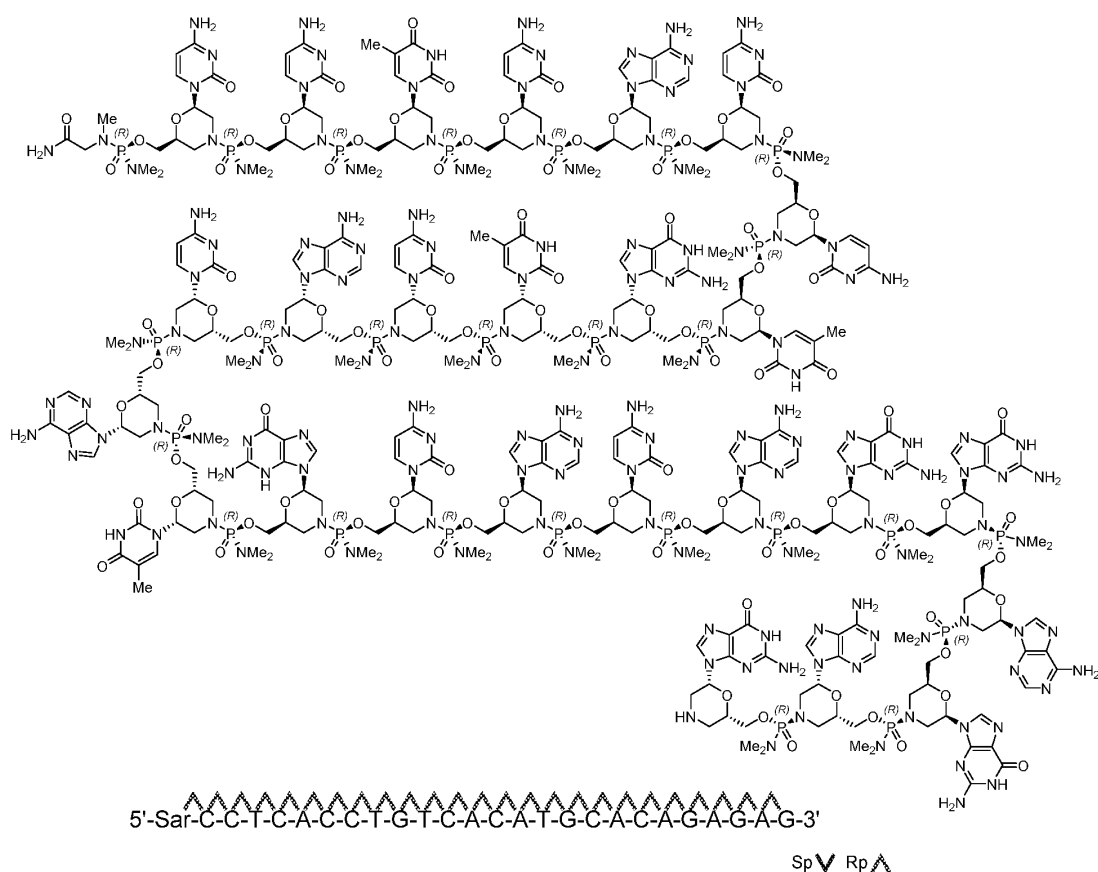
Found: 8397.98.

$T_m = 87.8^\circ\text{C}$ (T_m of stereorandom = 79.3°C). See Fig. 16.

5

See Fig. 14 for HPLC and HRMS data.

[0270]PMO-402:



ESI-TOF-MS Calcd.: 8398.20 for $C_{294}H_{462}N_{147}O_{98}P_{25}$

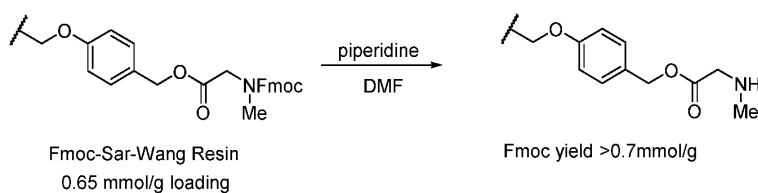
Found: 8397.99.

$T_m = 69.0\text{ }^{\circ}\text{C}$ (T_m of stereorandom = $79.3\text{ }^{\circ}\text{C}$). See Fig. 16.

5 See Fig. 15 for HPLC and HRMS data.

Example 10: Solid phase Synthesis of stereopure PMOs using peptide synthesizer

Deprotection of Fmoc on Sar-Wang resin:



10

[0271] Fmoc-SAR-Wang resin (purchased from Aapptec, RWG103, Lot#9953380, 0.65 mmol/g, 110-200 mesh) (1 g, 650 mmol) was treated with DMF (8 mL), allowed resin to swell for 2h and drained DMF. The resin was treated with 20% piperidine in DMF (6 mL), shaken for 3 minutes, removed solvent, and dried for 1 minute under N_2 gas (repeated the same sequence for 4

15

times). Finally, the resin was washed with DMF (5 mL x 5 times), washed with CH₂Cl₂ (5 mL x 5 times), and dried under vacuum using N₂ gas for overnight to give 0.8 g of resin.

[0272] *Calculation of resin loading:* To the collected piperidine solution was added 20% piperidine in DMF to make final volume of 40 mL. Now, 0.1 mL of solution was diluted 100 times with DMF and measured UV absorbance at 301 nm of the Fmoc group per gram. The loading amount of the resin was >700 μmol/g.

Conditions for UV measurement

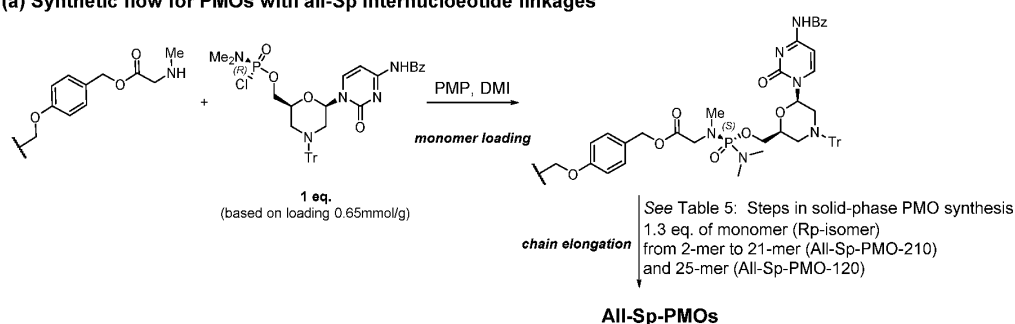
Solvent: 20% piperidine in DMF

Wave length: 301 nm

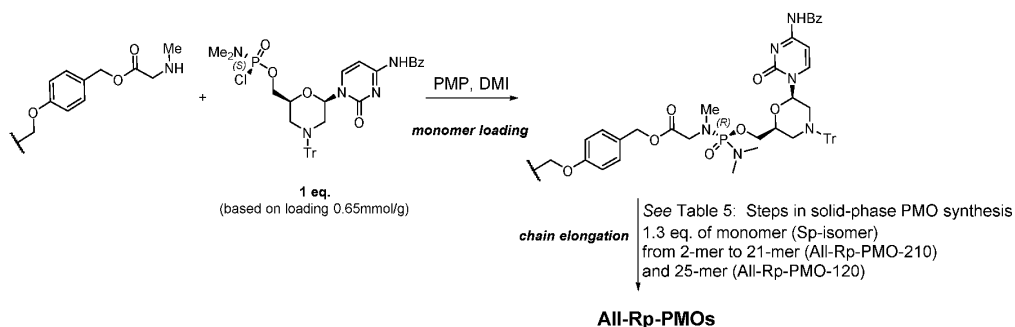
ε=7800

General procedure for solid-phase synthesis of PMOs:

(a) Synthetic flow for PMOs with all-Sp internucleotide linkages



(b) Synthetic flow for PMOs with all-Rp internucleotide linkages



[0273] Fmoc deprotected resin (1.10 g, loading: 0.650 mmol/g) was transferred into the peptide synthesizer reaction vessel, washed with CH₂Cl₂ (20 mL x 5 times), washed with acetonitrile (20 mL x 5 times), and dried. Stereopure cytosine dimethylphosphoramidochloridate (1 eq.) was added to flask as a solid. Then, 1,2,2,6,6-pentamethylpiperidine (PMP, 10.0 eq.) and anhydrous 1,3-dimethyl-2-imidazolidinone (DMI, 5.0 mL) were added to vessel and shaken at room temperature for 20 hours. LCMS of the reaction aliquot showed no monomer in the solution (indicates all monomer was loaded on resin). Then, steps 5 -9 in Table 9 were performed.

Table 9: Steps in solid-phase PMO synthesis

Step	Reaction	Reagent	
1	Detritylation	2% 3-cyanopyridine-TFA, 0.9% EtOH, 20% TFE/DCM (80 mL) then CH ₂ Cl ₂ (15 mL X 2)	7 cycles
2	Neutralization	10% Hunig's base in NMP	30 mL x 4 times
3	Wash	1,3-dimethyl-2-imidazolidinone (30 mL x 2 times) MeCN (25 mL x 10 times), CH ₂ Cl ₂ (25 mL x 15 times)	
4	Coupling	Monomer (1.3 eq), DMI (0.05 M), PMP (10 eq.)	20 h
5	Wash	MeCN (25 mL x 10 times), CH ₂ Cl ₂ (25 mL x 10 times)	
6	Capping	2,6-Lutidine (30% in MeCN, 6 mL) Ac ₂ O (20% in MeCN, 6 mL)	3 times
7	Wash	CH ₂ Cl ₂ (25 mL x 10 times)	
8	Ac ₂ O removal	10% Morpholine in NMP	40 mL x 4 times
9	Wash	CH ₂ Cl ₂ (30 mL x 10 times)	

Preparation of Detritylation Solution:

[0274] To a solution of 4-cyanopyridine (10.1 g; 1.055 eq) in dichloromethane (790 mL) is added trifluoroacetic acid (10.5 g; 1.0 eq), followed by 2,2,2-trifluoroethanol (198 mL) and ethanol (10 mL), and the solution is stirred for 3 hours.

[0275] After the first monomer loading on resin, the synthetic cycle (as shown in Table 9) was started. The synthesis had a series of iterative steps including deprotection/neutralization/coupling/capping. The required monomer (purities of monomers were characterized by HPLC-Mass before use) was added in each cycle to obtain the titled nucleotide sequence.

[0276] In each synthetic cycle, after coupling reaction (step 4, Table 9) a bit of resin was subjected to cleavage conditions (0.1 mL of 7N NH₃/MeOH, at 55°C, 4 h), and recorded RP HPLC-Mass for coupling efficiency (RP HPLC-Mass showed two peaks for methyl ester and amide in ~2:1 ratio. For complete conversion of methyl ester to amide, the cleavage reaction was left overnight stirring at 55°C). The cleavage protocol was iterated from 2-mer to 21-mer for PMO-324 and PMO-424 and to 25-mer for PMO-402 and PMO-502. The RP HPLC-Mass was recorded using conditions in Table 10.

Table 10. Analytical conditions for reaction monitoring in PMO synthesis.

Analytical	
Column	Acquity UPLC BEH C18 1.7 μ m 50 x 2.1 mm (Part number : 186002350)
Flow rate	0.8 mL/min
Column temperature	60 °C
Buffer A	10 mM ammonium bicarbonate in water
Buffer B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 99% B (6 min)

[0277] For example, Fig. 17 shows the UV chromatogram of trityl-protected 21-mer (all-Sp-Sar-
 5 CCTCACCTGTACATGCACAG-Tr) after cleavage from resin.

Cleavage from the resin and base deprotection:

[0278] After completion of desired oligonucleotide length, the synthesized PMO-loaded resin
 was dried, transferred to centrifugal bottle, and charged with 7N NH₃/MeOH (~ 0.5 mL/1 μ mol).
 The mixture was stirred at 50 - 55 °C for 60 hours. The reaction was cooled to room
 10 temperature, filtered the solids, and washed with methanol. The resulting filtrate was
 concentrated under reduced pressure to approximate final volume of ~20 mL, then, filtered any
 solids over 0.4 micron membrane filter. The filtrate was concentrated to dryness and weighed.
 The obtained crude residue was dissolved with 60 mL of solvent mixture of aq. 50 mM
 Et₃NHOAc (used cell culture water)/MeCN (1/1) with Et₃N (0.1%). The filtrate was purified by
 15 reversed phase HPLC conditions as shown in Table 11.

Table 11. Analytical and purification conditions for stereopure 3'-N-trityl protected PMOs.

Analytical	
Column	XBridge, Premier BEH C18 300 Å 2.5 μ m 150 x 2.1 mm (Part number : 186009894)
Flow rate	0.8 mL/min
Column temperature	60 °C
Solution A	10 mM ammonium bicarbonate in water
Solution B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 99% B (6 min)
Preparative	
Column	Waters, XBridge Prep C18 OBD 5 μ m, 19 x 100 mm (Part number : 186002978)
Flow rate	25.5 mL/min
Column temperature	room temperature
Solution A	10 mM ammonium bicarbonate in water
Solution B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 38% B (28 min)

Final detritylation:

[0279] To a flask containing the recovered 3'-N-Tr-PMO (1 eq.) was added freshly prepared 0.1 M aq. phosphoric acid (20 eq.) and the mixture was stirred at room temperature for 2 hours (a white turbid solution was formed within 10 minutes). Reaction completion was checked by two consecutive LCMS runs (shows starting material peak was converted to an earlier eluting peak. HPLC sample was prepared in water only). The reaction was basified by the addition of 28% ammonium hydroxide (40 eq.), stirred for 30 min, filtered the solids through membrane filter (0.45 μ m), and washed with water. The resulting filtrate was purified by reverse phase HPLC (Table 12).

10 **Table 12.** Analytical and purification conditions for fully deprotected stereopure PMOs

Analytical	
Column	XBridge, Premier BEH C18 300 Å 2.5 μ m 150 x 2.1 mm (Part number : 186009894)
Flow rate	0.8 mL/min
Column temperature	60 °C
Solution A	10 mM ammonium bicarbonate in water
Solution B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 99% B (6 min)
Preparative	
Column	Waters, XBridge Prep C18 OBD 5 μ m, 19 x 100 mm (Part number : 186002978)
Flow rate	25.5 mL/min
Column temperature	room temperature
Solution A	10 mM ammonium bicarbonate in water
Solution B	10 mM ammonium bicarbonate/MeOH/MeCN (10/10/80)
Gradient	5 to 28% B (28 min)

15 [0280] Each fraction was analyzed (on HPLC) and the product containing fractions were dried using Genevac. The final product was dissolved in endotoxin-free water, the solution was filtered through Amicon 3K filter to remove any inorganic salt impurities. The aqueous solution obtained was freeze-dried to give the title compound as a white cotton-like solid.

Analytical data for stereopure PMOs prepared by solid-phase synthesis:

[0281] PMO-424:

20 P^{31} NMR (D_2O , 162 MHz) δ 21.5, 18.7, 18.6, 18.5, 18.4, 18.3, 18.3, 18.1, 18.0, 17.9.
LRMS: Calcd. m/z for $[M+5H]^{5+}$ ion of $C_{246}H_{390}N_{119}O_{84}P_{21}$ ($m/z = 7009.02$): 1402.80;
found : 1402.62

[0282] PMO-324:

LRMS: Calcd. m/z for $[M+5H]^{5+}$ ion of $C_{294}H_{462}N_{119}O_{98}P_{25}$ ($m/z = 7009.02$): 1402.80;
found : 1403.4

[0283] PMO-402:

5 LRMS: Calcd. m/z for $[M+6H]^{6+}$ ion of $C_{294}H_{462}N_{119}O_{98}P_{25}$ ($m/z = 8396.92$): 1400.66;
found : 1401.2

Example 11: Evaluation of *in vivo* activity of PMO-402, PMO-502, PMO-324, and PMO-424

[0284] To examine the *in vivo* effect of PMO-402, PMO-502, PMO-324, and PMO-424 prepared
10 in Example 8, a study in hCD33 mice was performed with 100 μ g and 300 μ g doses,
administered by ICV. The study was performed in a manner identical to Example 5 with the
exception of the administration volume of 10 μ L. Skipping effect was assessed after 7-days. The
data represented as fold-change relative to PBS control is shown in Figs. 18 and 19.

Example 12: Additional Exemplary MOE-ASOs

[0285] Phosphorothioate oligonucleotides were designed for screening. All oligonucleotides
listed in Table 13 below contain ribonucleotides with phosphorothioate backbone except when
noted (e.g. solid line (-) = phosphodiester (PO) bond). All oligonucleotides listed in Table 13
below were synthesized with 5-methylcytosine ribonucleotide. All oligonucleotides listed in Table
20 13 below have stereorandom phosphorothioate internucleotide linkages, and thus are called
stereorandom oligonucleotides. All oligonucleotides listed in Table 13 are complementary to
Region 6: (SEQ ID NO:218).

Table 13. ASOs targeting *CD33*

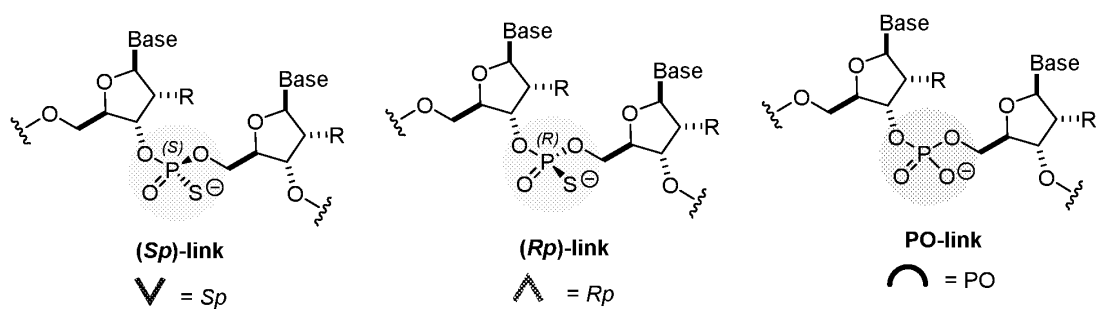
X (A, T, C, G) = 2'-MOE ribonucleotide, C = 5-Methyl cytosine, lower case letter = LNA (locked
25 nucleic acid), (-) = PO bond, fX = 2'-fluoro ribonucleotide, mX = 2'-OMe ribonucleotide

ASO#	Sequence (5' to 3')	SEQ ID No:	MW (free form calculated)	MS observed
MOE-245	CTCCATCCGAAAGAAGTATG	245	7966.9	7967.1
MOE-246	TCCATCCGAAAGAAGTATGA	246	7976.9	7976.9
MOE-247	CCATCCGAAAGAAGTATGAA	247	7985.9	7986.9
MOE-248	CATCCGAAAGAAGTATGAAC	248	7985.9	7986.4
MOE-249	TCCGAAAGAAGTATGAACCA	249	7985.9	7986.6
MOE-250	CCGAAAGAAGTATGAACCAT	250	7985.9	7986.2
MOE-251	ATCCGAAAGAAGTATGAA	251	7199.2	7199.9
MOE-252	CCGAAAGAAGTATGAACC	252	7188.1	7188.3

MOE-253	TCCGAAAGAAGTATGAAC	253	7189.2	7188.2
MOE-254	CCATCCGAAAGAAGTATG	254	7179.2	7179.1
MOE-255	TCCATCCGAAAGAAGTAT	255	7154.2	7154.2
MOE-256	GAAAGAAGTATGAACCAT	256	7199.2	7197.9
MOE-257	ATC-CGAAAGAAGTATGA-ACC	12	7953.8	7953.9
MOE-258	ATCC-GAAAGAAGTATG-AACC	12	7953.8	7953.3
MOE-259	ATCCG-AAAGAAGTAT-GAACC	12	7953.8	7953.4
MOE-260	ATCCG-AAAGAAGTA-TGAACC	12	7953.8	7953.4
MOE-261	ATCC-GAAAGA-AGTATG-AACC	12	7937.7	7937.3
MOE-262	ATCC-gAAAGAAGTATGAACC	12	7923.8	7924.3
MOE-263	ATCC-gAAAGAAGTATG-aACC	12	7861.7	7861.9
MOE-264	ATCC-gAAAGAAgTATG-aACC	12	7815.6	7815.8
MOE-265	CCGA-aAGAAGTATGAACC	252	7126.1	7126.5
MOE-266	CCGA-aAGAAGTATG-aACC	252	7064.0	7064.1
MOE-267	CCGA-aAGAAGtATG-aACC	252	7017.9	7018.0
MOE-268	CCG-AAAGAAGTATGA-ACC	252	7156.1	7156.7
MOE-269	CCGA-AAGAAGTATG-AACC	252	7156.1	7156.6
MOE-270	CCGAA-AGAA-GTATG-AACC	252	7140.0	7140.7
MOE-271	CCGAA-AGAAGTAT-GAACC	252	7156.1	7156.7
MOE-272	CCG-A-AAGAAGTATGAACC	252	7156.1	7156.7
MOE-273	CCG-AA-AGAAGTATGAACC	252	7156.1	7156.8
MOE-274	CCGAAAGAAGTATG-A-ACC	252	7156.1	7156.8
MOE-275	mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmC mC	12	6814.1	6814.7
MOE-276	fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTf GfAfAfCfC	12	6862.3	6862.9

[0286] All oligonucleotides listed in Table 14 below contain a 2'-O-MOE modified ribonucleotides and a hydroxyl group at the 5' end. Oligonucleotides in Table 14 contain stereopure phosphorothioate internucleotide linkages, and thus are called stereopure MOE oligonucleotides. All oligonucleotides listed in Table 14 are complementary to Region 6: (SEQ ID NO:218).

Table 14. Stereopure ASOs targeting *CD33*



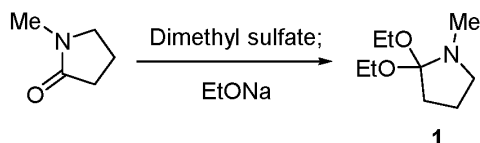
ASO#	Sequence (5' to 3')	SEQ ID No:
MOE-277	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-278	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-279	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-280	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-281	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-282	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-283	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-284	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-285	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-286	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-287	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-288	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-289	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-290	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-291	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252

MOE-292	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-293	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-294	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-295	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-296	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-297	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-298	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-299	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-300	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-301	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-303	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-304	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-305	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-306	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-307	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
MOE-308	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-309	5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	12
MOE-310	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252

MOE-311	5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'	252
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Example 13: Preparation of stereopure 2'-MOE phosphorothiolate oligonucleotides

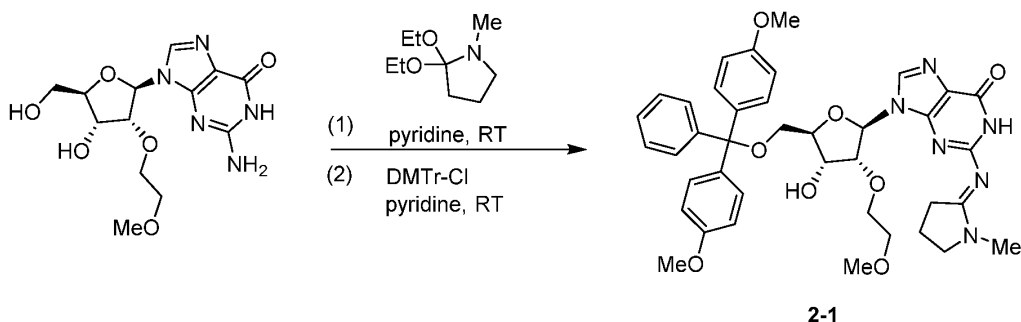
Protected 2'-O-MOE-3'-OH Monomers



- 5 [0287] **(1) 2,2-diethoxy-1-methylpyrrolidine:** A mixture of NMP (100 mL, 1039.008 mmol) and dimethyl sulfate (99 mL, 1039.008 mmol) was stirred and heated to 80°C (sand bath) overnight, then allowed to cool to rt. After cooling, the homogeneous liquid was washed with ether (2 X 100 mL) and the residual solvent was removed *in vacuo*. The obtained residue was dissolved in CH₂Cl₂ (400 mL), dried over anhydrous MgSO₄, filtered, washed with CH₂Cl₂ (100 mL) and
- 10 concentrated under reduced pressure to give 5-methoxy-1-methyl-3,4-dihydro-2H-pyrrol-1-ium as a brown color viscous liquid (solidified at -20 °C storage); ¹H NMR (400 MHz, CDCl₃) δ 4.35 - 4.40 (m, 3 H), 3.98 - 4.05 (m, 2 H), 3.69 - 3.73 (m, 3 H), 3.31 - 3.38 (m, 2 H), 3.19 - 3.22 (m, 3 H), 2.37 - 2.48 (m, 2 H).

- [0288] The crude product (obtained above) was added to a solution of sodium ethanolate (370 g, 1142.909 mmol, 21% sodium ethoxide in ethanol) at 50 to 55 °C over 1 hour by cannula or dropping funnel under N₂ atmosphere. After stirring at the same temperature for 3 hours, the reaction was cooled to room temperature. The precipitated white solid was filtered, washed with ethanol (50 mL) and the filtrate was concentrated (maintain water-bath temperature ~30 °C). Fractional distillation of crude residue under house vacuum at 55 - 65 °C gave 2,2-diethoxy-1-
- 15 g, 1142.909 mmol, 21% sodium ethoxide in ethanol) at 50 to 55 °C over 1 hour by cannula or dropping funnel under N₂ atmosphere. After stirring at the same temperature for 3 hours, the reaction was cooled to room temperature. The precipitated white solid was filtered, washed with ethanol (50 mL) and the filtrate was concentrated (maintain water-bath temperature ~30 °C). Fractional distillation of crude residue under house vacuum at 55 - 65 °C gave 2,2-diethoxy-1-
- 20 methylpyrrolidine (115 g, 66 % yield) as a pale yellow or colorless liquid. The pure product was stored at -20 °C; ¹H NMR (400 MHz, CDCl₃) δ 3.44 - 3.60 (m, 4 H), 2.83 - 2.91 (m, 3 H), 2.33 - 2.40 (m, 4 H), 1.90 - 1.98 (m, 2 H), 1.72 - 1.87 (m, 2 H), 1.15 - 1.22 (m, 6 H).

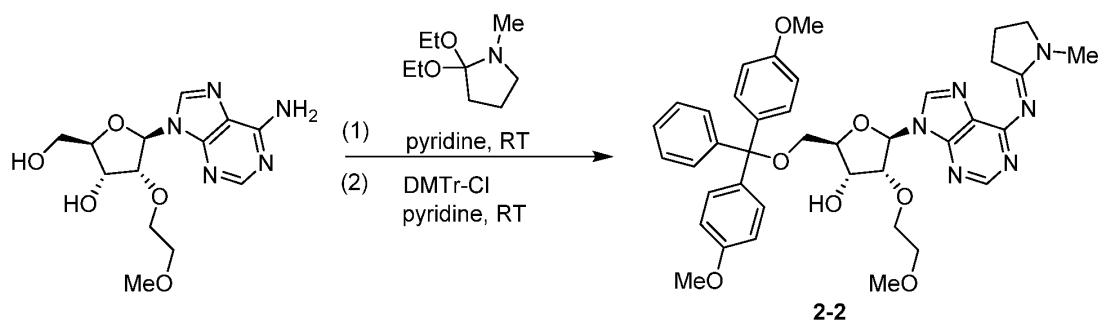
General Procedure 1: Pya (N-methylpyrrolidine) protection of 2'-O-MOE G, A, and mC



[0289] **(2-1)** 9-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-2-(1-methylpyrrolidin-2-ylidene)amino)-1,9-dihydro-6H-purin-6-one:

[0290] 2-amino-9-((2*R*,3*R*,4*R*,5*R*)-4-hydroxy-5-(hydroxymethyl)-3-(2-

- 5 methoxyethoxy)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (13.8 g, 40.431 mmol) was chased under vacuum with anhydrous pyridine (100 mL) for two times. To the concentrated residue was added anhydrous pyridine (114 mL, 1418.073 mmol) followed by 2,2-diethoxy-1-methylpyrrolidine (14.01 g, 80.862 mmol) slowly at room temperature. The reaction was stirred at room temperature overnight, changing from a white turbid solution to a brown clear solution.
- 10 Water (0.1 mL/6 mmol) was added, and the mixture was concentrated under vacuum, then chased with pyridine and MeCN 3 times. To the resulting residue were added pyridine (105 mL, 1298.197 mmol) and 1-[chloro-(4-methoxyphenyl)-phenylmethyl]-4-methoxybenzene (15.62 g, 46.108 mmol) at room temperature. After stirring at rt overnight, the reaction mixture was worked up with saturated NaHCO₃ (150 mL) and EtOAc (300 mL X 2), and the residue was
- 15 purified by a silica-gel column chromatography (100 g Star Silica, EtOAc/Hept 30 to 100% then EtOAc/MeOH 0 to 30%) to give **2-1** as a foamy solid in 77% yield; ¹H NMR (400 MHz, CDCl₃) δ 9.28 - 9.36 (m, 1 H), 7.67 - 7.72 (m, 1 H), 7.32 - 7.39 (m, 2 H), 7.16 - 7.28 (m, 6 H), 7.08 - 7.16 (m, 1 H), 6.69 - 6.78 (m, 4 H), 5.92 - 5.96 (m, 1 H), 4.29 - 4.37 (m, 2 H), 4.11 - 4.17 (m, 1 H), 3.74 - 3.82 (m, 1 H), 3.68 - 3.73 (m, 7 H), 3.55 - 3.63 (m, 1 H), 3.45 - 3.52 (m, 1 H), 3.34 - 3.41
- 20 (m, 3 H), 3.25 - 3.33 (m, 5 H), 3.01 - 3.09 (m, 2 H), 2.92 - 2.96 (m, 3 H), 1.90 - 2.00 (m, 2 H); MS (ESI, *m/z*) calculated for [C₃₉H₄₄N₅O₈ + H⁺] 725.33 found 725.4.



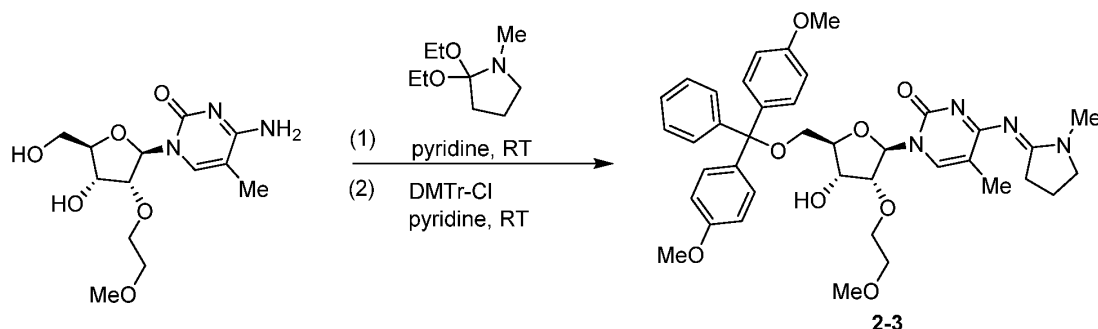
[0291] **(2-2)** (2*R*,3*R*,4*R*,5*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-

- 25 methoxyethoxy)-5-(6-1-methylpyrrolidin-2-ylidene)amino)-9H-purin-9-yl)tetrahydrofuran-3-ol:

[0292] Prepared according to general procedure 1, foamy solid, 89% yield; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.36 (d, *J* = 8.0 Hz, 2H), 7.40 - 7.31 (m, 2H), 7.29 - 7.16 (m, 7H), 6.87 - 6.77 (m, 4H), 6.07 (d, *J* = 4.8 Hz, 1H), 5.18 (d, *J* = 6.0 Hz, 1H), 4.69 (t, *J* = 5.2 Hz, 1H), 4.44 (q, *J* = 5.2

30 Hz, 1H), 4.11 - 4.05 (m, 1H), 3.76-3.71 (m, 7H), 3.62 (dt, *J* = 11.2, 4.8 Hz, 1H), 3.49 (t, *J* = 7.2 Hz, 2H), 3.42 (t, *J* = 4.8 Hz, 2H), 3.23 (d, *J* = 4.8 Hz, 2H), 3.14 (s, 3H), 3.04 (s, 3H), 2.85 (t, *J* =

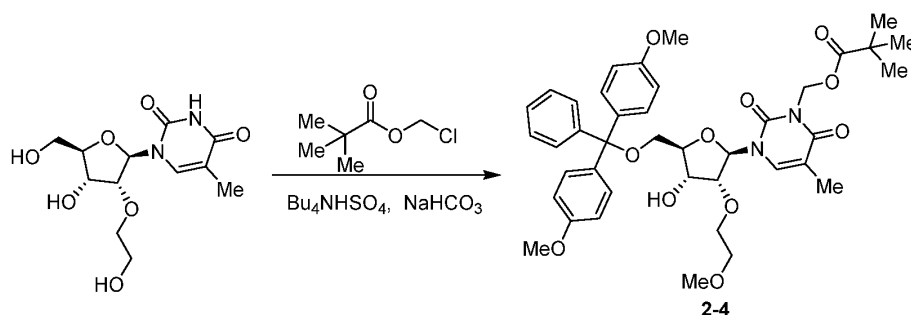
8.0 Hz, 2H), 2.02-1.93 (m, 2H); MS (ESI, m/z) calculated for $[C_{39}H_{44}N_6O_7 + H^+]$ 709.33 found 709.20.



- 5 [0293] **(2-3)** 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-4-(1-methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one:

- [0294] Prepared according to general procedure 1, 87% yield; foamy solid; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 - 7.81 (m, 1 H), 7.46 - 7.51 (m, 2 H), 7.34 - 7.41 (m, 4 H), 7.27 - 7.33 (m, 2 H), 7.20 - 7.27 (m, 1 H), 6.82 - 6.88 (m, 4 H), 5.99 - 6.04 (m, 1 H), 4.34 - 4.43 (m, 1 H), 4.25 - 4.33 (m, 1 H), 4.08 - 4.15 (m, 1 H), 3.99 - 4.05 (m, 1 H), 3.90 - 3.99 (m, 1 H), 3.74 - 3.83 (m, 6 H), 3.54 - 3.64 (m, 3 H), 3.42 - 3.50 (m, 3 H), 3.42 (s, 3 H), 3.29 - 3.34 (m, 1 H), 3.07 - 3.29 (m, 2 H), 3.03 - 3.07 (m, 3 H), 2.00 - 2.11 (m, 2 H), 1.53 - 1.58 (m, 3 H); MS (ESI, m/z) calculated for $[C_{39}H_{44}N_6O_8 + H^+]$ 699.33 found 699.25.

Pivaloylmethyl (POM) protection of T:



- [0295] **(2-4)** (3-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl pivalate:

[0296] Step 1: To 1-((2R,3R,4R,5R)-4-hydroxy-5-(hydroxymethyl)-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (14.2 g, 44.893

mmol) in pyridine (99 mL, 1228.96 mmol) was added 1-[chloro-(4-methoxyphenyl)-phenylmethyl]-4-methoxybenzene (18.25 g, 53.871 mmol) at room temperature. Upon completion, as monitored by UPLC-MS, saturated NaHCO₃ (80 mL) was added to the mixture, extracted with EtOAc (200 mL X 2), and purified by a silica-gel column chromatography (100 g, Star silica, EtOAc/Hept 10 to 100%) to give 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (25 g, 40.408 mmol) in 90% yield.

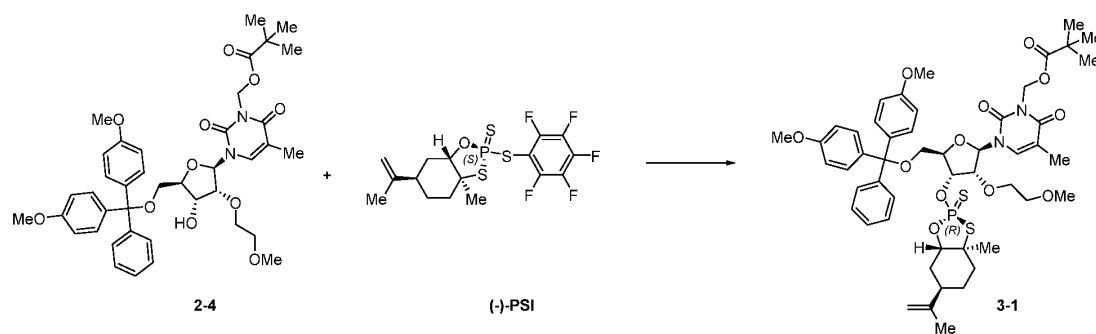
[0297] ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.37 (s, 1H), 7.49 (s, 1H), 7.39 (d, *J* = 7.6 Hz, 2H), 7.35 – 7.21 (m, 8H), 6.90 (d, *J* = 8.8 Hz, 4H), 5.85 (d, *J* = 4.8 Hz, 1H), 5.12 (d, *J* = 6.0 Hz, 1H), 4.23 (q, *J* = 5.2 Hz, 1H), 4.09 (t, *J* = 4.8 Hz, 1H), 4.02-3.95 (m, 1H), 3.79 – 3.67 (m, 8H), 3.48 (t, *J* = 4.7 Hz, 2H), 3.26-3.20 (m, 5H), 1.40 (s, 3H).

[0298] Step 2: To an aqueous solution of Na₂CO₃ (242 mL, 121.225 mmol) were added 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (25 g, 40.408 mmol) in DCM (250 mL, 3885.69 mmol), Tetrabutylammoniumhydrogensulfate (5.49 g, 16.163 mmol), and chloromethyl pivalate (7.30 g, 48.49 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. Some starting material remained unreacted by UPLC-Mass analysis, thus, added 700 mg of chloromethyl pivalate at room temperature. After stirring at rt for another 2 days, the mixture was worked up with saturated NaHCO₃ (50 mL) and extracted with EtOAc (100 mL X 3), and purified by a column chromatography (100 g snap, EtOAc/Hept 10 to 60%) to give (3-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl pivalate (23 g, 31.4 mmol, 78 % yield) along with recovered starting material (3.25 g).

[0299] ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.62 (s, 1H), 7.40 (d, *J* = 7.6 Hz, 2H), 7.36 – 7.20 (m, 7H), 6.90 (d, *J* = 8.8 Hz, 4H), 5.89 (d, *J* = 4.8 Hz, 1H), 5.84 – 5.73 (m, 2H), 5.17 (d, *J* = 6.0 Hz, 1H), 4.26 (q, *J* = 5.6 Hz, 1H), 4.12 (t, *J* = 4.8 Hz, 1H), 4.02-3.98 (m, 1H), 3.78-3.70 (m, 8H), 3.51-3.40 (m, 2H), 3.28-3.20 (m, 5H), 1.44 (s, 3H), 1.10 (s, 9H); MS (ESI, *m/z*) calculated for [C₄₀H₄₈N₂O₁₁ + Na⁺] 755.32 found 755.1.

2'-O-MOE-3'-PSI Activated Monomers

General Procedure 2¹: PSI activation



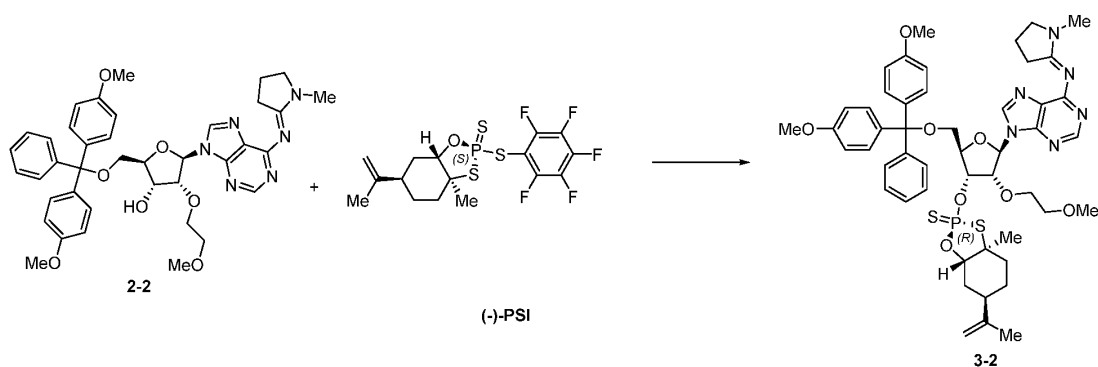
[0300] **(3-1)** (3-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2R,3aS,6R,7aS)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl pivalate:

5

[0301] (2S,3aS,6R,7aS)-3a-methyl-2-((perfluorophenyl)thio)-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-sulfide (3.70 g, 8.29 mmol) ((-)-PSI reagent) and (3-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl pivalate (4.50 g, 6.141 mmol) were dissolved in THF (20.47 mL, 6.141 mmol) and acetonitrile (20.47 mL, 6.141 mmol), and the solution was cooled in ice bath. To the mixture was added DBU (1.203 mL, 7.983 mmol) and it was stirred at 0 °C until the reaction was completed (0.5~2 h) as monitored by UPLC-MS. The reaction mixture was diluted by EtOAc was washed with saturated NaH₂PO₄ (aq.) solution, then saturated NaHCO₃ (aq.), dried over Na₂SO₄, and purified by a silica gel chromatography (50 g Star, Hept: EtOAc gradient to 70% to give **3-1** as a white solid (5.3 g, 88% yield).

[0302] ¹H NMR (400 MHz, CD₃CN) δ ppm 7.46 - 7.54 (3 H, m), 7.33 - 7.39 (6 H, m), 7.26 - 7.32 (1 H, m), 6.91 (4 H, d, *J*=8.75 Hz), 5.97 (1 H, d, *J*=6.38 Hz), 5.86 - 5.93 (2 H, m), 5.45 - 5.52 (1 H, m), 5.02 (1 H, s), 4.93 (1 H, s), 4.45 - 4.54 (2 H, m), 4.26 (1 H, d, *J*=2.88 Hz), 3.77 - 3.84 (8 H, m), 3.47 - 3.62 (2 H, m), 3.42 (1 H, dd, *J*=11.01, 2.88 Hz), 3.29 - 3.33 (1 H, m), 3.28 (3 H, s), 2.64 (1 H, br s), 2.25 - 2.32 (1 H, m), 2.12 - 2.14 (3 H, m), 2.07 (1 H, br dd, *J*=13.70, 4.44 Hz), 1.99 - 1.99 (1 H, m), 1.81 - 1.95 (2 H, m), 1.80 (3 H, s), 1.69 (3 H, s), 1.44 (3 H, s), 1.18 (9 H, s); ³¹P NMR (162 MHz, CD₃CN) δ ppm 101.69; MS (ESI, *m/z*) calculated for [C₅₀H₆₃N₂O₁₂PS₂ + Na⁺] 1001.35 found 1001.4.

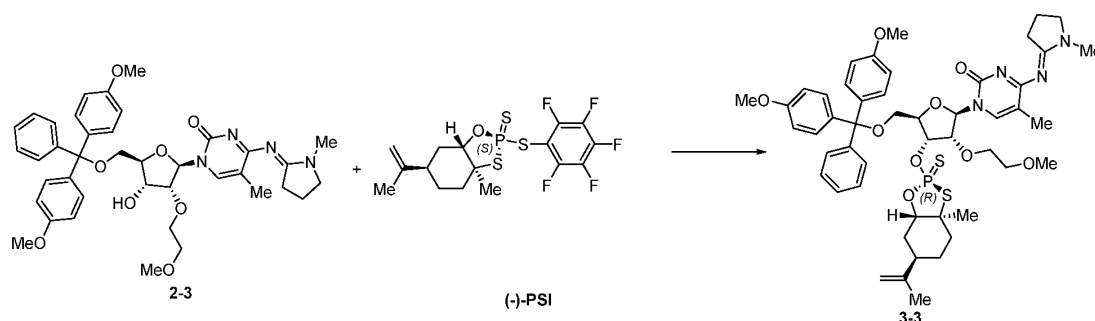
25



[0303] **(3-2)** (2R,3aS,6R,7aS)-2-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(6-(((*E*)-1-methylpyrrolidin-2-ylidene)amino)-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)-3a-methyl-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-sulfide:

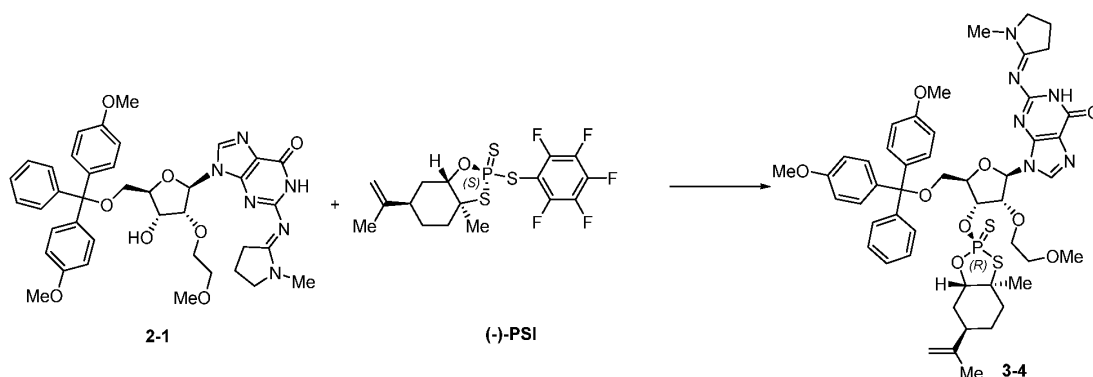
30

[0304] Prepared according to general procedure 2 with (-)-PSI reagent, 78% yield; foamy solid;
¹H NMR (400 MHz, CDCl₃) δ 8.40 - 8.46 (m, 1 H), 8.00 - 8.03 (m, 1 H), 7.36 - 7.41 (m, 2 H),
 7.24 - 7.30 (m, 4 H), 7.17 - 7.22 (m, 2 H), 7.08 - 7.16 (m, 1 H), 6.69 - 6.78 (m, 4 H), 6.05 (d,
 $J=7.5$ Hz, 1 H), 5.45 - 5.59 (m, 1 H), 5.04 (dd, $J=7.5$, 4.7 Hz, 1 H), 4.95 (s, 1 H), 4.78 - 4.93 (m,
 5 1 H), 4.50 (dt, $J=12.6$, 3.3 Hz, 1 H), 4.27 - 4.33 (m, 1 H), 3.59 - 3.79 (m, 10 H), 3.31 - 3.46 (m, 5
 H), 3.10 - 3.15 (m, 3 H), 3.06 - 3.10 (m, 3 H), 2.83 - 2.97 (m, 2 H), 2.47 - 2.54 (m, 1 H), 2.16 -
 2.24 (m, 1 H), 2.03 - 2.13 (m, 1 H), 1.94 - 2.03 (m, 2 H), 1.74 - 1.94 (m, 4 H), 1.66 - 1.69 (m, 3
 H), 1.60 - 1.65 (m, 3 H); ¹³C NMR (101 MHz, CDCl₃) δ 166.9, 160.9, 158.6, 158.5, 152.8,
 151.6, 144.8, 144.5, 140.1, 135.6, 135.6, 130.2, 130.1, 128.2, 128.0, 126.9, 126.6, 113.3,
 10 112.2, 86.8, 85.4, 85.4, 83.6, 83.5, 80.1, 80.0, 77.3, 76.9, 72.3, 70.6, 68.0, 65.7, 63.0, 58.9,
 55.2, 51.6, 38.9, 33.7, 33.7, 32.0, 30.1, 27.8, 27.6, 25.6, 23.5, 22.7, 21.8, 19.7; ³¹P NMR (162
 MHz, CDCl₃) δ 101.34; MS (ESI, m/z) calculated for [C₄₉H₅₉N₆O₈PS₂ + H⁺] 955.36 found 956.3.



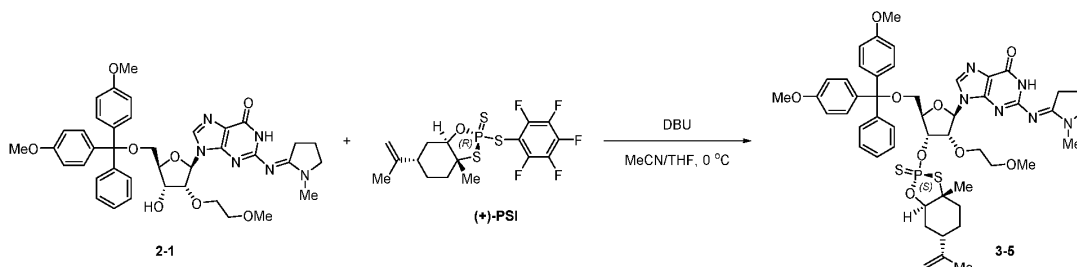
[0305] **(3-3)** 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-
 15 methoxyethoxy)-4-(((2R,3aS,6R,7aS)-3a-methyl-6-(prop-1-en-2-yl)-2-
 sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-4-((1-
 methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one:

[0306] Prepared according to general procedure 2 with (-)-PSI reagent, foamy solid, 70% yield;
 20 ¹H NMR (400 MHz, CD₃CN) δ ppm 7.59 (1 H, s), 7.50 (2 H, d, $J=7.38$ Hz), 7.32 - 7.41 (6 H, m),
 7.25 - 7.31 (1 H, m), 6.90 (4 H, d, $J=8.63$ Hz), 5.99 (1 H, d, $J=5.00$ Hz), 5.44 (1 H, dt, $J=12.98$,
 5.02 Hz), 5.02 (1 H, s), 4.93 (1 H, s), 4.47 (1 H, dt, $J=12.73$, 3.20 Hz), 4.34 (1 H, t, $J=5.07$ Hz),
 4.22 - 4.28 (1 H, m), 3.86 - 3.94 (1 H, m), 3.79 (6 H, s), 3.44 - 3.61 (4 H, m), 3.33 - 3.41 (3 H,
 m), 3.30 (3 H, s), 3.05 - 3.09 (1 H, m), 3.04 (3 H, s), 2.76 (1 H, s), 2.64 (1 H, br s), 2.20 - 2.30 (2
 25 H, m), 2.01 - 2.09 (3 H, m), 1.99 - 1.99 (2 H, m), 1.81 - 1.92 (1 H, m), 1.80 (3 H, s), 1.68 (3 H, s),
 1.55 (3 H, s); ³¹P NMR (162 MHz, CD₃CN) δ ppm 101.51; MS (ESI, m/z) calculated for
 [C₄₉H₆₁N₄O₉PS₂ + H⁺] 945.36 found 945.4.



[0307] **(3-4)** 9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2R,3aS,6R,7aS)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-2-((1-methylpyrrolidin-2-ylidene)amino)-1,9-dihydro-6H-purin-6-one:

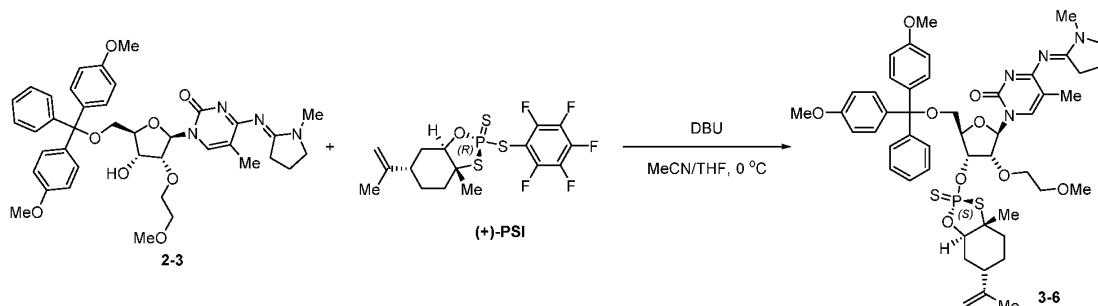
[0308] Prepared according to general procedure 2 with (-)-PSI reagent, foamy solid, 80% yield; ^1H NMR (400 MHz, CD_3CN) δ ppm 9.18 (1 H, br s), 7.74 (1 H, s), 7.43 (2 H, d, $J=7.38$ Hz), 7.22 - 7.33 (7 H, m), 6.85 (4 H, dd, $J=9.01$, 2.63 Hz), 5.89 (1 H, d, $J=5.50$ Hz), 5.48 (1 H, dt, $J=13.54$, 4.80 Hz), 4.98 (1 H, s), 4.92 (1 H, s), 4.85 (1 H, t, $J=5.38$ Hz), 4.50 (1 H, dt, $J=12.69$, 3.22 Hz), 4.23 (1 H, q, $J=4.09$ Hz), 3.79 (6 H, s), 3.67 - 3.77 (2 H, m), 3.43 - 3.51 (4 H, m), 3.32 (2 H, qd, $J=10.94$, 4.06 Hz), 3.21 (3 H, s), 3.03 (3 H, s), 2.99 - 3.02 (1 H, m), 2.63 (1 H, br s), 2.27 (1 H, br d, $J=13.13$ Hz), 2.12 - 2.15 (1 H, m), 2.01 - 2.06 (1 H, m), 1.99 - 1.99 (4 H, m), 1.79 - 1.93 (2 H, m), 1.77 (3 H, s), 1.68 (3 H, s); ^{31}P NMR (162 MHz, CD_3CN) δ ppm 101.36; MS (ESI, m/z) calculated for $[\text{C}_{49}\text{H}_{59}\text{N}_6\text{O}_9\text{PS}_2 + \text{H}^+]$ 971.35 found 971.4.



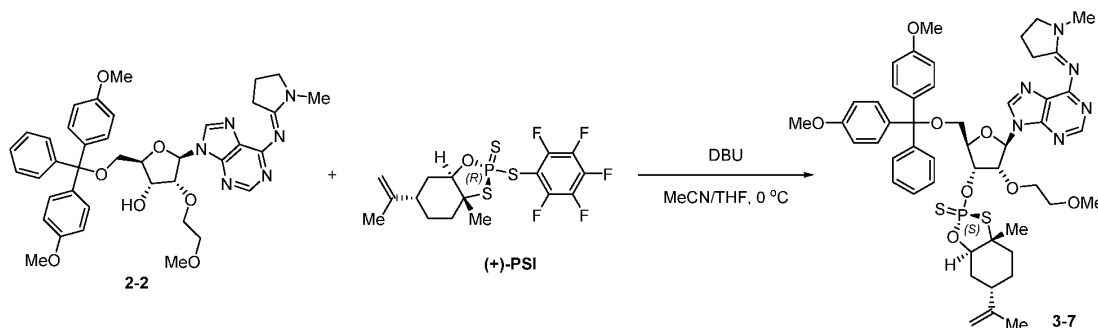
[0309] **(3-5)** 9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2S,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-2-((1-methylpyrrolidin-2-ylidene)amino)-1,9-dihydro-6H-purin-6-one:

[0310] Prepared according to general procedure 2 with (+)-PSI reagent, white foamy solid; ^1H NMR (400 MHz, CD_3CN , 296 K) δ (ppm) = 9.56 (br s, 1H), 7.74 (s, 1H), 7.44 (d, $J = 7.5$ Hz, 2H), 7.34 - 7.28 (m, 6H), 7.28 - 7.21 (m, 1H), 6.86 (dd, $J = 2.4$, 8.9 Hz, 4H), 5.89 (d, $J = 6.4$ Hz, 1H), 5.44 - 5.36 (m, 1H), 4.99 (s, 1H), 4.89 (s, 1H), 4.78 (t, $J = 5.8$ Hz, 1H), 4.45 (td, $J = 3.0$, 12.7 Hz, 1H), 4.27 (q, $J = 3.9$ Hz, 1H), 3.78 (s, 6H), 3.75 - 3.70 (m, 1H), 3.67 - 3.57 (m, 1H), 3.47 - 3.40 (m, 2H), 3.39 - 3.32 (m, 4H), 3.12 (s, 3H), 3.09 - 2.92 (m, 5H), 2.63 (br s, 1H), 2.30 - 2.15 (m,

- 2H), 2.04 (br dd, $J = 4.0, 12.9$ Hz, 1H), 2.00 - 1.90 (m, 4H), 1.82 (br s, 1H), 1.79 - 1.75 (m, 3H), 1.68 (s, 3H); ^{13}C NMR (101 MHz, CD_3CN , 298 K) δ (ppm) = 170.8, 160.1, 159.3, 158.3, 152.1, 147.2, 146.2, 137.9, 136.9, 136.9, 131.5, 131.4, 129.4, 129.3, 128.3, 114.5, 112.4, 87.9, 87.6, 87.3, 83.5, 83.4, 81.8, 78.2, 78.1, 73.1, 72.3, 66.4, 64.4, 59.4, 56.3, 52.4, 40.2, 34.9, 34.8, 32.5, 32.4, 28.7, 28.6, 24.2, 23.2, 22.3, 20.8; ^{31}P NMR (162 MHz, CD_3CN) δ 101.9; MS (ESI, m/z) calculated for $[\text{C}_{49}\text{H}_{59}\text{N}_6\text{O}_9\text{PS}_2 + \text{H}^+]$ 971.35 found 971.1.



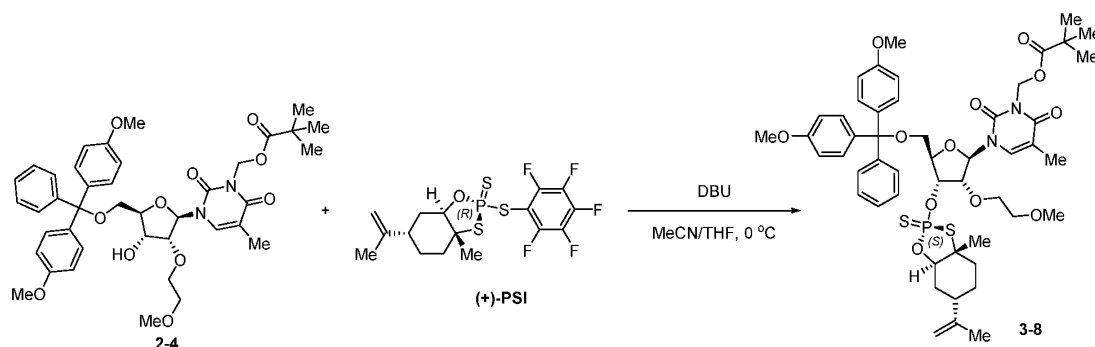
- [0311] **(3-6)** 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2S,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one: Prepared according to general procedure 2 with (+)-PSI reagent, white foamy solid; ^1H NMR (400 MHz, CD_3CN , 296 K) δ (ppm) = 7.57 (s, 1H), 7.50 (d, $J = 7.5$ Hz, 2H), 7.40 - 7.31 (m, 6H), 7.31 - 7.23 (m, 1H), 6.90 (d, $J = 8.9$ Hz, 4H), 6.05 (d, $J = 5.8$ Hz, 1H), 5.49 - 5.40 (m, 1H), 4.99 (s, 1H), 4.88 (s, 1H), 4.43 (td, $J = 3.1, 12.6$ Hz, 1H), 4.34 (t, $J = 5.4$ Hz, 1H), 4.26 (br d, $J = 3.4$ Hz, 1H), 3.85 - 3.72 (m, 8H), 3.54 - 3.45 (m, 4H), 3.38 (d, $J = 2.8$ Hz, 2H), 3.26 (s, 3H), 3.11 - 3.05 (m, 2H), 3.03 (s, 4H), 2.62 (br s, 1H), 2.22 (br d, $J = 12.3$ Hz, 1H), 2.13 - 2.01 (m, 3H), 2.01 - 1.92 (m, 2H), 1.92 - 1.79 (m, 2H), 1.76 (s, 3H), 1.68 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (101 MHz, CD_3CN , 298 K) δ (ppm) = 172.6, 170.0, 160.2, 147.2, 146.1, 138.4, 137.0, 136.8, 131.5, 131.5, 129.5, 129.4, 128.4, 114.6, 112.5, 88.7, 88.2, 87.8, 82.9, 82.9, 82.3, 82.2, 77.7, 77.6, 73.3, 71.8, 66.7, 63.9, 59.5, 56.3, 52.5, 40.2, 34.9, 34.8, 32.4, 31.8, 28.7, 28.6, 24.2, 23.2, 22.3, 20.8; ^{31}P NMR (162 MHz, CD_3CN) δ 101.8; MS (ESI, m/z) calculated for $[\text{C}_{49}\text{H}_{61}\text{N}_4\text{O}_9\text{PS}_2 + \text{H}^+]$ 945.36 found 946.5.



- [0312] **(3-7)** (2S,3aR,6S,7aR)-2-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(6-(((E)-1-methylpyrrolidin-2-

ylidene)amino)-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)-3a-methyl-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-sulfide:

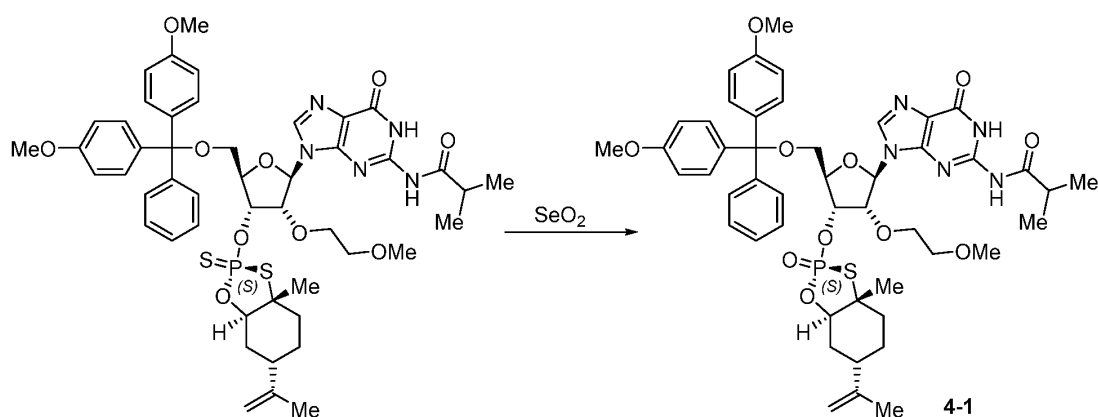
[0313] Prepared according to general procedure 2 with (+)-PSI reagent, white foamy solid; ^1H NMR (400 MHz, CD_3CN , 296 K) δ (ppm) = 8.35 (s, 1H), 8.06 (s, 1H), 7.47 (d, J = 7.4 Hz, 2H), 7.34 (dd, J = 1.6, 8.8 Hz, 4H), 7.30 (s, 2H), 7.26 - 7.19 (m, 1H), 6.85 (d, J = 8.9 Hz, 4H), 6.01 (d, J = 6.6 Hz, 1H), 5.60 - 5.53 (m, 1H), 5.10 (t, J = 5.7 Hz, 1H), 4.99 (s, 1H), 4.91 (s, 1H), 4.49 (td, J = 3.0, 12.6 Hz, 1H), 4.41 - 4.32 (m, 1H), 3.78 (s, 6H), 3.77 - 3.71 (m, 1H), 3.67 (br t, J = 6.4 Hz, 2H), 3.65 - 3.57 (m, 1H), 3.52 (t, J = 7.1 Hz, 2H), 3.45 (br d, J = 4.5 Hz, 1H), 3.40 - 3.34 (m, 3H), 3.09 (s, 3H), 3.07 (s, 3H), 2.93 (t, J = 7.9 Hz, 2H), 2.64 (br s, 1H), 2.27 (br d, J = 13.4 Hz, 1H), 2.18 - 2.06 (m, 1H), 2.06 - 2.01 (m, 2H), 2.00 - 1.86 (m, 1H), 1.78 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (101 MHz, CD_3CN , 297 K) δ (ppm) = 168.5, 162.1, 160.1, 153.5, 152.6, 147.2, 146.3, 142.2, 137.0, 131.5, 131.5, 129.4, 129.2, 129.2, 128.3, 128.0, 114.5, 112.5, 87.8, 87.7, 87.6, 83.9, 83.8, 80.8, 80.8, 78.4, 78.4, 72.9, 72.1, 68.7, 66.6, 64.2, 59.3, 56.3, 52.5, 40.2, 34.9, 34.8, 32.4, 31.3, 28.7, 28.6, 26.6, 24.2, 23.2, 22.3, 20.8; ^{31}P NMR (162 MHz, CD_3CN) δ 101.7; MS (ESI, m/z) calculated for $[\text{C}_{49}\text{H}_{59}\text{N}_6\text{O}_8\text{PS}_2 + \text{H}^+]$ 955.36 found 956.6.



[0314] **(3-8)** (3-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2S,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl pivalate:

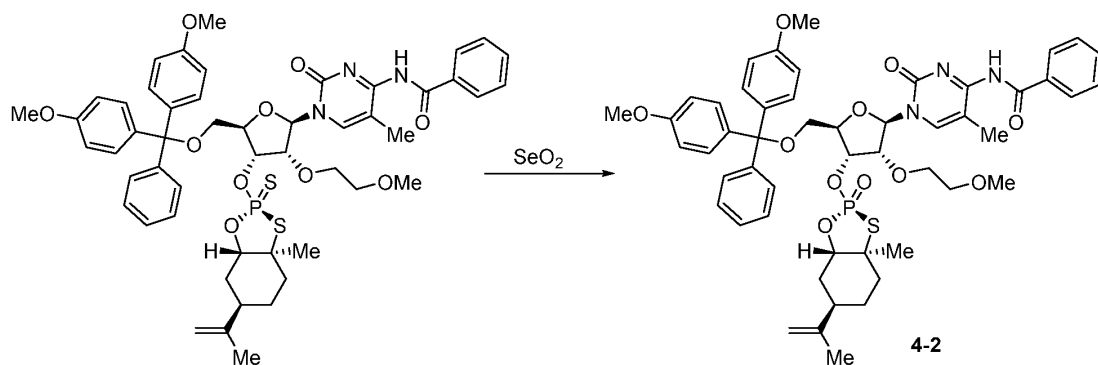
[0315] Prepared according to general procedure 2 with (+)-PSI reagent, foamy solid; ^1H NMR (400 MHz, CD_3CN) δ ppm 7.53 (1 H, s), 7.48 (2 H, d, J = 7.63 Hz), 7.33 - 7.39 (6 H, m), 7.26 - 7.32 (1 H, m), 6.92 (4 H, d, J = 8.76 Hz), 6.01 (1 H, d, J = 6.88 Hz), 5.86 - 5.92 (2 H, m), 5.45 (1 H, ddd, J = 11.60, 4.78, 2.75 Hz), 5.01 (1 H, s), 4.90 (1 H, s), 4.44 - 4.49 (2 H, m), 4.29 (1 H, br d, J = 2.63 Hz), 3.80 (6 H, s), 3.76 - 3.79 (1 H, m), 3.32 - 3.54 (4 H, m), 3.23 (3 H, s), 2.60 - 2.66 (1 H, m), 2.24 (2 H, br d, J = 12.76 Hz), 2.07 (1 H, br dd, J = 13.01, 3.75 Hz), 1.99 - 2.01 (2 H, m), 1.80 - 1.92 (2 H, m), 1.78 (3 H, s), 1.68 (3 H, s), 1.46 (3 H, s), 1.18 (9 H, s); ^{31}P NMR (162 MHz, CD_3CN) δ ppm 102.18; MS (ESI, m/z) calculated for $[\text{C}_{50}\text{H}_{63}\text{N}_2\text{O}_{12}\text{PS}_2 + \text{Na}^+]$ 1001.35 found 1001.1.

General Procedure 3: PO-PSI monomer from PS-PSI monomer



[0316] **(4-1)** N-(9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2S,3aR,6S,7aR)-3a-methyl-2-oxido-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide:

[0317] To N-(9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2S,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (1 g, 1.042 mmol) in MeCN (15.00 mL, 15 vol) was added SeO₂ (1.0 eq., 0.116 g, 1.042 mmol) in ice-bath. Additional SeO₂ (0.116 g, 1.042 mmol) was added at 0 °C until the reaction was completed at 0°C. Total 3 equivalents of SeO₂ were used. Upon completion as monitored by UPLC-MS, the mixture was filtered over celite and dry SiO₂ (EtOAc/THF). The filtrate was washed with saturated NaHCO₃ (10 mL), dried over Na₂SO₄, filtered (dry SiO₂) and concentrated. The residue was purified by a silica-gel column chromatography (50g, Hept/EtOAc, 20 to 100 then EtOAc/THF 0 to 100%) to give the **4-1** (0.55 g, 56% yield). MS (ESI, *m/z*) calculated for [C₄₈H₅₈N₅O₁₁PS - H⁺] 942.36 found 942.53.

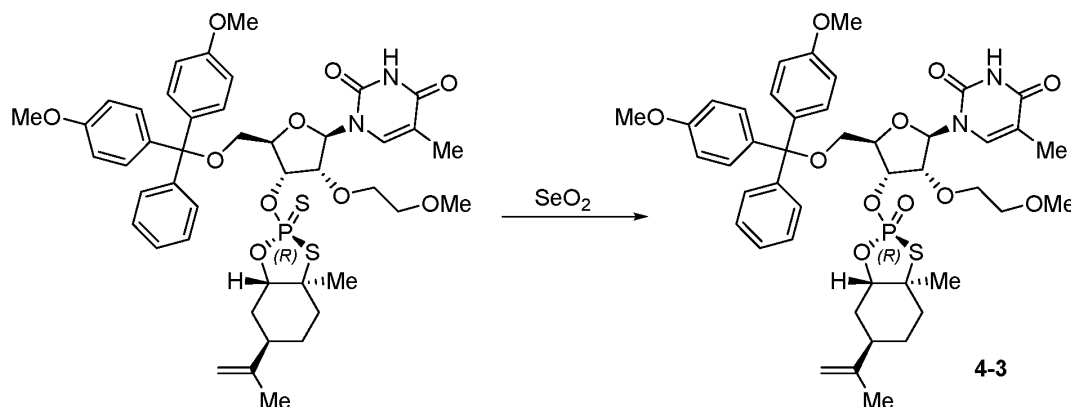


[0318] **(4-2)** N-(1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2R,3aS,6R,7aS)-3a-methyl-2-oxido-6-(prop-1-en-2-

yl)hexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide:

[0319] Prepared according to general procedure 3, white foamy solid; 59% yield; ^{31}P NMR (162 MHz, acetonitrile- d_3) δ 40.36; MS (ESI, m/z) calculated for $[\text{C}_{51}\text{H}_{58}\text{N}_3\text{O}_{11}\text{PS} + \text{H}^+]$ 952.35 found

5 952.35.

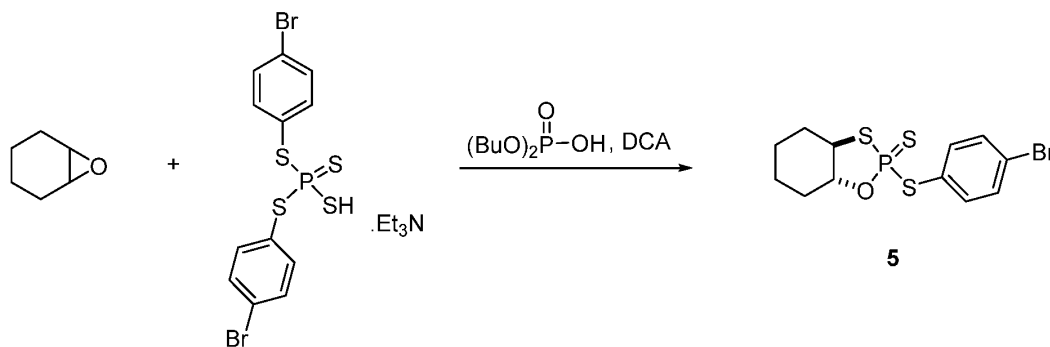


[0320] **(4-3)** 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((2R,3aS,6R,7aS)-3a-methyl-2-oxido-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione:

10

[0321] Prepared according to general procedure 3, white foamy solid; 46% yield; ^{31}P NMR (162 MHz, acetonitrile- d_3) δ 40.42; MS (ESI, m/z) calculated for $[\text{C}_{44}\text{H}_{53}\text{N}_2\text{O}_{11}\text{PS} + \text{Na}^+]$ 871.30 found 871.28.

PO-PSI reagent from cyclohexyl epoxide:



15

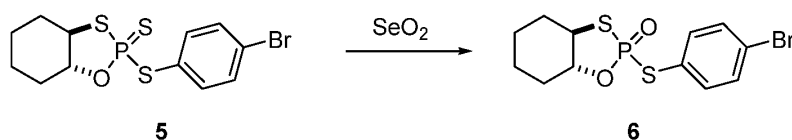
[0322] **(5)** rac-2-((4-bromophenyl)thio)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-sulfide:

[0323] A solution of triethylamine bis(4-bromophenyl) phosphorotetrathioate (50.0 g, 87.2 mmol) and cyclohexene oxide (13.2 mL, 131 mmol) in chloroform (175 mL) was treated with dibutyl phosphate (16.2 mL, 87.2 mmol) and dichloroacetic acid (10.8 mL, 131 mmol). After stirring at room temperature for 15 hours, the mixture was concentrated *in vacuo*. The residue was diluted with water (125 mL) and *n*-heptane (125 mL), cooled with an ice bath, and stirred at

20

0 °C for 2 hours. The resulting precipitate was filtered, and washed subsequently with water (100 mL) and *n*-heptane (125 mL). The filter cake was dissolved in CH₂Cl₂ (200 mL) and the aqueous layer was removed. The organic layer was concentrated *in vacuo* to ca. 50 mL and treated with *n*-heptane (75 mL). The mixture was stirred at room temperature for 20 min and

- 5 concentrated *in vacuo* to ca. 50 mL. The resulting precipitate was filtered, washed with *n*-heptane (20 mL), and dried over N₂ purge for 2 hours to give the title compound (30.1 g, 91%).
 [0324] ¹H NMR (400 MHz, CDCl₃, 296 K) (a 1:2 mixture of diastereomers) δ (ppm) = 7.58 - 7.51 (m, 8H), 7.47 - 7.41 (m, 4H), 4.04 (dt, *J* = 3.9, 10.7 Hz, 1H), 3.65 - 3.56 (m, 4H), 2.27 - 2.12 (m, 6H), 1.89 (m, 3H), 1.81 (m, 3H), 1.75 - 1.58 (m, 3H), 1.49 - 1.25 (m, 8H), 1.23 - 1.16 (m, 1H),
 10 1.07 - 0.86 (m, 1H); ³¹P NMR (162 MHz, CDCl₃, 296 K) δ (ppm) = 107.01 (s, 1P), 103.23 (s, 2P); MS (ESI) *m/z*: [M+H]⁺ calcd for C₁₂H₁₅BrOPS₃ 380.91; Found 380.84.

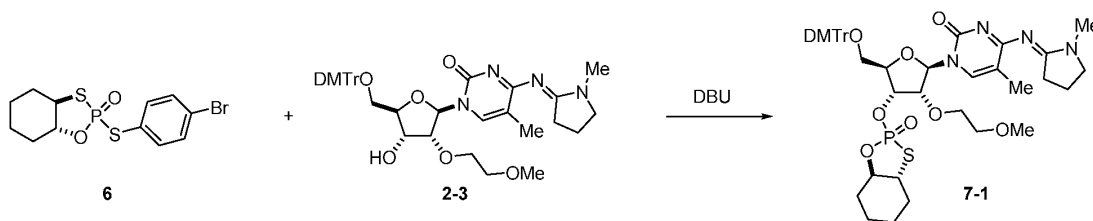


[0325] **(6)** rac-2-((4-bromophenyl)thio)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-oxide:

- 15 [0326] A solution of (3aR,7aR)-2-((4-bromophenyl)thio)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-sulfide (10.0 g, 26.2 mmol) in CH₂Cl₂ (170 mL) was treated with SeO₂ (2.91 g, 26.2 mmol) and stirred at room temperature for 2 hours. Additional SeO₂ (2.91 g, 26.2 mmol) was added and stirring was continued at rt for additional 19 hours. The reaction mixture was filtered through a dry silica gel pad and rinsed
 20 with CH₂Cl₂. The filtrate was washed with 10% NaH₂PO₄ (70.0 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was treated with *n*-heptane (46 mL) and the resulting slurry was stirred at room temperature for 20 minutes. The precipitate was filtered, washed with *n*-heptane (20 mL) and dried over N₂ purge to give the title compound (6.18 g, 64.5%).

- [0327] ¹H NMR (400 MHz, CDCl₃, 296 K) (ca. 1:2 mixture of two diastereomers) δ (ppm) = 7.58 - 7.48 (m, 12H), 4.10 (dt, *J* = 4.1, 10.8 Hz, 1H), 3.60 (dt, *J* = 3.6, 10.8 Hz, 2H), 3.37 (dt, *J* = 3.9, 10.8 Hz, 2H), 2.43 - 2.36 (m, 1H), 2.25 - 2.07 (m, 5H), 1.98 - 1.83 (m, 4H), 1.83 - 1.73 (m, 3H), 1.63 - 1.48 (m, 3H), 1.46 - 1.23 (m, 8H), 1.11 - 0.99 (m, 1H); ³¹P NMR (162 MHz, CDCl₃, 297 K) δ (ppm) = 62.54 (s, 1P), 56.98 (s, 2P); MS (ESI) *m/z*: [M+H]⁺ calcd for C₁₂H₁₅BrO₂PS₂ 364.94; Found 364.97.

30 General Procedure 4: PO-PSI monomer



[0328] **(7-1)** 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((3aR,7aR)-2-oxohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one:

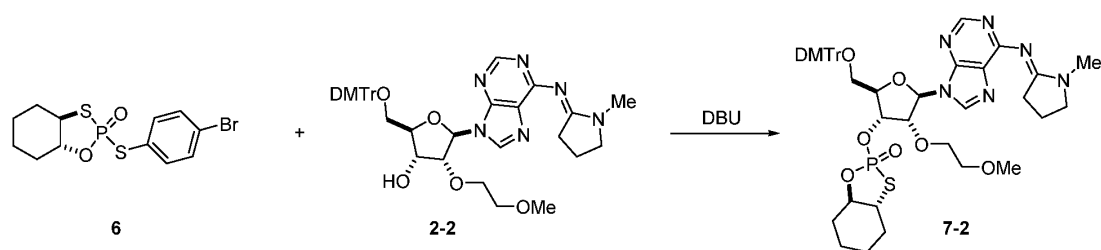
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[0329] 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one (4.30 g, 6.15 mmol) and (3aR,7aR)-2-((4-bromophenyl)thio)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-oxide (3.15 g, 8.62 mmol) was azeotroped three times with acetonitrile (43 mL). The residue was dissolved in acetonitrile (43 mL), cooled to 0 °C, and treated with DBU (1.6 mL, 8.3 mmol). The mixture was stirred at 0 °C for 2 hours, quenched with saturated NaH₂PO₄ (40 mL), and diluted with ethyl acetate (50 mL). The organic layer was separated, and the aqueous layer was extracted twice with ethyl acetate (50 mL). The organic layers were combined, washed with sat. NaHCO₃ (20 mL), dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate in *n*-heptane = 17% to 100% and then THF in ethyl acetate = 0% to 100%) to give the title compound (3.07 g, 57.1%) as a foaming solid.

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[0330] MS (ESI) *m/z*: [M+H]⁺ calcd for C₄₅H₅₆N₄O₁₀PS 875.3; Found 875.1.

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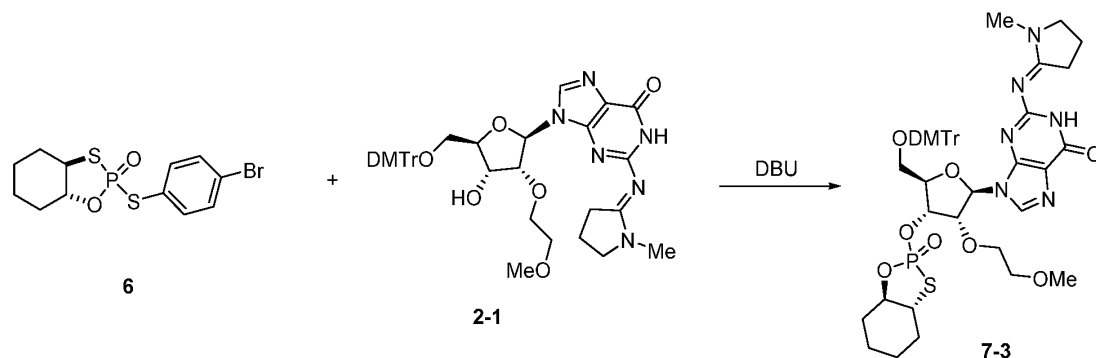
[0331] **(7-2)** (3aR,7aR)-2-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(6-(((E)-1-methylpyrrolidin-2-ylidene)amino)-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-oxide:

[0332] (2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(6-(((E)-1-methylpyrrolidin-2-ylidene)amino)-9H-purin-9-yl)tetrahydrofuran-3-ol (2.70 g, 3.81 mmol) and (3aR,7aR)-2-((4-bromophenyl)thio)hexahydrobenzo[d][1,3,2]oxathiaphosphole 2-oxide (1.95 g, 5.33 mmol) was azeotroped three times with acetonitrile (25.4 mL) in the rotary evaporator. The residue was dissolved in acetonitrile (25.4 mL), cooled to 0 °C, and treated with DBU (0.78 mL, 5.1 mmol). The mixture was stirred at 0 °C for 2 hours, quenched with saturated NaH₂PO₄ (30 mL), and diluted with ethyl acetate (30 mL). The organic layer was separated, and the aqueous layer was extracted twice with ethyl acetate (30 mL). The organic layers were combined, washed with saturated NaHCO₃ (20 mL), dried over MgSO₄, and concentrated *in*

30

vacuo. The residue was purified by column chromatography (ethyl acetate in *n*-heptane = 17% to 100%, and then THF in ethyl acetate = 0% to 100%) to give the title compound (2.10 g, 62.3%) as a foamy solid.

[0333] MS (ESI) m/z : $[M+H]^+$ calcd for $C_{45}H_{54}N_6O_9PS$ 884.33 Found 884.45.



5

[0334] **(7-3)** 9-(((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-methoxyethoxy)-4-(((3aR,7aR)-2-oxidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-2-((1-methylpyrrolidin-2-ylidene)amino)-1,9-dihydro-6H-purin-6-one:

[0335] Prepared according to general procedure 4, a white foamy solid; 80% yield; MS (ESI, m/z) calculated for $[C_{45}H_{53}N_6O_{10}PS + H^+]$ 901.33 found 901.1.

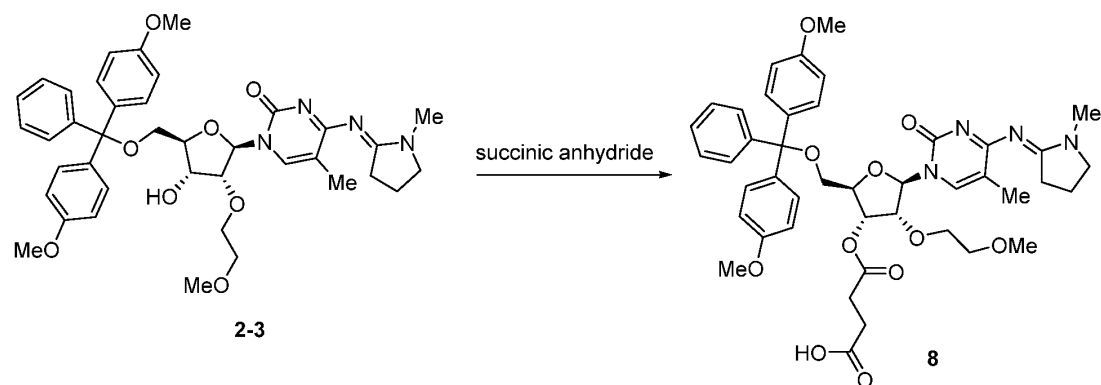
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General Procedure 5: Synthesis of Monomer Succinates

[0336] To the protected nucleoside (1.0 eq.) and succinic anhydride (1.5 eq.) were added DCM (8 vol) and Et_3N (3.0 eq.) at room temperature. The mixture was stirred overnight at room temperature. To the mixture was added phosphate buffer (pH 7, 6 vol) and extracted with DCM (8 vol) 3 times. Then the organic layers were concentrated and purified by a column chromatography (Heptane/EtOAc, 10 to 100%).

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(5-methyl-C-MOE succinate):



[0337] **(8)** 4-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(5-methyl-4-((E)-1-methylpyrrolidin-2-ylidene)amino)-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)oxy)-4-oxobutanoic acid:

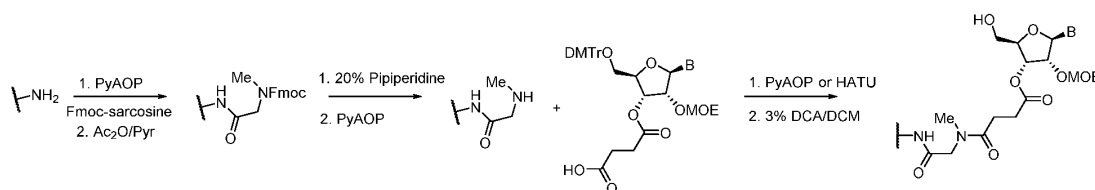
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- [0338] To 1-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-(2-methoxyethoxy)tetrahydrofuran-2-yl)-5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)pyrimidin-2(1H)-one (5 g, 7.155 mmol) and succinic anhydride (1.074 g, 10.732 mmol) in DCM (40.0 mL, 621.71 mmol) was added Et₃N (2.99 mL, 21.465 mmol) at room temperature. The mixture was stirred overnight at room temperature. To the mixture was added phosphate buffer (pH 7, 30 mL) and extracted with DCM (50 mL X 3). Then the organic layers were concentrated and purified by a column chromatography (Hept/EtOAc, 10 to 100%) to give 4-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)oxy)-4-oxobutanoic acid (4.92 g, 6.16 mmol, 86 % yield).
- [0339] ¹H NMR (400 MHz, CD₃CN) δ ppm 7.59 (1 H, s), 7.47 (2 H, d, *J*=7.50 Hz), 7.31 - 7.39 (6 H, m), 7.24 - 7.31 (1 H, m), 6.91 (4 H, d, *J*=8.63 Hz), 6.04 (1 H, d, *J*=5.25 Hz), 5.35 (1 H, t, *J*=5.13 Hz), 4.35 (1 H, t, *J*=5.32 Hz), 4.14 - 4.25 (1 H, m), 3.79 (6 H, s), 3.74 - 3.78 (1 H, m), 3.66 (1 H, dt, *J*=11.44, 4.28 Hz), 3.44 - 3.52 (4 H, m), 3.33 - 3.40 (2 H, m), 3.26 (3 H, s), 3.05 - 3.11 (2 H, m), 3.04 (3 H, s), 2.50 - 2.65 (4 H, m), 2.00 - 2.08 (2 H, m), 1.99 (1 H, s), 1.61 (3 H, s); MS (ESI, *m/z*) Calculated for [C₄₃H₅₀N₄O₁₁+H⁺] 799.35; Found 799.9.

General Procedure 6: Solid Phase Synthesis of Stereo-controlled PS MOE ASO

- [0340] A general procedure for automated solid-phase synthesis of stereo-controlled PS-oligonucleotides was modified from the reported procedures in Knouse et al., "Unlocking P(V): Reagents for chiral phosphorothioate synthesis," *Science* **2018**, 361 (6408), 1234-1238; and Huang et al., "A P(V) platform for oligonucleotide synthesis," *Science* **2021**, 373 (6560), 1265-1270.

Automated Solid-Phase Oligonucleotide Synthesis: Part 1. Loading to Resin: Preparation of 1mer



- [0341] TentaGel S-NH₂ (AC354610050, ACROS Organics, loading 0.2 to 0.3 mmol/g) (4 g, ~ 1 mmol) was placed in a 50 mL solid phase reaction flask and washed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3). To the resin were added N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-methylglycine (3.11 g, 10.00 mmol) in DMF (5.00 mL) and ((3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)oxy)tri(pyrrolidin-1-yl)phosphonium hexafluorophosphate(V) (5.21 g, 10.00 mmol) in DMF (5 mL) followed by N-4-methylmorpholine (2199 mL, 20.00 mmol) at room temperature. It was shaken at 400 rpms. After 24 hours, the liquid was drained and the

resin was rinsed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3). To the resin was added premixed pyridine (4.85 mL, 60.00 mmol) and Ac₂O (0.944 mL, 10.00 mmol) at room temperature. After 3 minutes, the solution was drained and premixed pyridine (4.85 mL, 60.00 mmol) and Ac₂O (0.944 mL, 10.00 mmol) was added at room temperature. After 3 minutes, the liquid was drained and the resin was washed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3).

[0342] Then, the resin was treated with 30 mL of 20% piperidine in DMF and the solution was collected after 3 minutes. This process was repeated 5 times and the resin was washed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3). A solution of 20% piperidine in DMF was added to the collected solution to make 300 mL in a volumetric flask. An aliquot of this solution was diluted 10-fold with 20% piperidine in DMF and the UV absorbance of the piperidine–fulvene adduct was measured ($\lambda = 301$ nm, $\epsilon = 7800$ M⁻¹cm⁻¹, A = 2.41) to give 230 μ mol/g as an estimated loading.

[0343] The resin was washed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3). To the resin was added 4-(((2R,3R,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(2-methoxyethoxy)-5-(5-methyl-4-(((E)-1-methylpyrrolidin-2-ylidene)amino)-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)oxy)-4-oxobutanoic acid (1.5 eq., 1.198 g, 1.5 mmol) in DMF (5 mL) followed by ((3H-[1,2,3]Triazolo[4,5-b]pyridin-3-yl)oxy)tri(pyrrolidin-1-yl)phosphonium hexafluorophosphate(V) (1.7 eq., 0.886 g, 1.7 mmol) in DMF (5 mL) and N-4-Methylmorpholine (2 eq. 0.258 g, 2 mmol). The mixture was shaken at room temperature for 3 days and washed with DMF (10 mL X 3), DCM (10 mL X 3) and DMF (10 mL X 3).

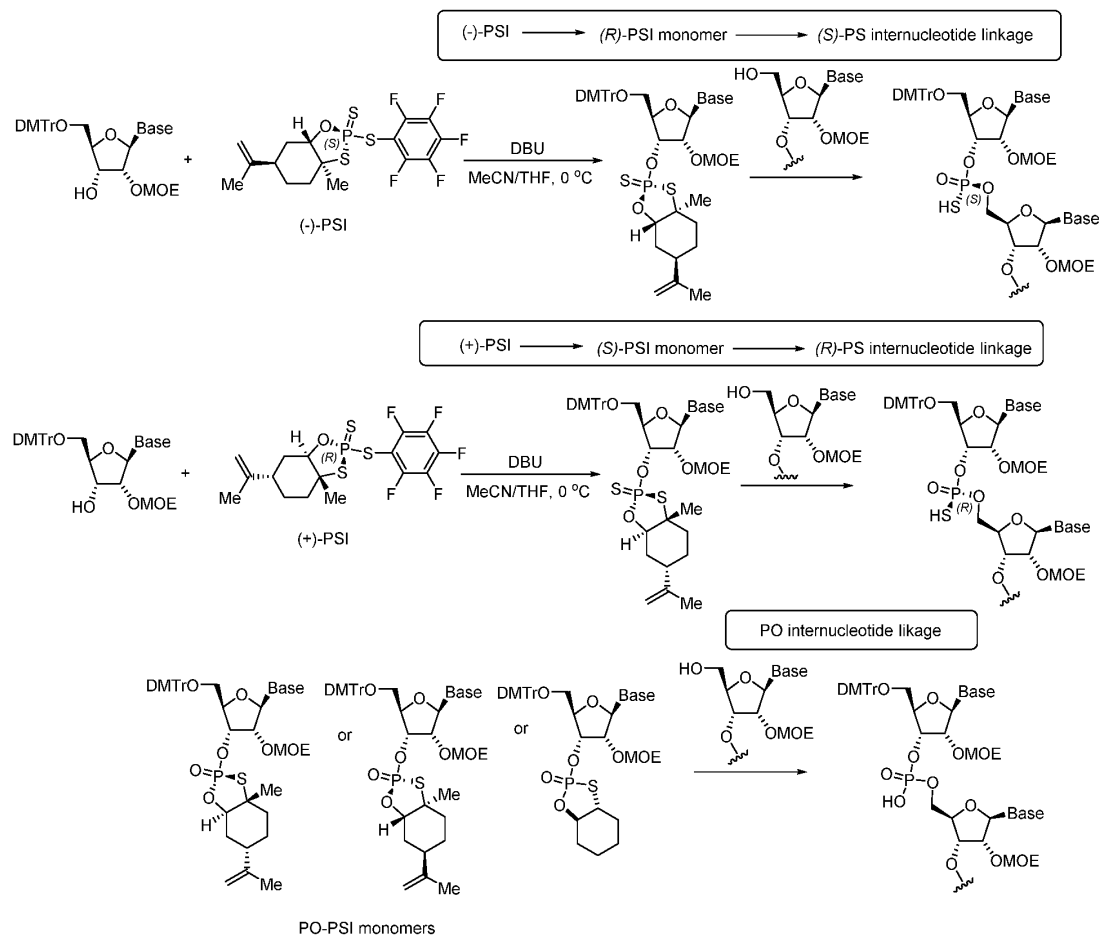
[0344] The resin was washed with DMF (10 mL X 3), DCM (10 mL X 3). It was treated for 2 minutes with 3% dichloroacetic acid (DCA) in DCM (20 mL) followed by DCM (20 mL) washing to remove the DMTr group. The process was repeated (> 5 times) until no color was observed. Then the resin was washed with DCM (10 mL X 3), DMF (10 mL X 3) and MeCN (10 mL X 3).

[0345] The combined deprotection solutions were diluted with 3% DCA in DCM. The UV absorbance of the DMTr cation was measured ($\lambda = 410$ nm, $\epsilon = 30,400$ M⁻¹cm⁻¹) to quantify the loading (0.2 mmol/g).

Part 2. Automated synthesis on K & A H-8-SE Oligo Synthesizer

[0346] The prepared 5'-O-DMTr-nucleotide-loaded TentaGel-SAR (20 μ mol, 200 μ mol/g) was packed in an empty 6 mL syringe column (Biocomma Limited, Cat# RSSC-6) and washed with MeCN. The stereopure oligonucleotides were synthesized on K & A H-8-SE Oligo Synthesizer following the cycles shown in Table 15 using stereopure PSI monomers and PO-PSI monomers. As shown in the schemes below: *Sp* phosphorothioate linkage was obtained using *Rp*-PSI-

monomers that were prepared from (-)-PSI reagent; *Rp* phosphorothioate linkage was obtained using *Sp*-PSI-monomers that were synthesized from (+)-PSI, and PO internucleotide linkages were obtained using PO-PSI monomers¹.



- 5 [0347] Monomers in the synthesis of *Sp*, *Rp* phosphorothioate and PO (phosphodiester) internucleotide linkages.

Table 15. Protocol for automated solid phase synthesis of MOE PS oligonucleotides

	Step	Comment	Reagent/solvent	Time	cycles
1	Deblock	PhMe wash between cycles	3% DCA in PhMe	1 min	5
2	Wash		MeCN	3 min	5
3	Base Wash*		2,6-lutidine/DBU/MeCN (2/1/20)	3 min	2
4	Wash		MeCN	3 min	5
5	Coupling		Monomer (>10 eq., 0.1 N), 2,6-lutidine (>50 eq.), DBU (13 eq.) (1 N/0.3 N)**	16 hr	1

6	Wash		MeCN	3 min	5
7	Capping	solvent (MeCN)	20% Ac ₂ O, 30% 2,6-lutidine, 20% N-Me-imidazole (1/1/1)	1 min	2
8	Wash		MeCN	3 min	5

*Base wash solution: MeCN/2,6-lutidine/DBU = 20/2/1 (v/v/v)

**Coupling: monomer (0.2 mmol) in MeCN (2 mL, 0.1 M) and base solution [2,6-lutidine 1.1 mL (1 M), DBU 0.5 mL (0.3 M), MeCN 10 mL] (0.9 mL) (>10 eq. DBU and >40 eq. 2,6-lutidine) was transferred to the column. It was shaken for 16 hours, it was drained and washed, and conversion was analyzed by RP HPLC-Mass after cleavage from a bit of resin (28% NH₄OH/EtOH/NH₄OAc (9/2/1, v/v/w), 65°C, 4 hours). >95% conversion was achieved while lower conversions were observed either without 2,6-lutidine or shorter reaction time: no 2,6-lutidine, DBU (15 eq.): ~50% conversion or Monomer (10 eq.), 2,6-lutidine (50 eq.), DBU (15 eq.), 8h: ~80% conversion.

[0348] **Analytical HPLC Method 1**-RP HPLC-Mass: Column: Acquity UPLC BEH C18 1.7 µm 2.1x50 mm (Part Number: 186002350); Solvents: Buffer A (10 mM ammonium bicarbonate in water), Buffer B (100 mM ammonium bicarbonate/MeOH/MeCN = 10/10/80); temperature: 60°C; Flow rate: 0.8 mL/min; Gradient: 5 ~ 99% B gradient (6 min).

15 **Part 3. Cleavage from Resin and Deprotection:**

[0349] After completion of the last cycle (DMTr-On), the resin in cleavage solution (28% NH₄OH/NH₄OAc/EtOH (10/1/1, ~1 mL/1 µmol) was heated at 65°C for 2 days in a closed bottle. It was cooled to room temperature, filtered, and then concentrated. The failed sequences were removed and DMTr group was deprotected by the below C18 cartridge protocol. The collected fractions were concentrated and purified by an Ion-Pairing Reverse-Phase (IR-RP) HPLC.

C18 column protocol:

[0350] Sep-Pak cartridge [Waters, Sep-Pak Vac 35cc (10 g) C18 Cartridge] was equilibrated with MeOH (2 CV), MeCN (2 CV) followed by 2 N Et₃NHOAc (2 column volumes (CV)). The crude sample in 0.1 N Et₃NHOAc was loaded on a cartridge. The cartridge was washed with 2 N NaCl/MeCN (5/1, v/v) to elute truncated sequences, and 3% TFA in water (150 mL), then water (50 mL). The crude DMTr-off PS-oligonucleotide was eluted with 50 mL of acetonitrile–water (1:1, v/v) containing 0.5% of 28% NH₄OH. The solution containing crude DMTr-off oligonucleotide was dried under vacuum. The weight was measured by Nanodrop (RNA-40) and ³¹P NMR was taken. It was analyzed by RP-HPLC, IEX-HPLC and UPLC/MS.

30 [0351] **Analytical HPLC Method 2**-Ion-pairing RP HPLC-Mass: Column: XBridge Premier BEH C18 (2.5 µm, 150 x 2.1 mm); Temperature: 60°C; Flow rate: 1 mL/minute; Detection wavelength: 260 nm; Solvents: buffer A: 100 mM HFIP/ 8.6 mM Et₃N (H₂O), buffer B: 100% MeOH; Gradient: 5% to 30% B gradient (15 minutes).

- [0352] **Analytical HPLC Method 3**-Ion-pairing RP HPLC-Mass: Column: XBridge Premier BEH C18 (300Å, 2.5 µm, 150 x 2.1 mm); Temperature: 60°C, Flow rate: 0.5 mL/minute; Detection wavelength: 260 nm; Solvents: Buffer A: 100 mM n-C₆H₁₃NH₃OAc (H₂O/MeCN 9/1) Buffer B: 100 mM C₆H₁₃NH₃OAc (H₂O/MeCN 1/1); Gradient: 80% to 100% B gradient (15 minutes).
- 5 [0353] **Analytical HPLC Method 4**-Ion-pairing RP HPLC-Mass: Column: XBridge Premier BEH C18 (300Å, 2.5 µm, 150 x 2.1 mm); Temperature: 60°C, Flow rate: 0.5 mL/min. Detection wavelength: 260 nm; Solvents: buffer A: 10 mM n-Hexylamine/50mM HFIP in water, buffer B: MeCN; Gradient: 23 ~ 28% Buffer B gradient (15 minutes).
- Part 4. HPLC purification and desalting:**
- 10 [0354] The crude material after SepPak treatment was purified by a ion-pairing RP HPLC by the following methods using sterile water (WFI from Baxter, VWR cat. 68000-955).
- [0355] **Preparative HPLC Method 1:** Column: XBridge Prep C18 OBD Prep (10 µm, 19 x 250 mm); Flow rate: 30 mL/minute. Detection wavelength: 260 nm; Solvents: buffer A: 8.6 mM TEA/100 mM HFIP in water, Buffer B: MeOH; Gradient: 10~37% Buffer B gradient (30 minute).
- 15 [0356] **Preparative HPLC Method 2:** Column: Xbridge BEH C18 (10 µm, 10 x 250mm); Flow rate: 14 mL/minute. Detection wavelength: 260 nm; Solvents: buffer A: 100 mM C₆H₁₃NH₃OAc (H₂O/MeCN 9/1), Buffer B: 100 mM C₆H₁₃NH₃OAc (H₂O/MeCN 1/1); Gradient: 50% to 75% gradient (26 min)
- 20 [0357] **Preparative HPLC Method 3:** Column: XBridge C18 OBD Prep (300 Å, 5 µm, 19x250 mm); Flow rate: 30 mL/minute; Detection wavelength: 260 nm; Solvents: buffer A: 10 mM HA/50 mM HFIP in water, Buffer B: MeCN; Gradient: 23 ~ 28% Buffer B gradient (30 minutes).
- 25 [0358] The fractions containing the desired compound were concentrated and dissolved with 0.2 N NaCl in EtOH/water (1/4). The resulting solution was desalted by membrane filtration by using a 3000MW cut-off (3K centrifugal membrane tube, Amicon Ultra-15, Ultracel-3K (3400 rpm, 45 minutes) (cat.UFC900396 from Sigma-Aldrich) or Macrosep Devices (cat. MAP003C38) from PALL, 3400 rpm, 40 minutes, 15 mL WFI X 3). The final desalted solution was filtered (0.2
- 30 micron sterile syringe filter). The absorbance of the diluted solution was measured at 260 nm on a Nanodrop UV-Vis spectrophotometer to give a yield (7 ~ 15% yield) and endotoxin level was confirmed to be less than 0.06 EU/mg by a kinetic chromogenic LAL method (Charles River, Endosafe® nexgen-PTS).
- 35 **Part 5. T_m measurement with reverse complementary RNA and NMR**
- [0359] T_m measurement device: Shimadzu UV-2700 UV-Vis Spectrophotometer

[0360] Protocol 1: ASO samples were prepared at a concentration of 400 μ M using deionized water. IDT's reverse complementary RNA (rcRNA) was dissolved to 400 μ M using UltraPure Distilled water. 10 μ L aliquots of each stock solutions were diluted to 1 mL using ultra pure distilled water and their actual concentrations were measured by UV-Vis Spectrophotometer. Test samples (500 μ L) were prepared containing 4.0 μ M ASO with 4.0 μ M rcRNA in buffer (100 mM NaCl, 10 mM Na phosphate pH 7.0 with 0.1 mM EDTA). Test samples were incubated in a 1 mL cuvette and heated from 15 $^{\circ}$ C to 105 $^{\circ}$ C at 0.5 $^{\circ}$ C/minute. UV absorbance increase due to strand melting was monitored at 260 nm. Prior to the experiment, the samples were melted and reannealed by heating from 25 $^{\circ}$ C to 95 $^{\circ}$ C at 5 $^{\circ}$ C/minute and cooling to starting temperatures to ensure complete annealing. Shimadzu Tm Analysis software was used to calculate the Tm (curve inflection point: 50% melting) using the derivative function.

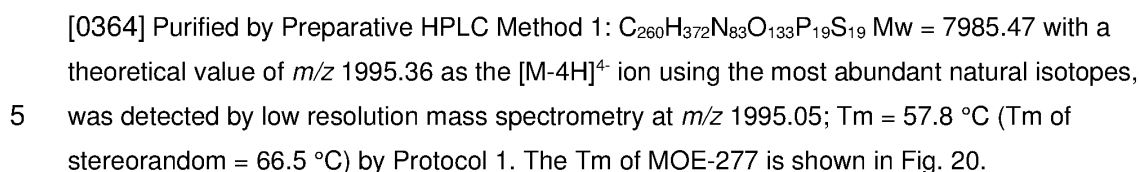
[0361] Protocol 2: ASO samples were prepared at a concentration of 200 μ M using PBS and then followed the same procedure as protocol 1 with adjusted amount.

[0362] ^{31} P NMR (162 MHz)³ was taken in stock phosphate buffer (100 mM, pD = 7.4) that was prepared with 135.5 mg of K₂DPO₄ and 31.2 mg of KD₂PO₄ in 10 mL D₂O after C18 purification and deprotection of DMTr. See Evstigneev et al., "Hexamer oligonucleotide topology and assembly under solution phase NMR and theoretical modeling scrutiny," *Biopolymers* **2010**, 93 (12), 1023-1038.

Exemplary Compounds

[0363] All nucleotides are 2'-MOE unless specified and "C" represent 5'-Methyl cytosine.

A. Compound MOE-277: 20mer, all Sp



5'-A-T-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'



5

C. Compound MOE-279: 20mer, 4Rp



10

D. Compound MOE-280: 20mer, 5Rp



Purified by Preparative HPLC Method 1: C₂₆₀H₃₇₂N₈₃O₁₃₃P₁₉S₁₉ Mw = 7985.47 with a theoretical value of *m/z* 1995.36 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was

detected by low resolution mass spectrometry at m/z 1994.88. T_m = 62.7°C (T_m of stereorandom = 66.5 °C) by Protocol 1.

E. Compound MOE-281: 20mer, 7Rp



[0367] Purified by Preparative HPLC Method 1: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1995.06. T_m = 62.3°C (T_m of stereorandom = 66.5 °C) by Protocol 1.

F. Compound MOE-282: 20mer, 7Rp



[0368] Purified by Preparative HPLC Method 1: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1994.81. T_m = 63.5°C (T_m of stereorandom = 66.5 °C) by Protocol 1.

G. Compound MOE-283: 20mer, 9Rp



[0369] Purified by Preparative HPLC Method 1: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1995.43. T_m = 64.8°C (T_m of stereorandom = 66.5 °C) by Protocol 1.

H. Compound MOE-284: 20mer, 10Rp



[0370] Purified by Preparative HPLC Method 1: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1994.96; T_m = 66.2°C (T_m of stereorandom = 66.5 °C) by Protocol 1.

I. Compound MOE-285: 20mer, 9Rp



- [0371] Purified by Preparative HPLC Method 1: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1995.50; Tm = 63.4°C (Tm of stereorandom = 66.5 °C) by Protocol 1.

J. Compound MOE-286: 20mer, 13Rp



- [0372] Purified by Preparative HPLC Method 2: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1994.55.

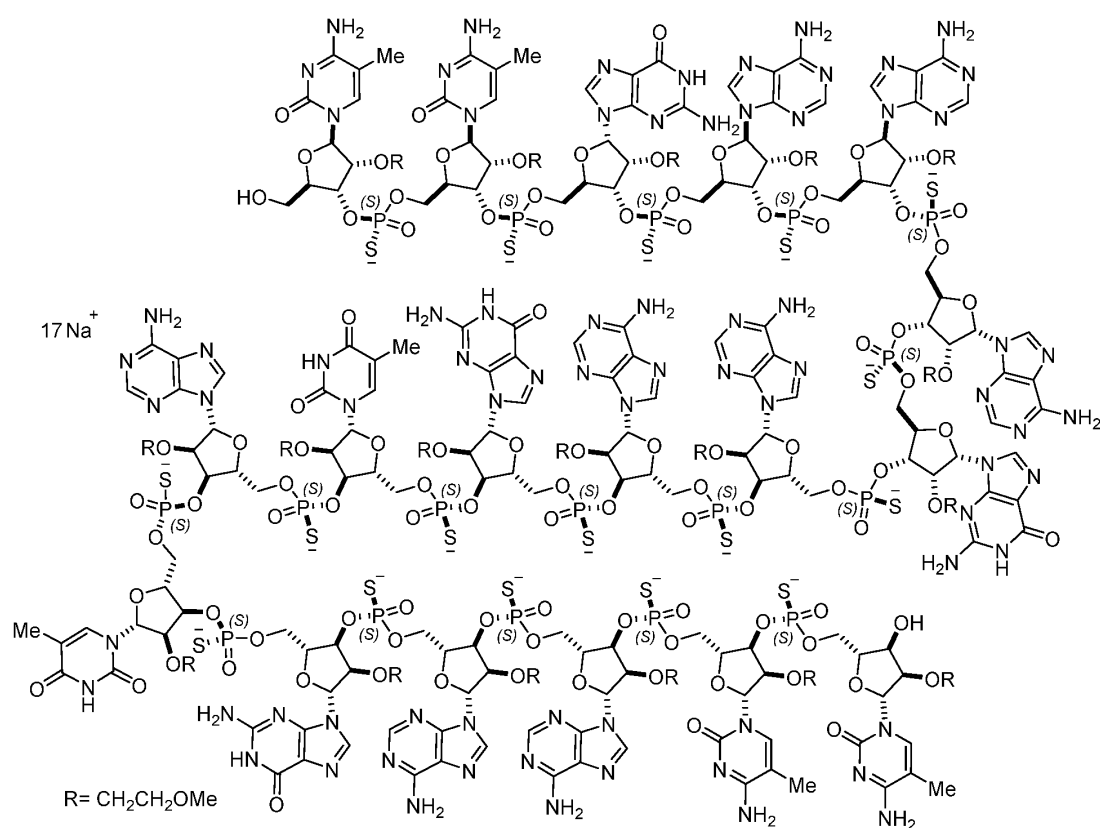
K. Compound MOE-287: 20mer, 3Rp



- [0373] Purified by Preparative HPLC Method 2: $C_{260}H_{372}N_{83}O_{133}P_{19}S_{19}$ Mw = 7985.47 with a theoretical value of m/z 1995.36 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1995.08; Tm = 59.7°C (Tm of stereorandom = 66.5 °C) by Protocol 1.
- [0374] Fig. 20 shows the Tms of MOE-012, MOE-277, and MOE-278. Fig. 21 shows the Tms of MOE-277 to MOE-287.

L. Compound MOE-288: 18mer, all Sp



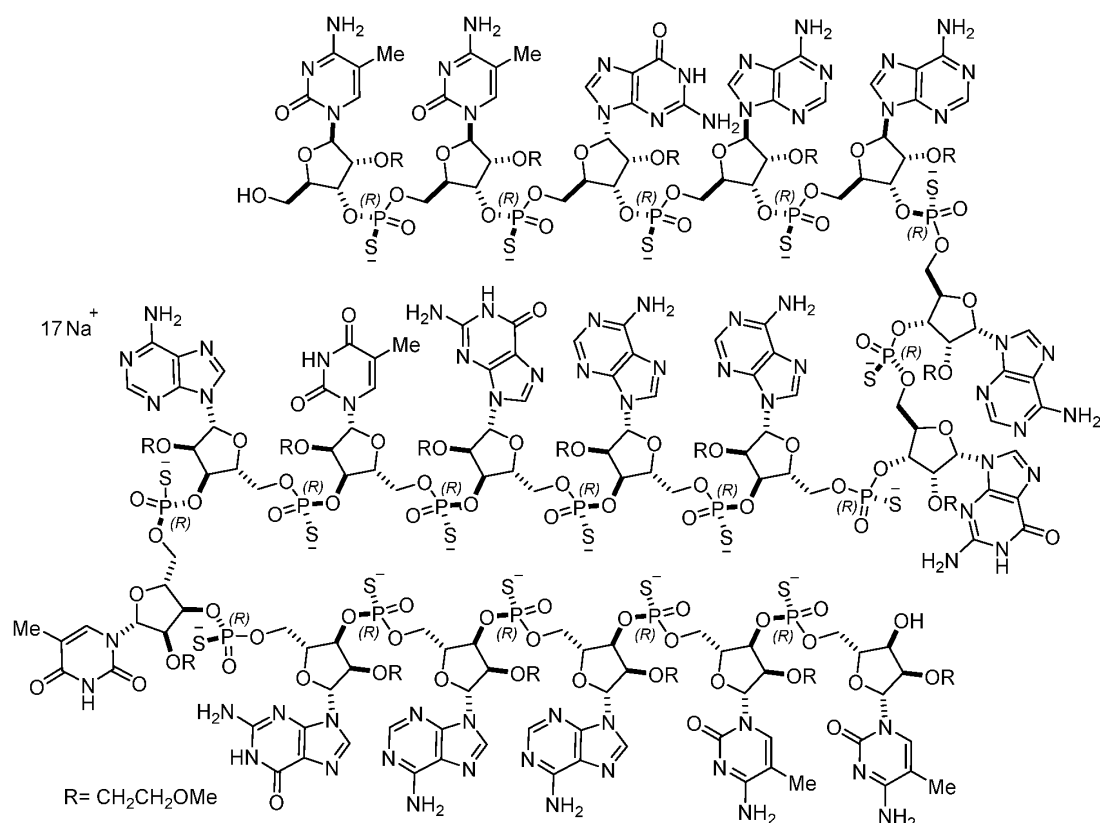


- [0375] Purified by Preparative HPLC Method 3: $\text{C}_{234}\text{H}_{335}\text{N}_{76}\text{O}_{119}\text{P}_{17}\text{S}_{17}$ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the $[\text{M}-4\text{H}]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1795.45; Tm = 58.4°C (Tm of
- 5 stereorandom = 65.9 °C) by Protocol 2.

[0376] ^{31}P NMR (162 MHz) δ ppm 56.09, 55.82, 55.78, 55.56, 55.52, 55.32, 55.21, 55.15, 55.06

[0377] **M. Compound MOE-289:** 18mer, all Rp

5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'



[0378] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of *m/z* 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at *m/z* 1795.38; T_m = 70.4°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0379] ³¹P NMR (162 MHz) δ ppm 56.09, 55.82, 55.78, 55.56, 55.52, 55.32, 55.21, 55.15, 55.06

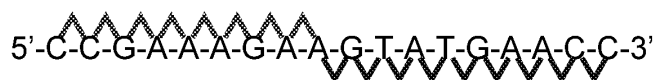
N. Compound MOE-290: 18mer, 11Rp



10 [0380] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of *m/z* 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at *m/z* 1795.57; T_m = 66.6°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0381] ³¹P NMR (162 MHz) δ ppm 58.53, 58.22, 58.08, 57.82, 57.60, 57.40, 57.12, 55.67, 55.54, 55.30, 55.12

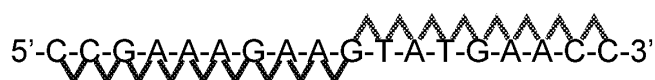
O. Compound MOE-291: 18mer, 8Rp



[0382] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1795.31; T_m = 62.5°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

- 5 [0383] ³¹P NMR (162 MHz) δ ppm 57.07, 56.83, 56.71, 56.54, 56.31, 55.16, 54.81, 54.33, 54.24, 54.12, 54.23

P. Compound MOE-292: 18mer, 8Rp



- 10 [0384] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1794.95; T_m = 62.6°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0385] ³¹P NMR (162 MHz) δ ppm 60.01, 59.40, 59.36, 58.87, 58.50, 58.14, 57.67, 57.37, 57.15, 56.66, 56.48, 55.83, 55.55, 55.26

- 15 [0386] Fig. 22 shows the T_ms of MOE-288 to MOE-292. Fig. 23 shows an example of overlay HPLC chromatogram (MOE-252 and MOE-288 to MOE-292 by Analytical HPLC Method 4.

Q. Compound MOE-293: 18mer, 4Rp



- 20 [0387] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1795.95; T_m = 59.5°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0388] ³¹P NMR (162 MHz) δ ppm 56.79, 56.19, 55.09, 54.93, 54.85, 54.67, 54.53

R. Compound MOE-294: 18mer, 6Rp

- 25 $5'-C-C-G-A-A-A-G-A-A-G-T-A-T-G-A-A-C-C-3'$

[0389] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₁₉P₁₇S₁₇ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1795.54; T_m = 59.7°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

- 30 [0390] ³¹P NMR (162 MHz) δ ppm 57.84, 57.43, 57.17, 56.92, 56.80, 55.98, 55.86, 55.62, 55.58, 55.46, 55.27, 55.11, 55.06, 55.00

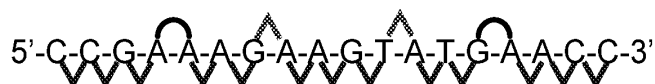
S. Compound MOE-295: 18mer, 4Rp



[0391] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{119}P_{17}S_{17}$ Mw = 7186.34 with a theoretical value of m/z 1795.59 as the $[M-4H]^+$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1795.82; Tm = 60.6°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

- 5 [0392] ^{31}P NMR (162 MHz) δ ppm 57.71, 57.25, 57.06, 56.11, 55.79, 55.68, 55.48, 55.35, 55.21, 55.11

T. Compound MOE-296: 18mer, 2Rp/2PO



- 10 [0393] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{121}P_{17}S_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[M-4H]^+$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.56; Tm = 61.3°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

[0394] ^{31}P NMR (162 MHz) δ ppm 58.26, 58.20, 57.84, 57.65, 57.49, 57.40, 57.16, 56.97, 0.33

U. Compound MOE-297: 18mer, 4Rp/2PO



[0395] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{121}P_{17}S_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[M-4H]^+$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.35; Tm = 62.7°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

- 20 [0396] ^{31}P NMR (162 MHz) δ ppm 57.24, 57.04, 56.42, 56.36, 55.83, 55.69, 55.56, 55.36, 55.17, 55.07, 54.64, -1.03, -1.13

V. Compound MOE-298: 18mer, 2Rp/2PO



- 25 [0397] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{121}P_{17}S_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[M-4H]^+$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.40; Tm = 61.6°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

[0398] ^{31}P NMR (162 MHz) δ ppm 57.20, 57.08, 56.77, 56.55, 56.17, 56.10, -0.72

W. Compound MOE-299: 20mer, 2Rp/2PO



[0399] Purified by Preparative HPLC Method 3: $C_{260}H_{372}N_{83}O_{135}P_{19}S_{17}$ Mw = 7950.51 with a theoretical value of m/z 1986.62 as the $[M-4H]^+$ ion using the most abundant natural isotopes,

was detected by low resolution mass spectrometry at m/z 1987.01; $T_m = 61.5^\circ\text{C}$ (T_m of stereorandom = 69.6°C) by Protocol 1.

[0400] ^{31}P NMR (162 MHz) δ ppm 57.48, 57.22, 56.14, 55.90, 55.65, 55.77, 55.38, 55.30, 55.25, 55.21, 55.06, 54.94, -0.95, -0.99

- 5 [0401] Fig. 24 shows the T_m s of MOE-252 and MOE-293 to MOE-298. Fig. 25 shows the T_m s of MOE-029 and MOE-299.

X. Compound MOE-300: 18mer, 6Rp/2PO



- 10 [0402] Purified by Preparative HPLC Method 3: $\text{C}_{234}\text{H}_{335}\text{N}_{76}\text{O}_{121}\text{P}_{17}\text{S}_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[\text{M}-4\text{H}]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.49; $T_m = 63.2^\circ\text{C}$ (T_m of stereorandom = 65.9°C) by Protocol 2.

[0403] ^{31}P NMR (162 MHz) δ ppm 58.39, 58.07, 57.85, 57.69, 56.36, 56.06, 55.78, 55.70, 55.57, 55.38, 55.33, 55.29, -0.97

- 15 **Y. Compound MOE-301:** 18mer, 5Rp/2PO



- 20 [0404] Purified by Preparative HPLC Method 3: $\text{C}_{234}\text{H}_{335}\text{N}_{76}\text{O}_{121}\text{P}_{17}\text{S}_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[\text{M}-4\text{H}]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.66; $T_m = 62.2^\circ\text{C}$ (T_m of stereorandom = 65.9°C) by Protocol 2.

[0405] ^{31}P NMR (162 MHz) δ ppm 58.57, 58.02, 57.81, 57.65, 56.31, 56.02, 55.73, 55.64, 55.52, 55.33, 55.27, 55.25, 55.11, -1.00

Z. Compound MOE-303: 18mer, 3PO



- 25 [0406] Purified by Preparative HPLC Method 3: $\text{C}_{234}\text{H}_{335}\text{N}_{76}\text{O}_{122}\text{P}_{17}\text{S}_{14}$ Mw = 7137.40 with a theoretical value of m/z 1783.25 as the $[\text{M}-4\text{H}]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1782.76; $T_m = 59.5^\circ\text{C}$ (T_m of stereorandom = 65.9°C) by Protocol 2.

- 30 [0407] ^{31}P NMR (162 MHz) δ ppm 56.36, 56.11, 55.93, 55.84, 55.69, 55.64, 55.53, 55.38, -0.88, -0.97

AA. Compound MOE-304: 18mer, 5PO



[0408] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{124}P_{17}S_{12}$ Mw = 7106.45 with a theoretical value of m/z 1775.61 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1775.83; Tm = 61.6°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

- 5 [0409] ^{31}P NMR (162 MHz, Solvent) δ ppm 56.29, 55.75, 55.71, 55.65, 55.53, 55.42, -0.62, -0.79, -0.89, -0.98

BB. Compound MOE-305: 18mer, 4PO



- 10 [0410] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{123}P_{17}S_{13}$ Mw = 7122.43 with a theoretical value of m/z 1779.50 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1779.42; Tm = 61.4°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

[0411] ^{31}P NMR (162 MHz) δ ppm 55.54, 55.49, 55.72, 55.26, 55.14, 55.11, 54.98, -1.02, -1.06, -1.14, -1.47

- 15 **CC. Compound MOE-306:** 18mer, 3PO



- [0412] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{122}P_{17}S_{14}$ Mw = 7137.40 with a theoretical value of m/z 1783.25 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1783.54; Tm = 60.3°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

20 [0413] ^{31}P NMR (162 MHz) δ ppm 56.18, 55.88, 55.53, 55.37, 55.30, 55.64, 55.16, 55.07, -0.84, -0.90, -0.95

DD. Compound MOE-307: 18mer, 2PO



- 25 [0414] Purified by Preparative HPLC Method 3: $C_{234}H_{335}N_{76}O_{121}P_{17}S_{15}$ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the $[M-4H]^{4-}$ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1788.19; Tm = 59.0°C (Tm of stereorandom = 65.9 °C) by Protocol 2.

- 30 [0415] ^{31}P NMR (162 MHz) δ ppm 56.03, 55.84, 55.70, 55.56, 55.45, 55.27, 55.24, 55.11, 54.95, -1.10, -1.20

EE. Compound MOE-308: 20mer, 2PO



[0416] Purified by Preparative HPLC Method 3: C₂₆₀H₃₇₂N₈₃O₁₃₅P₁₉S₁₇ Mw = 7950.51 with a theoretical value of m/z 1986.62 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1986.92; T_m = 58.5°C (T_m of stereorandom = 69.6 °C) by Protocol 1.

5 [0417]

[0418] ³¹P NMR (162 MHz) δ ppm 55.91, 55.76, 55.52, 55.20, 55.10, 54.99, 54.89, -0.99, -1.05, -1.09

FF. Compound MOE-309: 20mer, 4PO



10 [0419] Purified by Preparative HPLC Method 3: C₂₆₀H₃₇₂N₈₃O₁₃₇P₁₉S₁₅ Mw = 7919.50 with a theoretical value of m/z 1978.87 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1978.66; T_m = 62.8°C (T_m of stereorandom = 69.6 °C) by Protocol 1.

[0420]

15 [0421] ³¹P NMR (162 MHz) δ ppm 55.83, 55.65, 55.50, 55.41, 55.19, 55.02, 55.11, 54.77, -1.04, -1.11, -1.15, -1.55

GG. Compound MOE-310: 18mer, 5R/2PO



20 [0422] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₂₁P₁₇S₁₅ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.64; T_m = 63.4°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0423] ³¹P NMR (162 MHz) δ ppm 58.22, 57.54, 56.08, 55.88, 55.41, 55.26, 55.17, 55.10, 55.04, 54.95, -1.02

25 **HH. Compound MOE-311:** 18mer, 4R/2PO



30 [0424] Purified by Preparative HPLC Method 3: C₂₃₄H₃₃₅N₇₆O₁₂₁P₁₇S₁₅ Mw = 7154.38 with a theoretical value of m/z 1787.59 as the [M-4H]⁴⁻ ion using the most abundant natural isotopes, was detected by low resolution mass spectrometry at m/z 1787.34; T_m = 61.0°C (T_m of stereorandom = 65.9 °C) by Protocol 2.

[0425] ³¹P NMR (162 MHz) δ ppm 58.21, 57.66, 56.05, 55.89, 55.57, 55.48, 55.38, 55.28, 55.26, 55.05, 54.96, -1.03, -1.25

Example 14: *In vitro* assay to assess skipping efficiency of phosphorothioate (PS) oligonucleotides in mouse BMDMs.

- [0426] Freshly isolated mouse BMDM cells were cultured and maintained using appropriate media (Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum) plus recombinant murine CSF. The Assay was performed in 96 well plate format, seeding about 30,000 cells per well and treating with the ASO at a concentration of 1 μ M, 3 μ M, and 10 μ M without addition of lipofectamine. Cells were incubated at 37°C in a cell culture incubator for 48 hours before isolating the total RNA. Total RNA was isolated and converted to cDNA per vendor protocol, then Taqman gene expression assays were used to quantify Exon-2: skipped *CD33* (Forward primer: CGCTGCTGCTACTGCTG (SEQ ID NO:207); Reverse Primer: TTCTAGAGTGCCAGGGATGA (SEQ ID NO:208); and probe: TGTGGGCAGACTTGACCCACAG (SEQ ID NO:209)) and un-skipped *CD33* (Forward primer: GGATGGAGAGAGGAAGTA (SEQ ID NO:210); Reverse Primer: GTGCCAGGGATGAGGATTT (SEQ ID NO:211); and probe: TGCATGTGACAGACTTGACCCACA (SEQ ID NO:212)) mRNA transcripts. Mouse house-keeping gene HPRT1 (Assay ID: Hs02800695_m1; ThermoFisher Scientific) expressions was used to normalize the target transcript expressions. Non-targeting (NTC) MOE sequence CCTTCCTGAAGGTTCTCC (SEQ ID NO: 257) was used (Mullick et al. (2011) J. Lipid Res. 52, 885). The *in vitro* skipping data for select ASOs listed in Tables 13 and 14 is shown in Tables 16, 17, 18, 19, 20 and 21.

Table 16. *In vitro* % *CD33* Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for non-targeting control ASO (NTC) is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10 μ M	% D2 Skipping at 3 μ M	% D2 Skipping at 1 μ M
NTC	14.53	14.22	16.67
MOE-245	49.82	50.36	43.74
MOE-246	38.21	27.62	27.53
MOE-247	49.35	30.45	26.93
MOE-248	39.88	23.61	23.61
MOE-249	50.10	32.40	28.12
MOE-250	50.03	36.50	31.20
MOE-251	51.65	31.55	28.51
MOE-252	47.31	31.88	28.61
MOE-253	37.21	24.99	21.68
MOE-254	48.19	31.22	27.38
MOE-255	35.16	26.89	27.46

Table 17. *In vitro* % *CD33* Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for non-targeting control ASO (NTC) is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10μM	% D2 Skipping at 3μM	% D2 Skipping at 1μM
NTC	12.29	12.87	13.10
MOE-256	66.49	49.30	40.29
MOE-257	68.67	40.30	35.85
MOE-258	72.87	45.15	39.69
MOE-259	69.53	42.52	35.47
MOE-260	68.57	43.35	35.01
MOE-261	65.92	38.36	32.69

Table 18. *In vitro* % CD33 Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for non-targeting control ASO (NTC) is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10μM	% D2 Skipping at 3μM	% D2 Skipping at 1μM
NTC	14.36	14.07	15.28
MOE-262	68.80	41.56	34.94
MOE-263	61.75	42.95	39.23
MOE-264	64.99	42.68	38.54
MOE-265	66.12	40.89	35.98
MOE-266	68.07	42.00	38.88
MOE-267	63.18	42.88	37.10

- 5 **Table 19.** *In vitro* % CD33 Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for non-targeting control ASO (NTC) is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10μM	% D2 Skipping at 3μM	% D2 Skipping at 1μM
NTC	13.90	13.27	16.43
MOE-277	64.10	37.96	29.41
MOE-278	34.60	21.94	21.37
MOE-279	71.80	45.73	38.89
MOE-280	62.39	39.16	31.20
MOE-281	62.76	43.98	36.66
MOE-282	63.53	35.93	49.15
MOE-283	52.25	41.27	38.59
MOE-284	44.54	34.82	30.51
MOE-285	47.44	37.69	34.09
MOE-286	32.65	25.61	26.96

MOE-287	48.50	39.66	38.68
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Table 20. *In vitro* % *CD33* Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for blank PBS is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10μM	% D2 Skipping at 3μM	% D2 Skipping at 1μM
PBS	13.97	13.97	13.97
MOE-268	68.05	47.09	34.43
MOE-269	67.10	44.97	32.63
MOE-270	61.13	43.03	30.94
MOE-271	68.60	48.46	36.29
MOE-272	74.17	54.61	40.17
MOE-273	73.23	50.45	39.60
MOE-274	69.18	46.22	37.73
MOE-275	44.96	32.26	20.87
MOE-276	70.31	58.36	41.69

- 5 **Table 21.** *In vitro* % *CD33* Exon-2 skipping data for select ASOs in mouse BMDMs. Skipping for non-targeting control ASO (NTC) is shown as control for the individual experiment.

ASO number	% D2 Skipping at 10μM	% D2 Skipping at 3μM	% D2 Skipping at 1μM
NTC	10.16	8.49	8.63
MOE-288	78.23	60.11	36.87
MOE-289	50.20	32.50	24.91
MOE-290	68.08	43.32	35.52
MOE-291	73.67	52.99	37.79
MOE-292	71.01	55.63	40.32

- 10 [0427] Select ASO sequences were tested for their efficacy in inducing skipping of Exon-2 in a *CD33* gene transcript in U-118 MG glioblastoma cells *in vitro*. The experimental details are identical to those used in Example 4 (Evaluation of splice modulation properties of *CD33* Exon-2 targeting oligonucleotides/*In vitro* assay methods/Evaluation of MOE-ASO Sequences). In this experiment, MOE-ASOs were evaluated at 3.33nM, 10nM and 30nM concentrations. The data is shown in Table 22.

- 15 **Table 22.** *In vitro* % D2 skipping data for select ASOs in U118-MG cells Skipping for non-targeting control ASO (NTC) and water are shown as control.

ASO	% D2 skipping at 30nM	% D2 skipping at 10nM	% D2 skipping at 3.33nM
MOE-252	41.82	31.99	16.92
MOE-277	37.63	31.54	23.93
MOE-278	52.14	37.51	19.02
MOE-279	50.02	37.48	31.68
MOE-280	56.77	40.14	25.78
MOE-281	48.42	54.49	31.16
MOE-282	36.11	36.55	20.57
MOE-283	48.42	43.26	27.82
MOE-284	51.64	38.60	24.11
MOE-285	48.53	35.77	25.33
MOE-275	74.89	63.06	44.09
MOE-288	35.20	28.44	27.02
MOE-289	45.72	28.84	18.90
MOE-290	46.64	35.73	23.58
MOE-291	52.54	42.30	30.26
MOE-292	51.13	36.48	24.71
MOE-293	47.16	39.80	26.46
MOE-294	61.46	42.57	30.78
MOE-295	54.12	38.56	27.56
MOE-296	49.18	31.38	23.25
MOE-297	59.64	43.28	26.22
MOE-298	57.83	39.06	20.83
MOE-299	55.34	47.80	38.21
NTC	14.52	15.33	20.26
Water	17.84		

Example 15: *In vivo* assay methods.

- [0428] Humanized CD33 mouse models were used to study *CD33* Exon-2 skipping ASOs. CRISPR/Cas9 mediated gene editing was used to replace murine *CD33* with human genomic
- 5 *CD33*, including the signal peptide. Murine 3' and 5' untranslated regions were retained. For *in vivo* experiments, mixed gender cohorts of human CD33 mouse lines on a C57BL/6 background were used, mice were 12-24 weeks old at the time of dosing.
- [0429] ASOs were administered via intracerebroventricular injection at the appropriate dose into the right lateral ventricle in a 10 μ L bolus on day 1. Mice were necropsied 14 days after the

injection, unless noted otherwise. At necropsy, mice were transcardially perfused with PBS under avertin anesthesia. Brains were rapidly removed from the skull, and the cortex and hippocampus were dissected from the injected hemisphere for exon skipping evaluation. For RNA isolation, frozen tissue was added with 9X volume of Trizol and homogenized for 3 minutes. 500 μ L of the Trizol lysate was transferred to a 1 mL deep well plate. 100 μ L of chloroform was added to each sample, shaken vigorously, and centrifuged at 4000xg for 5 minutes. The supernatant (250 μ L) was transferred to the binding plate from SV96 total RNA extraction kit (Promega) and RNA was extracted per the same protocol. Total RNA was isolated and converted to cDNA per SV96 protocol (Promega), then Taqman gene expression assays were used to quantify Exon-2 skipped *CD33* mRNA transcripts. Mouse house-keeping gene HPRT1 expression was used to normalize the target transcript expressions. The data can be expressed as fold change of Exon-2 skipped *CD33* mRNA as compared with PBS treated group. Alternatively, the data can be expressed as the amount (%) of Exon-2 skipped *CD33* mRNA *in vivo* relative to PBS control. The *in vivo* skipping data for select sequences listed in Tables 13 and 14 is shown in Figs. 22-28. *In vivo* dose response for MOE-279 is shown in Fig. 29. Duration of effect of MOE-277 after a single ICV dose of 100 μ g is shown in Fig. 30.

Example 16: Hybridization ELISA for determining concentration of ASOs in brain tissues.

[0430] Concentrations of ASO was quantified in mouse cortex and hippocampus using a hybridization-based immunoassay method (HELISA). Two single-stranded DNA oligonucleotides with complementary sequences to **MOE-277** were designed as Detection probe: TCTTTCGGAT/3'-Bio (TCTTTCGGAT (SEQ ID NO:258)); and Capture probe: 5'-DigN/GGTTTCATACT (GGTTTCATACT (SEQ ID NO: 259))(Integrated DNA Technologies, Coralville, IA).

[0431] Tissues were lysed in TRIzol, 1:10 (Thermo Fisher Scientific, Waltham, MA), and were diluted in hybridization buffer (1:100, 1M NaCl in TE-Buffer and 0.1% Tween20). MOE-277 was spiked in diluted tissue homogenate to prepare standard curves and quality control (QC) samples. 35 μ L of diluted samples, standards and QCs were transferred to a 96-well PCR plate. 35 μ L of detection probe solution (100nM in hybridization buffer), was added to the PCR plate containing standards and samples. Sample and detection probe were hybridized on a thermal cycler under the following conditions: 95 $^{\circ}$ C for 10 minutes, 37 $^{\circ}$ C for 60 minutes, and a final hold at 4 $^{\circ}$ C.

[0432] MSD Gold 96-well Streptavidin SECTOR plate (Meso Scale Diagnostics, LLC., Rockville, MD) was blocked with 150 μ L of Casein in TBS blocker (Thermo Fisher Scientific, Waltham, MA) at room temperature for 1.5 hours. After washing, 25 μ L of capture probe (200nM in hybridization buffer), was added to the MSD plate and incubated at 37 $^{\circ}$ C, 300 rpm for 1 hour. After the wash step, 25 μ L of samples, standards and QCs were transferred to an MSD plate in duplicate, and were incubated at 37 $^{\circ}$ C for 1 hour on a shaking platform (300 rpm). The plate

was then washed 3 times and incubated for 1 hour with 50 μ L of 1 μ g/mL ruthenium labeled anti-digoxigenin antibody in Casein in TBS Blocking Buffer and 0.05% Tween20.

[0433] After the final wash, 150 μ L of 2X MSD Read Buffer T (Meso Scale Diagnostics, LLC., Rockville, MD) was added and the plate was read on an MSD Sector S 600 instrument (Meso Scale Diagnostics, LLC., Rockville, MD). A nonlinear regression analysis was performed to calculate the concentrations of reference compound from the signal intensities via interpolation from a calibration curve using 4-parameter logistic (4PL) model (weighting factor = 1/Y²) in Discovery Workbench 4.012.1 (Meso Scale Diagnostics, LLC., Rockville, MD). PK data analysis for duration of MOE-277 is shown in Fig. 31.

- 10 [0434] Those having ordinary skill in the art will appreciate that the disclosure can be modified in ways not specifically described herein. The disclosure is not to be limited in scope by the specific embodiments described herein, which are for illustrative purposes only. The disclosure includes any modifications and variations, including all functionally equivalent productions, compositions, and methods.
- 15 [0435] The entire disclosures of all publications cited herein are hereby incorporated by reference. No admission is made that any such publication constitutes prior art or is part of the common general knowledge of those having ordinary skill in the art.

What is claimed is:

1. An antisense oligonucleotide of 16-30 nucleotides in length, wherein the antisense oligonucleotide is complementary to a portion of SEQ ID NO:1, and wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a
- 5 Standard Exon-Skipping Efficiency Assay for the antisense oligonucleotide.
2. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 18-30 nucleotides in length.
3. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 21-30 nucleotides in length.
- 10 4. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 21-25 nucleotides in length.
5. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 18-21 nucleotides in length.
6. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 18-25
- 15 nucleotides in length.
7. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 25-30 nucleotides in length.
8. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 21 nucleotides in length.
- 20 9. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide is 25 nucleotides in length.
10. The antisense oligonucleotide of any of claims 1-9, wherein the antisense oligonucleotide is complementary to a portion of:
 - a. SEQ ID NO:213;
 - 25 b. SEQ ID NO:214;
 - c. SEQ ID NO:215;
 - d. SEQ ID NO:217;
 - e. SEQ ID NO:218;
 - f. SEQ ID NO:219; and/or
 - 30 g. SEQ ID NO:220.
11. The antisense oligonucleotide of any of claims 1-10, wherein the antisense oligonucleotide comprises a non-natural sugar moiety, a non-natural internucleotide linkage, or a non-natural sugar moiety and a non-natural internucleotide linkage.
12. The antisense oligonucleotide of claim 11, wherein the antisense oligonucleotide
- 35 comprises modified sugar moieties.
13. The antisense oligonucleotide of claim 12, wherein the modified sugar moieties comprise 2'-O-methoxyethyl ribose (2'-O-MOE).

14. The antisense oligonucleotide of claim 13, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs.
15. The antisense oligonucleotide of any of claims 10-14, wherein the antisense
5 oligonucleotide comprises non-natural internucleotide linkages.
16. The antisense oligonucleotide of claim 15, wherein the non-natural internucleotide linkages are stereopure.
17. The antisense oligonucleotide of claim 16, wherein the non-natural internucleotide linkages are all Sp.
- 10 18. The antisense oligonucleotide of claim 16, wherein the non-natural internucleotide linkages are all Rp.
19. The antisense oligonucleotide of claim 16, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
- 15 20. The antisense oligonucleotide of claim 15, wherein the non-natural internucleotide linkages are stereorandom.
21. The antisense oligonucleotide of claim 12, wherein the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs).
22. The antisense oligonucleotide of claim 21, wherein the antisense oligonucleotide has a
20 *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs.
23. The antisense oligonucleotide of claim 21 or 22, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.
24. The antisense oligonucleotide of claim 23, wherein the non-natural internucleotide
25 linkages are stereopure.
25. The antisense oligonucleotide of claim 24, wherein the non-natural internucleotide linkages are all Sp.
26. The antisense oligonucleotide of claim 24, wherein the non-natural internucleotide linkages are all Rp.
- 30 27. The antisense oligonucleotide of claim 24, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
28. The antisense oligonucleotide of claim 23, wherein the non-natural internucleotide linkages are stereorandom.
29. The antisense oligonucleotide of any of claims 1-28, wherein the antisense
35 oligonucleotide comprises modified nucleobases.
30. A composition comprising the antisense oligonucleotide of any of claims 1-29 and optionally a pharmaceutically acceptable carrier or excipient.
31. An antisense oligonucleotide comprising all or a portion of:

- a. PMO-002 (5'-CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:2);
- b. PMO-003 (5'-CCTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);
- c. PMO-036 (5'-TTGTAAGTGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
- d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
- 5 e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTACTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
- g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
- 10 j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- l. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- m. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- n. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- 15 o. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- p. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- q. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- r. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- s. MOE-136 (5'-AAAGAAGTATGAACCATAT-3') (SEQ ID NO:136);
- 20 t. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- u. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- v. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO:15);
- w. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- x. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- 25 y. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- z. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- aa. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).
32. An antisense oligonucleotide comprising all or a portion of:
- a. PMO-221 (5'- CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);
- 30 b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
- c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
- d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);
- e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
- f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
- 35 g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
- h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
- i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
- j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);

- k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
- l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
- m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
- n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:234);
- 5 o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
- p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
- q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
- r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
- s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
- 10 t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
- u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
- v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
- w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
- x. PMO-244 (5'- TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
- 15 y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
RRRRRRRRRRRRRRRRRRRRRR
- z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSSSS
- aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
- 20 Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRRRR; or
- bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSSSS.
33. An antisense oligonucleotide comprising all or a portion of:
- a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
- 25 b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
- c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
- d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);
- e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- 30 g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- 35 l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);

- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- 5 t. MOE-264 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- 10 y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- 15 dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID NO:012);
- gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- 20 SSSSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- RRRRRRRRRRRRRRRRRRRR;
- ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSSRSSSRSSSRSSSRSSS;
- 25 jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSSRSSSRSSSRSSSRSSS;
- kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSSRSRSRSRSRSRSRSSS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- 30 SSSSSRRRRRRRRSSSSSSS;
- mm. MOE-283 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSRRSRRRSRRRSRSSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSRRSRRRSRRRSRSSS;
- 35 oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSSSRRRRRRRRRRSSSSS;
- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
- SSRRRRRRRRRRRRRRSSS;

- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRSSSSSSSSRSRSSSSS;
- rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSSS;
- 5 ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRRRR;
- tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRRRRRRRRRRRSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
10 RRRRRRRRSSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSRRRRRRRR;
- ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSSRSSRSSRSSS;
- 15 xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSRSRSRSRSRSRSSS;
- yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SRSSSRSSSRSSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
20 SSSOSSRSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSOSRSRSRSSOSSS;
- bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSOSSS;
- 25 ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSSOSSRRSSSRSSOSSS;
- ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRORRRROSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SRRORRRROSSSSSSSSS;
- fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOOOSSSSSSSSSSSSS;
- ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
OOOOOSSSSSSSSSSSSS;
- 35 hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSSOSSOSSOSSS;
- iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSSOSSSSSOSSS;

- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSSOSSS;
- kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSOSSS;
- 5 III. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSOSSOSSSSOSSS;
- mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSSOSSS; or
- nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRRROSSSSSSSSOSSS.
- 10 34. An antisense oligonucleotide selected from the group consisting of:
- a. PMO-002 (5'-CCTCACCTGTACATGCACAGAGAG-3') (SEQ ID NO:2);
- b. PMO-003 (5'-CCTGTACATGCACAGAGAGCTGGG-3') (SEQ ID NO:3);
- c. PMO-036 (5'-TTGTAAGTATTTGGTACTTCCTC-3') (SEQ ID NO:36);
- 15 d. PMO-037 (5'-ACTGTATTTGGTACTTCCTCTCTCC-3') (SEQ ID NO:37);
- e. PMO-004 (5'-ATTTGGTACTTCCTCTCTCCATCCG-3') (SEQ ID NO:4);
- f. PMO-038 (5'-GTAAGTTCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:38);
- g. PMO-039 (5'-TCCTCTCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:39);
- h. PMO-005 (5'-TCTCCATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:5);
- 20 i. PMO-082 (5'-TAGTAGGGTATGGGATGGAAGAAAG-3') (SEQ ID NO:82);
- j. PMO-083 (5'-GGGTATGGGATGGAAGAAAGTGCAG-3') (SEQ ID NO:83);
- k. PMO-006 (5'-TGGGATGGAAGAAAGTGCAGGGCAC-3') (SEQ ID NO:6);
- l. MOE-009 (5'-CACATGCACAGAGAGCTGGG-3') (SEQ ID NO:9);
- m. MOE-128 (5'-GCACAGAGAGCTGGGGAGAT-3') (SEQ ID NO:128);
- 25 n. MOE-010 (5'-GAGAGCTGGGGAGATTTGTA-3') (SEQ ID NO:10);
- o. MOE-132 (5'-ACTGTATTTGGTACTTCCTC-3') (SEQ ID NO:132);
- p. MOE-135 (5'-TCCTCTCTCCATCCGAAAGA-3') (SEQ ID NO:135);
- q. MOE-011 (5'-TCTCCATCCGAAAGAAGTAT-3') (SEQ ID NO:11);
- r. MOE-012 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12);
- 30 s. MOE-136 (5'-AAAGAAGTATGAACCATTAT-3') (SEQ ID NO:136);
- t. MOE-013 (5'-ATGCTCAGGGAGCAGTTGTT-3') (SEQ ID NO:13);
- u. MOE-014 (5'-GAGTCTCCTCCTGTACTTCT-3') (SEQ ID NO:14);
- v. MOE-015 (5'-CGCACAAACCCTCCTGTACC-3') (SEQ ID NO:15);
- w. MOE-183 (5'-AAACCCTCCTGTACCGTCAC-3') (SEQ ID NO:183);
- 35 x. MOE-184 (5'-CTCCTGTACCGTCACTGACT-3') (SEQ ID NO:184);
- y. MOE-190 (5'-CAGCCAGAAATTTGGATCCA-3') (SEQ ID NO:190);
- z. MOE-196 (5'-CCCTGTGGGGAAACGAGGGT-3') (SEQ ID NO:196); or
- aa. MOE-197 (5'-TGGGGAAACGAGGGTCAGCT-3') (SEQ ID NO:197).

35. An antisense oligonucleotide selected from the group consisting of:
- a. PMO-221 (5'- CCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:221);
 - b. PMO-222 (5'- TCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:222);
 - c. PMO-223 (5'- CTCACCTGTCACATGCACAGAGA-3') (SEQ ID NO:223);
 - 5 d. PMO-224 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224);
 - e. PMO-225 (5'- ACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:225);
 - f. PMO-226 (5'- TCACCTGTCACATGCACAGAG-3') (SEQ ID NO:226);
 - g. PMO-227 (5'- TCACCTGTCACATGCACAGAGAGCT-3') (SEQ ID NO:227);
 - h. PMO-228 (5'- CCTGTGCCTCACCTGTCACATGCAC-3') (SEQ ID NO:228);
 - 10 i. PMO-229 (5'- GTGCCTCACCTGTCACATGCACAGA-3') (SEQ ID NO:229);
 - j. PMO-230 (5'- TGCCTCACCTGTCACATGCACAGAG-3') (SEQ ID NO:230);
 - k. PMO-231 (5'- CTCACCTGTCACATGCACAGAGAGC-3') (SEQ ID NO:231);
 - l. PMO-232 (5'- CACCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:232);
 - m. PMO-233 (5'- ACCTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:233);
 - 15 n. PMO-234 (5'- CTGTCACATGCACAGAGAGCTGGGG-3') (SEQ ID NO:234);
 - o. PMO-235 (5'- CCTGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:235);
 - p. PMO-236 (5'- TGTCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:236);
 - q. PMO-237 (5'- CTGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:237);
 - r. PMO-238 (5'- TGTCACATGCACAGAGAGCTGG-3') (SEQ ID NO:238);
 - 20 s. PMO-239 (5'- TCACATGCACAGAGAGCTGGG-3') (SEQ ID NO:239);
 - t. PMO-240 (5'- TGTCACATGCACAGAGAGCTG-3') (SEQ ID NO:240);
 - u. PMO-241 (5'- CTGTATTTGGTACTTCCTCTCTCCA-3') (SEQ ID NO:241);
 - v. PMO-242 (5'- TGTATTTGGTACTTCCTCTCTCCAT-3') (SEQ ID NO:242);
 - w. PMO-243 (5'- GTATTTGGTACTTCCTCTCTCCATC-3') (SEQ ID NO:243);
 - 25 x. PMO-244 (5'-TATTTGGTACTTCCTCTCTCCATCC-3') (SEQ ID NO:244);
 - y. PMO-324 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
RRRRRRRRRRRRRRRRRRRRRR;
 - z. PMO-424 (5'- CCTCACCTGTCACATGCACAG-3') (SEQ ID NO:224); Stereopattern:
SSSSSSSSSSSSSSSSSSSSSS;
 - 30 aa. PMO-402 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: RRRRRRRRRRRRRRRRRRRRRRRRRRRRR; or
 - bb. PMO-502 (5'- CCTCACCTGTCACATGCACAGAGAG-3') (SEQ ID NO:002);
Stereopattern: SSSSSSSSSSSSSSSSSSSSSSSSSSSSS.
36. An antisense oligonucleotide selected from the group consisting of:
- 35 a. MOE-245 (5'-CTCCATCCGAAAGAAGTATG-3') (SEQ ID NO:245);
 - b. MOE-246 (5'-TCCATCCGAAAGAAGTATGA-3') (SEQ ID NO:246);
 - c. MOE-247 (5'-CCATCCGAAAGAAGTATGAA-3') (SEQ ID NO:247);
 - d. MOE-248 (5'-CATCCGAAAGAAGTATGAAC-3') (SEQ ID NO:248);

- e. MOE-249 (5'-TCCGAAAGAAGTATGAACCA-3') (SEQ ID NO:249);
- f. MOE-250 (5'-CCGAAAGAAGTATGAACCAT-3') (SEQ ID NO:250);
- g. MOE-251 (5'-ATCCGAAAGAAGTATGAA-3') (SEQ ID NO:251);
- h. MOE-252 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252);
- 5 i. MOE-253 (5'-TCCGAAAGAAGTATGAAC-3') (SEQ ID NO:253);
- j. MOE-254 (5'-CCATCCGAAAGAAGTATG-3') (SEQ ID NO:254);
- k. MOE-255 (5'-TCCATCCGAAAGAAGTAT-3') (SEQ ID NO:255);
- l. MOE-256 (5'- GAAAGAAGTATGAACCAT-3') (SEQ ID NO:256);
- m. MOE-257 (5'- ATC-CGAAAGAAGTATGA-ACC-3') (SEQ ID NO:012);
- 10 n. MOE-258 (5'- ATCC-GAAAGAAGTATG-AACC-3') (SEQ ID NO:012);
- o. MOE-259 (5'- ATCCG-AAAGAAGTAT-GAACC-3') (SEQ ID NO:012);
- p. MOE-260 (5'- ATCCG-AAAGAAGTA-TGAACC-3') (SEQ ID NO:012);
- q. MOE-261 (5'- ATCC-GAAAGA-AGTATG-AACC-3') (SEQ ID NO:012);
- r. MOE-262 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- 15 s. MOE-263 (5'- ATCC-gAAAGAAGTATG-aACC-3') (SEQ ID NO:012);
- t. MOE-264 (5'- ATCC-gAAAGAAgTATG-aACC-3') (SEQ ID NO:012);
- u. MOE-265 (5'-CCGA-aAGAAGTATGAACC-3') (SEQ ID NO:252);
- v. MOE-266 (5'-CCGA-aAGAAGTATG-aACC-3') (SEQ ID NO:252);
- w. MOE-267 (5'-CCGA-aAGAAGtATG-aACC-3') (SEQ ID NO:252);
- 20 x. MOE-268 (5'-CCG-AAAGAAGTATGA-ACC-3') (SEQ ID NO:252);
- y. MOE-269 (5'-CCGA-AAGAAGTATG-AACC-3') (SEQ ID NO:252);
- z. MOE-270 (5'-CCGAA-AGAA-GTATG-AACC-3') (SEQ ID NO:252);
- aa. MOE-271 (5'-CCGAA-AGAAGTAT-GAACC-3') (SEQ ID NO:252);
- bb. MOE-272 (5'-CCG-A-AAGAAGTATGAACC-3') (SEQ ID NO:252);
- 25 cc. MOE-273 (5'-CCG-AA-AGAAGTATGAACC-3') (SEQ ID NO:252);
- dd. MOE-274 (5'-CCGAAAGAAGTATG-A-ACC-3') (SEQ ID NO:252);
- ee. MOE-275 (5'-mAmTfCfCfGfAfAfAfGfAfAfGfTfAfTfGfAfAmCmC-3') (SEQ ID NO:012);
- ff. MOE-276 (5'- fAfTfCfCfGmAmAmAmGmAmAmGmTmAfTfGfAfAfCfC-3') (SEQ ID NO:012);
- 30 gg. MOE-277 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSS;
- hh. MOE-278 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRR;
- ii. MOE-279 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;
- 35 jj. MOE-280 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS;

- kk. MOE-281 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSRSRSRSRSRSRSSSS;
- ll. MOE-282 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSSSSRRRRRRRSSSSSS;
- 5 mm. MOE-283 (5'- ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- nn. MOE-284 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRSRRRSRRRSRSSS;
- oo. MOE-285 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
10 SSSSRRRRRRRRRSSSSS;
- pp. MOE-286 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRRRRRRRRRRRRSSS;
- qq. MOE-287 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:012); Stereopattern:
SSRSSSSSSSRSSSSS;
- 15 rr. MOE-288 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSSSSSSSSSS;
- ss. MOE-289 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRRRRRRRRR;
- tt. MOE-290 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252; Stereopattern:
20 SSSRRRRRRRRRRSSS;
- uu. MOE-291 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
RRRRRRRRSSSSSSSSS;
- vv. MOE-292 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSSSSSSRRRRRRRR;
- 25 ww. MOE-293 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSSRSSRSSRSSS;
- xx. MOE-294 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSSRSRSRSRSRSSS;
- yy. MOE-295 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
30 SRSSSRSSSRSSRSSS;
- zz. MOE-296 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSSRSSSRSSOSSS;
- aaa. MOE-297 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SSOSRSRSRSSOSSS;
- 35 bbb. MOE-298 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern:
SOSSSRSSSRSSOSSS;
- ccc. MOE-299 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern:
SSOSSSRSSSRSSOSSS;

- ddd. MOE-300 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: RRRORRRROSSSSSSSSSS;
- eee. MOE-301 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SRRORRRROSSSSSSSSSS;
- 5 fff. MOE-303 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOOSSSSSSSSSSSSSSSS;
- ggg. MOE-304 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: OOOOSSSSSSSSSSSSSSSS;
- hhh. MOE-305 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSOSSOSSOSSSS;
- 10 iii. MOE-306 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SOSSSSOSSSSSSOSSSS;
- jjj. MOE-307 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSOSSSSSSSSSSOSSSS;
- 15 kkk. MOE-308 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSSSSSSSOSSSS;
- lll. MOE-309 (5'-ATCCGAAAGAAGTATGAACC-3') (SEQ ID NO:12); Stereopattern: SSSOSSSSOSSOSSOSSSS;
- mmm. MOE-310 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252); Stereopattern: SSORRRRRSSSSSSOSSSS; or
- 20 nnn. MOE-311 (5'-CCGAAAGAAGTATGAACC-3') (SEQ ID NO:252). Stereopattern: RRRROSSSSSSSSOSSSS.
37. The antisense oligonucleotide of claim 31 or 34, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises phosphorodiamidate morpholino oligomers or according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs when the antisense oligonucleotide comprises methoxyethyl ribose oligomers.
- 25 38. A composition comprising the antisense oligonucleotide of any of claims 31-37 and optionally a pharmaceutically acceptable carrier or excipient.
39. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that is complementary to a portion of SEQ ID NO:1, hybridizes to a target region of the *CD33* gene, and induces Exon-2 skipping during pre-mRNA splicing of the
- 35 *CD33* gene, and wherein the Exon-2 skipping efficiency of the antisense oligonucleotide is 30% or greater according to a Standard Exon-Skipping Efficiency Assay for ASOs.
40. The method of claim 39, wherein the antisense oligonucleotide is 18-30 nucleotides in length.

41. The method of claim 39, wherein the antisense oligonucleotide is 21-30 nucleotides in length.
42. The method of claim 39, wherein the antisense oligonucleotide is 21-25 nucleotides in length.
- 5 43. The method of claim 39, wherein the antisense oligonucleotide is 18-21 nucleotides in length.
44. The method of claim 39, wherein the antisense oligonucleotide is 18-25 nucleotides in length.
45. The method of claim 39, wherein the antisense oligonucleotide is 25-30 nucleotides in
10 length.
46. The method of claim 39, wherein the antisense oligonucleotide is 21 nucleotides in length.
47. The method of claim 39, wherein the antisense oligonucleotide is 25 nucleotides in length.
- 15 48. The method of any of claims 39-47, wherein the antisense oligonucleotide is complementary to a portion of:
- a. SEQ ID NO:213;
- b. SEQ ID NO:214;
- c. SEQ ID NO:215;
- 20 d. SEQ ID NO:217;
- e. SEQ ID NO:218;
- f. SEQ ID NO:219; and/or
- g. SEQ ID NO:220.
49. The method of any of claims 39-48, wherein the antisense oligonucleotide comprises a
25 non-natural sugar moiety, a non-natural internucleotide linkage, or a non-natural sugar moiety and a non-natural internucleotide linkage.
50. The method of claim 49, wherein the antisense oligonucleotide comprises modified sugar moieties.
51. The method of claim 50, wherein the modified sugar moieties comprise 2'-O-methoxyethyl ribose (2'-O-MOE).
30
52. The method of claim 51, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs.
53. The method of any of claims 49-52, wherein the antisense oligonucleotide comprises
35 non-natural internucleotide linkages.
54. The method of claim 53, wherein the non-natural internucleotide linkages are stereopure.
55. The method of claim 54, wherein the non-natural internucleotide linkages are all Sp.

56. The method of claim 54, wherein the non-natural internucleotide linkages are all Rp.
57. The method of claim 54, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
58. The method of claim 53, wherein the non-natural internucleotide linkages are stereorandom.
59. The method of claim 50, wherein the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs).
60. The method of claim 59, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs.
61. The method of claim 59 or 60, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.
62. The method of claim 61, wherein the non-natural internucleotide linkages are stereopure.
63. The method of claim 62, wherein the non-natural internucleotide linkages are all Sp.
64. The method of claim 62, wherein the non-natural internucleotide linkages are all Rp.
65. The method of claim 62, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
66. The method of claim 61, wherein the non-natural internucleotide linkages are stereorandom.
67. The method of any of claims 39-66, wherein the antisense oligonucleotide comprises modified nucleobases.
68. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: PMO-002 (SEQ ID NO:2); PMO-003 (SEQ ID NO:3); PMO-036 (SEQ ID NO:36); PMO-037 (SEQ ID NO:37); PMO-004 (SEQ ID NO:4); PMO-038 (SEQ ID NO:38); PMO-039 (SEQ ID NO:39); PMO-005 (SEQ ID NO:5); PMO-082 (SEQ ID NO:82); PMO-083 (SEQ ID NO:83); PMO-006 (SEQ ID NO:6); PMO-096 (SEQ ID NO:96); PMO-007 (SEQ ID NO:7); PMO-097 (SEQ ID NO:97); PMO-008 (SEQ ID NO:8); MOE-009 (SEQ ID NO:9); MOE-128 (SEQ ID NO:128); MOE-010 (SEQ ID NO:10); MOE-132 (SEQ ID NO:132); MOE-135 (SEQ ID NO:135); MOE-011 (SEQ ID NO: 11); MOE-012 (SEQ ID NO:12); MOE-136 (SEQ ID NO:136); MOE-013 (SEQ ID NO:13); MOE-014 (SEQ ID NO:14); MOE-015 (SEQ ID NO:15); MOE-183 (SEQ ID NO:183); MOE-184 (SEQ ID NO:184); MOE-190 (SEQ ID NO:190); MOE-196 (SEQ ID NO:196); or MOE-197 (SEQ ID NO:197).
69. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: PMO-221 (SEQ ID NO:221); PMO-222 (SEQ ID NO:222); PMO-223 (SEQ ID NO:223); PMO-224 (SEQ ID NO:224); PMO-

- 225 (SEQ ID NO:225); PMO-226 (SEQ ID NO:226); PMO-227 (SEQ ID NO:227); PMO-228 (SEQ ID NO:228); PMO-229 (SEQ ID NO:229); PMO-230 (SEQ ID NO:230); PMO-231 (SEQ ID NO:231); PMO-232 (SEQ ID NO:232); PMO-233 (SEQ ID NO:233); PMO-234 (SEQ ID NO:234); PMO-235 (SEQ ID NO:235); PMO-236 (SEQ ID NO:236); PMO-237 (SEQ ID NO:237); PMO-238 (SEQ ID NO:238); PMO-239 (SEQ ID NO:239); PMO-240 (SEQ ID NO:240); PMO-241 (SEQ ID NO:241); PMO-242 (SEQ ID NO:242); PMO-243 (SEQ ID NO:243); PMO-244 (SEQ ID NO:244); PMO-324 (SEQ ID NO:224); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; PMO-424 (SEQ ID NO:224); Stereopattern: SSSSSSSSSSSSSSSSSSSSS; PMO-402 (SEQ ID NO:002); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; or PMO-502 (SEQ ID NO:002); Stereopattern: SSSSSSSSSSSSSSSSSSSSS.
70. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: MOE-245 (SEQ ID NO:245); MOE-246 (SEQ ID NO:246); MOE-247 (SEQ ID NO:247); MOE-248 (SEQ ID NO:248); MOE-249 (SEQ ID NO:249); MOE-250 (SEQ ID NO:250); MOE-251 (SEQ ID NO:251); MOE-252 (SEQ ID NO:252); MOE-253 (SEQ ID NO:253); MOE-254 (SEQ ID NO:254); MOE-255 (SEQ ID NO:255); MOE-256 (SEQ ID NO:256); MOE-257 (SEQ ID NO:012); MOE-258 (SEQ ID NO:012); MOE-259 (SEQ ID NO:012); MOE-260 (SEQ ID NO:012); MOE-261 (SEQ ID NO:012); MOE-262 (SEQ ID NO:012); MOE-263 (SEQ ID NO:012); MOE-264 (SEQ ID NO:012); MOE-265 (SEQ ID NO:252); MOE-266 (SEQ ID NO:252); MOE-267 (SEQ ID NO:252); MOE-268 (SEQ ID NO:252); MOE-269 (SEQ ID NO:252); MOE-270 (SEQ ID NO:252); MOE-271 (SEQ ID NO:252); MOE-272 (SEQ ID NO:252); MOE-273 (SEQ ID NO:252); MOE-274 (SEQ ID NO:252); MOE-275 (SEQ ID NO:012); MOE-276 (SEQ ID NO:012); MOE-277 (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSSSS; MOE-278 (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; MOE-279 (SEQ ID NO:012); Stereopattern: SSSRSSRSSRSSRSSRSS; MOE-280 (SEQ ID NO:012); Stereopattern: SSSRSSRSSRSSRSSRSS; MOE-281 (SEQ ID NO:012); Stereopattern: SSSRSSRSSRSSRSSRSS; MOE-282 (SEQ ID NO:012); Stereopattern: SSSSSRRRRRRRRSSSSSS; MOE-283 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRSS; MOE-284 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRSS; MOE-285 (SEQ ID NO:012); Stereopattern: SSSSSRRRRRRRRRRSSSS; MOE-286 (SEQ ID NO:012); Stereopattern: SSSRRRRRRRRRRRRRRSS; MOE-287 (SEQ ID NO:012); Stereopattern: SSRSSSSSSSSSRSSSSSS; MOE-288 (SEQ ID NO:252); Stereopattern: SSSSSSSSSSSSSSSSSSS; MOE-289 (SEQ ID NO:252); Stereopattern: RRRRRRRRRRRRRRRRRRR; MOE-290 (SEQ ID NO:252); Stereopattern: SSSRRRRRRRRRRRRSS; MOE-291 (SEQ ID NO:252); Stereopattern:

RRRRRRRRRSSSSSSSSSS; MOE-292 (SEQ ID NO:252); Stereopattern:
 SSSSSSSSSRRRRRRRRR; MOE-293 (SEQ ID NO:252); Stereopattern:
 SSSRSSRSSRRSSRSS; MOE-294 (SEQ ID NO:252); Stereopattern:
 SSSRSRSRSRSRSS; MOE-295 (SEQ ID NO:252); Stereopattern:
 5 SRSSSRSSSRSSSRSS; MOE-296 (SEQ ID NO:252); Stereopattern:
 SSSOSSRSSRRSSOSS; MOE-297 (SEQ ID NO:252); Stereopattern:
 SSSOSRSRSRSSOSS; MOE-298 (SEQ ID NO:252); Stereopattern:
 SOSSSRSSRRSSOSS; MOE-299 (SEQ ID NO:12); Stereopattern:
 SSSOSSRRSSRRSSOSS; MOE-300 (SEQ ID NO:252); Stereopattern:
 10 RRRORRRROSSSSSSSSSS; MOE-301 (SEQ ID NO:252); Stereopattern:
 SRRORRRROSSSSSSSSSS; MOE-303 (SEQ ID NO:252); Stereopattern:
 SSOOSSSSSSSSSSSSSS; MOE-304 (SEQ ID NO:252); Stereopattern:
 OOOOSSSSSSSSSSSSSS; MOE-305 (SEQ ID NO:252); Stereopattern:
 SSOSSSOSSOSSOSS; MOE-306 (SEQ ID NO:252); Stereopattern:
 15 SOSSSSOSSSSSSOSS; MOE-307 (SEQ ID NO:252); Stereopattern:
 SSOSSSSSSSSSSOSS; MOE-308 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSSSSSSSOSS; MOE-309 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSOSSOSSOSS; MOE-310 (SEQ ID NO:252); Stereopattern:
 SSORRRRRSSSSOSS; or MOE-311 (SEQ ID NO:252). Stereopattern:
 20 RRRRRROSSSSSSOSS.

71. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the group consisting of: PMO-002 (SEQ ID NO:2); PMO-003 (SEQ ID NO:3); PMO-036 (SEQ ID NO:36); PMO-037 (SEQ ID NO:37); PMO-
 25 004 (SEQ ID NO:4); PMO-038 (SEQ ID NO:38); PMO-039 (SEQ ID NO:39); PMO-005 (SEQ ID NO:5); PMO-082 (SEQ ID NO:82); PMO-083 (SEQ ID NO:83); PMO-006 (SEQ ID NO:6); PMO-096 (SEQ ID NO:96); PMO-007 (SEQ ID NO:7); PMO-097 (SEQ ID NO:97); PMO-008 (SEQ ID NO:8); MOE-009 (SEQ ID NO:9); MOE-128 (SEQ ID NO:128); MOE-010 (SEQ ID NO:10); MOE-132 (SEQ ID NO:132); MOE-135 (SEQ ID NO:135); MOE-011 (SEQ ID NO: 11); MOE-
 30 012 (SEQ ID NO:12); MOE-136 (SEQ ID NO:136); MOE-013 (SEQ ID NO:13); MOE-014 (SEQ ID NO:14); MOE-015 (SEQ ID NO:15); MOE-183 (SEQ ID NO:183); MOE-184 (SEQ ID NO:184); MOE-190 (SEQ ID NO:190); MOE-196 (SEQ ID NO:196); or MOE-197 (SEQ ID NO:197).

72. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the group consisting of: PMO-221 (SEQ ID NO:221); PMO-222 (SEQ ID NO:222); PMO-223 (SEQ ID NO:223); PMO-224 (SEQ ID NO:224); PMO-225 (SEQ ID NO:225); PMO-226 (SEQ ID NO:226); PMO-227 (SEQ ID

NO:227); PMO-228 (SEQ ID NO:228); PMO-229 (SEQ ID NO:229); PMO-230 (SEQ ID NO:230); PMO-231 (SEQ ID NO:231); PMO-232 (SEQ ID NO:232); PMO-233 (SEQ ID NO:233); PMO-234 (SEQ ID NO:234); PMO-235 (SEQ ID NO:235); PMO-236 (SEQ ID NO:236); PMO-237 (SEQ ID NO:237); PMO-238 (SEQ ID NO:238); PMO-239 (SEQ ID NO:239); PMO-240 (SEQ ID NO:240); PMO-241 (SEQ ID NO:241); PMO-242 (SEQ ID NO:242); PMO-243 (SEQ ID NO:243); PMO-244 (SEQ ID NO:244); PMO-324 (SEQ ID NO:224); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; PMO-424 (SEQ ID NO:224); Stereopattern: SSSSSSSSSSSSSSSSSSSSS; PMO-402 (SEQ ID NO:002); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; or PMO-502 (SEQ ID NO:002); Stereopattern: SSSSSSSSSSSSSSSSSSSSS.

73. A method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing a nucleic acid molecule into a cell, wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the group consisting of: MOE-245 (SEQ ID NO:245); MOE-246 (SEQ ID NO:246); MOE-247 (SEQ ID NO:247); MOE-248 (SEQ ID NO:248); MOE-249 (SEQ ID NO:249); MOE-250 (SEQ ID NO:250); MOE-251 (SEQ ID NO:251); MOE-252 (SEQ ID NO:252); MOE-253 (SEQ ID NO:253); MOE-254 (SEQ ID NO:254); MOE-255 (SEQ ID NO:255); MOE-256 (SEQ ID NO:256); MOE-257 (SEQ ID NO:012); MOE-258 (SEQ ID NO:012); MOE-259 (SEQ ID NO:012); MOE-260 (SEQ ID NO:012); MOE-261 (SEQ ID NO:012); MOE-262 (SEQ ID NO:012); MOE-263 (SEQ ID NO:012); MOE-264 (SEQ ID NO:012); MOE-265 (SEQ ID NO:252); MOE-266 (SEQ ID NO:252); MOE-267 (SEQ ID NO:252); MOE-268 (SEQ ID NO:252); MOE-269 (SEQ ID NO:252); MOE-270 (SEQ ID NO:252); MOE-271 (SEQ ID NO:252); MOE-272 (SEQ ID NO:252); MOE-273 (SEQ ID NO:252); MOE-274 (SEQ ID NO:252); MOE-275 (SEQ ID NO:012); MOE-276 (SEQ ID NO:012); MOE-277 (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSSSS; MOE-278 (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRRRR; MOE-279 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-280 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-281 (SEQ ID NO:012); Stereopattern: SSSRSRSRSRSRSRSRSRS; MOE-282 (SEQ ID NO:012); Stereopattern: SSSSSRRRRRRRRSSSSSS; MOE-283 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRSSS; MOE-284 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRSSS; MOE-285 (SEQ ID NO:012); Stereopattern: SSSSSRRRRRRRRRRSSSSS; MOE-286 (SEQ ID NO:012); Stereopattern: SSSRRRRRRRRRRRRRRSS; MOE-287 (SEQ ID NO:012); Stereopattern: SSRSSSSSSSSSRSSSSSSS; MOE-288 (SEQ ID NO:252); Stereopattern: SSSSSSSSSSSSSSSSSSSS; MOE-289 (SEQ ID NO:252); Stereopattern: RRRRRRRRRRRRRRRRRRR; MOE-290 (SEQ ID NO:252); Stereopattern: SSSRRRRRRRRRRRRSSS; MOE-291 (SEQ ID NO:252); Stereopattern:

- RRRRRRRRSSSSSSSSSS; MOE-292 (SEQ ID NO:252); Stereopattern:
 SSSSSSSSSRRRRRRRRR; MOE-293 (SEQ ID NO:252); Stereopattern:
 SSSRSSRSSRSSRSS; MOE-294 (SEQ ID NO:252); Stereopattern:
 SSSRSRSRSRSRSS; MOE-295 (SEQ ID NO:252); Stereopattern:
 5 SRSSSRSSSRSSRSS; MOE-296 (SEQ ID NO:252); Stereopattern:
 SSSOSSRSSRSSOSS; MOE-297 (SEQ ID NO:252); Stereopattern:
 SSSOSRSRSRSSOSS; MOE-298 (SEQ ID NO:252); Stereopattern:
 SOSSSRSSRSSOSS; MOE-299 (SEQ ID NO:12); Stereopattern:
 SSSOSSRSSRSSOSS; MOE-300 (SEQ ID NO:252); Stereopattern:
 10 RRRORRRROSSSSSSSSSS; MOE-301 (SEQ ID NO:252); Stereopattern:
 SRRORRRROSSSSSSSSSS; MOE-303 (SEQ ID NO:252); Stereopattern:
 SSOOSSSSSSSSSSSSSS; MOE-304 (SEQ ID NO:252); Stereopattern:
 OOOOSSSSSSSSSSSSSS; MOE-305 (SEQ ID NO:252); Stereopattern:
 SSOSSOSSOSSOSSOSS; MOE-306 (SEQ ID NO:252); Stereopattern:
 15 SOSSSSOSSSSSSOSSSS; MOE-307 (SEQ ID NO:252); Stereopattern:
 SSOSSSSSSSSSSOSSSS; MOE-308 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSSSSSSSOSSSS; MOE-309 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSOSSOSSOSSSS; MOE-310 (SEQ ID NO:252); Stereopattern:
 SSORRRRRSSSSSSOSSSS; or MOE-311 (SEQ ID NO:252). Stereopattern:
 20 RRRRRROSSSSSSSSOSSS.
74. The method of claim 68 or 71, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises phosphorodiamidate morpholino oligomers or according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs when the
 25 antisense oligonucleotide comprises methoxyethyl ribose oligomers.
75. The method of any of claims 39-74, wherein the cell is an animal cell.
76. The method of claim 75, wherein the cell is a human cell.
77. The method of any of claims 36-76, wherein the method is performed *in vitro*.
78. The method of any of claims 36-76, wherein the method is performed *in vivo*.
- 30 79. A method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 1.
80. The method of claim 79, wherein the antisense oligonucleotide is 18-30 nucleotides in length.
- 35 81. The method of claim 79, wherein the antisense oligonucleotide is 21-30 nucleotides in length.
82. The method of claim 79, wherein the antisense oligonucleotide is 21-25 nucleotides in length.

83. The method of claim 79, wherein the antisense oligonucleotide is 18-21 nucleotides in length.
84. The method of claim 79, wherein the antisense oligonucleotide is 18-25 nucleotides in length.
- 5 85. The method of claim 79, wherein the antisense oligonucleotide is 25-30 nucleotides in length.
86. The method of claim 79, wherein the antisense oligonucleotide is 21 nucleotides in length.
87. The method of claim 79, wherein the antisense oligonucleotide is 25 nucleotides in length.
- 10 88. The method of any of claims 79-87, wherein the antisense oligonucleotide is complementary to a portion of:
- a. SEQ ID NO:213;
 - b. SEQ ID NO:214;
 - 15 c. SEQ ID NO:215;
 - d. SEQ ID NO:217;
 - e. SEQ ID NO:218;
 - f. SEQ ID NO:219; and/or
 - g. SEQ ID NO:220.
- 20 89. The method of any of claims 79-88, wherein the antisense oligonucleotide comprises a non-natural sugar moiety, a non-natural internucleotide linkage, or a non-natural sugar moiety and a non-natural internucleotide linkage.
90. The method of claim 89, wherein the antisense oligonucleotide comprises modified sugar moieties.
- 25 91. The method of claim 90, wherein the modified sugar moieties comprise 2'-O-methoxyethyl ribose (2'-O-MOE).
92. The method of claim 91, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs.
- 30 93. The method of any of claims 89-92, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.
94. The method of claim 93, wherein the non-natural internucleotide linkages are stereopure.
95. The method of claim 94, wherein the non-natural internucleotide linkages are all Sp.
- 35 96. The method of claim 94, wherein the non-natural internucleotide linkages are all Rp.
97. The method of claim 94, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.

98. The method of claim 93, wherein the non-natural internucleotide linkages are stereorandom.
99. The method of claim 890, wherein the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs).
- 5 100. The method of claim 999, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs.
101. The method of claim 99 or 9100, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.
- 10 102. The method of claim 9101, wherein the non-natural internucleotide linkages are stereopure.
103. The method of claim 102, wherein the non-natural internucleotide linkages are all Sp.
104. The method of claim 102, wherein the non-natural internucleotide linkages are all Rp.
105. The method of claim 102, wherein the non-natural internucleotide linkages are
- 15 independently selected from Sp and Rp.
106. The method of claim 101, wherein the non-natural internucleotide linkages are stereorandom.
107. The method of any of claims 79-106, wherein the antisense oligonucleotide comprises modified nucleobases.
- 20 108. A method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide, wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: PMO-002 (SEQ ID NO:2); PMO-003 (SEQ ID NO:3); PMO-036 (SEQ ID NO:36); PMO-037 (SEQ ID NO:37); PMO-004 (SEQ ID NO:4); PMO-038 (SEQ ID NO:38); PMO-039 (SEQ ID
- 25 NO:39); PMO-005 (SEQ ID NO:5); PMO-082 (SEQ ID NO:82); PMO-083 (SEQ ID NO:83); PMO-006 (SEQ ID NO:6); PMO-096 (SEQ ID NO:96); PMO-007 (SEQ ID NO:7); PMO-097 (SEQ ID NO:97); PMO-008 (SEQ ID NO:8); MOE-009 (SEQ ID NO:9); MOE-128 (SEQ ID NO:128); MOE-010 (SEQ ID NO:10); MOE-132 (SEQ ID NO:132); MOE-135 (SEQ ID NO:135); MOE-011 (SEQ ID NO: 11); MOE-012 (SEQ ID NO:12); MOE-136 (SEQ ID NO:136); MOE-013
- 30 (SEQ ID NO:13); MOE-014 (SEQ ID NO:14); MOE-015 (SEQ ID NO:15); MOE-183 (SEQ ID NO:183); MOE-184 (SEQ ID NO:184); MOE-190 (SEQ ID NO:190); MOE-196 (SEQ ID NO:196); or MOE-197 (SEQ ID NO:197).
109. A method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide,
- 35 wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: PMO-221 (SEQ ID NO:221); PMO-222 (SEQ ID NO:222); PMO-223 (SEQ ID NO:223); PMO-224 (SEQ ID NO:224); PMO-225 (SEQ ID NO:225); PMO-226 (SEQ ID NO:226); PMO-227 (SEQ ID NO:227); PMO-228 (SEQ ID NO:228); PMO-229 (SEQ ID NO:229); PMO-230

- (SEQ ID NO:230); PMO-231 (SEQ ID NO:231); PMO-232 (SEQ ID NO:232); PMO-233 (SEQ ID NO:233); PMO-234 (SEQ ID NO:234); PMO-235 (SEQ ID NO:235); PMO-236 (SEQ ID NO:236); PMO-237 (SEQ ID NO:237); PMO-238 (SEQ ID NO:238); PMO-239 (SEQ ID NO:239); PMO-240 (SEQ ID NO:240); PMO-241 (SEQ ID NO:241); PMO-242 (SEQ ID NO:242); PMO-243 (SEQ ID NO:243); PMO-244 (SEQ ID NO:244); PMO-324 (SEQ ID NO:224); Stereopattern: RRRRRRRRRRRRRRRRRRRR; PMO-424 (SEQ ID NO:224); Stereopattern: SSSSSSSSSSSSSSSSSSSS; PMO-402 (SEQ ID NO:002); Stereopattern: RRRRRRRRRRRRRRRRRRRR; or PMO-502 (SEQ ID NO:002); Stereopattern: SSSSSSSSSSSSSSSSSSSS.
110. A method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide, wherein the nucleic acid molecule is an antisense oligonucleotide that comprises all or a portion of: MOE-245 (SEQ ID NO:245); MOE-246 (SEQ ID NO:246); MOE-247 (SEQ ID NO:247); MOE-248 (SEQ ID NO:248); MOE-249 (SEQ ID NO:249); MOE-250 (SEQ ID NO:250); MOE-251 (SEQ ID NO:251); MOE-252 (SEQ ID NO:252); MOE-253 (SEQ ID NO:253); MOE-254 (SEQ ID NO:254); MOE-255 (SEQ ID NO:255); MOE-256 (SEQ ID NO:256); MOE-257 (SEQ ID NO:012); MOE-258 (SEQ ID NO:012); MOE-259 (SEQ ID NO:012); MOE-260 (SEQ ID NO:012); MOE-261 (SEQ ID NO:012); MOE-262 (SEQ ID NO:012); MOE-263 (SEQ ID NO:012); MOE-264 (SEQ ID NO:012); MOE-265 (SEQ ID NO:252); MOE-266 (SEQ ID NO:252); MOE-267 (SEQ ID NO:252); MOE-268 (SEQ ID NO:252); MOE-269 (SEQ ID NO:252); MOE-270 (SEQ ID NO:252); MOE-271 (SEQ ID NO:252); MOE-272 (SEQ ID NO:252); MOE-273 (SEQ ID NO:252); MOE-274 (SEQ ID NO:252); MOE-275 (SEQ ID NO:012); MOE-276 (SEQ ID NO:012); MOE-277 (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSSS; MOE-278 (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRRR; MOE-279 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-280 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-281 (SEQ ID NO:012); Stereopattern: SSSRSRSRSRSRSRSRSRS; MOE-282 (SEQ ID NO:012); Stereopattern: SSSSSSRSSSRSSSRSSS; MOE-283 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRS; MOE-284 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRS; MOE-285 (SEQ ID NO:012); Stereopattern: SSSSSRRSSSRSSSRSSS; MOE-286 (SEQ ID NO:012); Stereopattern: SSSRRRRRRRRRRRRRRSS; MOE-287 (SEQ ID NO:012); Stereopattern: SSRSSSSSSSSSRSSSSS; MOE-288 (SEQ ID NO:252); Stereopattern: SSSSSSSSSSSSSSSSSS; MOE-289 (SEQ ID NO:252); Stereopattern: RRRRRRRRRRRRRRRRRR; MOE-290 (SEQ ID NO:252); Stereopattern: SSSRRRRRRRRRRRRSSS; MOE-291 (SEQ ID NO:252); Stereopattern: RRRRRRRRRSSSSSSSSS; MOE-292 (SEQ ID NO:252); Stereopattern:

- SSSSSSSSRRRRRRRR; MOE-293 (SEQ ID NO:252); Stereopattern:
 SSSRRSSRRSSRRSS; MOE-294 (SEQ ID NO:252); Stereopattern:
 SSSRSRSRSRSRSRSS; MOE-295 (SEQ ID NO:252); Stereopattern:
 SRSSRRSSRRSSRRSS; MOE-296 (SEQ ID NO:252); Stereopattern:
 5 SSSOSSRRSSRRSSOSS; MOE-297 (SEQ ID NO:252); Stereopattern:
 SSSOSRSRSRRSSOSS; MOE-298 (SEQ ID NO:252); Stereopattern:
 SOSSRRSSRRSSOSS; MOE-299 (SEQ ID NO:12); Stereopattern:
 SSSOSSRRSSRRSSOSS; MOE-300 (SEQ ID NO:252); Stereopattern:
 RRRORRRROSSSSSSSS; MOE-301 (SEQ ID NO:252); Stereopattern:
 10 SRRORRRROSSSSSSSS; MOE-303 (SEQ ID NO:252); Stereopattern:
 SSOOSSSSSSSSSSSS; MOE-304 (SEQ ID NO:252); Stereopattern:
 OOOOSSSSSSSSSSSS; MOE-305 (SEQ ID NO:252); Stereopattern:
 SSOSSOSSOSSOSS; MOE-306 (SEQ ID NO:252); Stereopattern:
 SOSSSSOSSSSSSOSS; MOE-307 (SEQ ID NO:252); Stereopattern:
 15 SSOSSSSSSSSSSOSS; MOE-308 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSSSSSSSOSS; MOE-309 (SEQ ID NO:12); Stereopattern:
 SSSOSSOSSOSSOSSOSS; MOE-310 (SEQ ID NO:252); Stereopattern:
 SSORRRRRSSSSOSS; or MOE-311 (SEQ ID NO:252). Stereopattern:
 RRRRRROSSSSSSOSS.
- 20 111. A method of treating a subject having a neurodegenerative disease comprising
 administering to said subject a therapeutically effective amount of the antisense oligonucleotide,
 wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the
 group consisting of: PMO-002 (SEQ ID NO:2); PMO-003 (SEQ ID NO:3); PMO-036 (SEQ ID
 NO:36); PMO-037 (SEQ ID NO:37); PMO-004 (SEQ ID NO:4); PMO-038 (SEQ ID NO:38);
 25 PMO-039 (SEQ ID NO:39); PMO-005 (SEQ ID NO:5); PMO-082 (SEQ ID NO:82); PMO-083
 (SEQ ID NO:83); PMO-006 (SEQ ID NO:6); PMO-096 (SEQ ID NO:96); PMO-007 (SEQ ID
 NO:7); PMO-097 (SEQ ID NO:97); PMO-008 (SEQ ID NO:8); MOE-009 (SEQ ID NO:9); MOE-
 128 (SEQ ID NO:128); MOE-010 (SEQ ID NO:10); MOE-132 (SEQ ID NO:132); MOE-135 (SEQ
 ID NO:135); MOE-011 (SEQ ID NO: 11); MOE-012 (SEQ ID NO:12); MOE-136 (SEQ ID
 30 NO:136); MOE-013 (SEQ ID NO:13); MOE-014 (SEQ ID NO:14); MOE-015 (SEQ ID NO:15);
 MOE-183 (SEQ ID NO:183); MOE-184 (SEQ ID NO:184); MOE-190 (SEQ ID NO:190); MOE-
 196 (SEQ ID NO:196); or MOE-197 (SEQ ID NO:197).
112. A method of treating a subject having a neurodegenerative disease comprising
 administering to said subject a therapeutically effective amount of the antisense oligonucleotide,
 35 wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the
 group consisting of: PMO-221 (SEQ ID NO:221); PMO-222 (SEQ ID NO:222); PMO-223 (SEQ
 ID NO:223); PMO-224 (SEQ ID NO:224); PMO-225 (SEQ ID NO:225); PMO-226 (SEQ ID
 NO:226); PMO-227 (SEQ ID NO:227); PMO-228 (SEQ ID NO:228); PMO-229 (SEQ ID

- NO:229); PMO-230 (SEQ ID NO:230); PMO-231 (SEQ ID NO:231); PMO-232 (SEQ ID NO:232); PMO-233 (SEQ ID NO:233); PMO-234 (SEQ ID NO:234); PMO-235 (SEQ ID NO:235); PMO-236 (SEQ ID NO:236); PMO-237 (SEQ ID NO:237); PMO-238 (SEQ ID NO:238); PMO-239 (SEQ ID NO:239); PMO-240 (SEQ ID NO:240); PMO-241 (SEQ ID NO:241); PMO-242 (SEQ ID NO:242); PMO-243 (SEQ ID NO:243); PMO-244 (SEQ ID NO:244); PMO-324 (SEQ ID NO:224); Stereopattern: RRRRRRRRRRRRRRRRRRRR; PMO-424 (SEQ ID NO:224); Stereopattern: SSSSSSSSSSSSSSSSSSSS; PMO-402 (SEQ ID NO:002); Stereopattern: RRRRRRRRRRRRRRRRRRRR; or PMO-502 (SEQ ID NO:002); Stereopattern: SSSSSSSSSSSSSSSSSSSS.
113. A method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide, wherein the nucleic acid molecule is an antisense oligonucleotide that is selected from the group consisting of: MOE-245 (SEQ ID NO:245); MOE-246 (SEQ ID NO:246); MOE-247 (SEQ ID NO:247); MOE-248 (SEQ ID NO:248); MOE-249 (SEQ ID NO:249); MOE-250 (SEQ ID NO:250); MOE-251 (SEQ ID NO:251); MOE-252 (SEQ ID NO:252); MOE-253 (SEQ ID NO:253); MOE-254 (SEQ ID NO:254); MOE-255 (SEQ ID NO:255); MOE-256 (SEQ ID NO:256); MOE-257 (SEQ ID NO:012); MOE-258 (SEQ ID NO:012); MOE-259 (SEQ ID NO:012); MOE-260 (SEQ ID NO:012); MOE-261 (SEQ ID NO:012); MOE-262 (SEQ ID NO:012); MOE-263 (SEQ ID NO:012); MOE-264 (SEQ ID NO:012); MOE-265 (SEQ ID NO:252); MOE-266 (SEQ ID NO:252); MOE-267 (SEQ ID NO:252); MOE-268 (SEQ ID NO:252); MOE-269 (SEQ ID NO:252); MOE-270 (SEQ ID NO:252); MOE-271 (SEQ ID NO:252); MOE-272 (SEQ ID NO:252); MOE-273 (SEQ ID NO:252); MOE-274 (SEQ ID NO:252); MOE-275 (SEQ ID NO:012); MOE-276 (SEQ ID NO:012); MOE-277 (SEQ ID NO:012); Stereopattern: SSSSSSSSSSSSSSSSSSSS; MOE-278 (SEQ ID NO:012); Stereopattern: RRRRRRRRRRRRRRRRRRRR; MOE-279 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-280 (SEQ ID NO:012); Stereopattern: SSSRSSSRSSSRSSSRSSS; MOE-281 (SEQ ID NO:012); Stereopattern: SSSRSRSRSRSRSRSRSRS; MOE-282 (SEQ ID NO:012); Stereopattern: SSSSSSRSSSRSSSRSSS; MOE-283 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRS; MOE-284 (SEQ ID NO:012); Stereopattern: SSSRRSRRSRRSRRSRRS; MOE-285 (SEQ ID NO:012); Stereopattern: SSSSSRRSSSRSSSRSSS; MOE-286 (SEQ ID NO:012); Stereopattern: SSSRRRRRRRRRRRRRRSS; MOE-287 (SEQ ID NO:012); Stereopattern: SSRSSSSSSSSSRSSSRSSS; MOE-288 (SEQ ID NO:252); Stereopattern: SSSSSSSSSSSSSSSSSS; MOE-289 (SEQ ID NO:252); Stereopattern: RRRRRRRRRRRRRRRRRR; MOE-290 (SEQ ID NO:252); Stereopattern: SSSRRRRRRRRRRRRSSS; MOE-291 (SEQ ID NO:252); Stereopattern: RRRRRRRRRSSSSSSSSS; MOE-292 (SEQ ID NO:252); Stereopattern:

- SSSSSSSSRRRRRRRR; MOE-293 (SEQ ID NO:252); Stereopattern:
 SSSRRSSRRSSRRSS; MOE-294 (SEQ ID NO:252); Stereopattern:
 SSSRRSSRRSSRRSS; MOE-295 (SEQ ID NO:252); Stereopattern:
 SRSSRRSSRRSS; MOE-296 (SEQ ID NO:252); Stereopattern:
 5 SSSOSSRRSSOSS; MOE-297 (SEQ ID NO:252); Stereopattern:
 SSSOSRRSSOSS; MOE-298 (SEQ ID NO:252); Stereopattern:
 SOSSRRSSOSS; MOE-299 (SEQ ID NO:12); Stereopattern:
 SSSOSSRRSSOSS; MOE-300 (SEQ ID NO:252); Stereopattern:
 RRRORRRSSSSSSSS; MOE-301 (SEQ ID NO:252); Stereopattern:
 10 SRRORRRSSSSSSSS; MOE-303 (SEQ ID NO:252); Stereopattern:
 SSOOSSSSSSSSSS; MOE-304 (SEQ ID NO:252); Stereopattern:
 OOOOSSSSSSSSSS; MOE-305 (SEQ ID NO:252); Stereopattern:
 SSOSSOSSOSSOSS; MOE-306 (SEQ ID NO:252); Stereopattern:
 SOSSOSSOSSOSS; MOE-307 (SEQ ID NO:252); Stereopattern:
 15 SSOSSOSSOSSOSS; MOE-308 (SEQ ID NO:12); Stereopattern:
 SSSOSSSSSSSSOSS; MOE-309 (SEQ ID NO:12); Stereopattern:
 SSSOSSOSSOSSOSS; MOE-310 (SEQ ID NO:252); Stereopattern:
 SSORRRSSSSOSS; or MOE-311 (SEQ ID NO:252). Stereopattern:
 RRRORRRSSSSOSS.
- 20 114. The method of any of claims 79-113, wherein the neurodegenerative disease is Alzheimer's Disease.
115. An antisense oligonucleotide according to claim 1 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell the antisense oligonucleotide of claim 1,
- 25 wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.
116. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 18-30 nucleotides in length.
117. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 21-30 nucleotides in length.
- 30 118. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 21-25 nucleotides in length.
119. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 18-21 nucleotides in length.
- 35 120. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 18-25 nucleotides in length.
121. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 25-30 nucleotides in length.

122. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 21 nucleotides in length.
123. The antisense oligonucleotide of claim 115, wherein the antisense oligonucleotide is 25 nucleotides in length.
- 5 124. The antisense oligonucleotide of any of claims 115-123, wherein the antisense oligonucleotide is complementary to a portion of:
- a. SEQ ID NO:213;
 - b. SEQ ID NO:214;
 - c. SEQ ID NO:215;
 - 10 d. SEQ ID NO:217;
 - e. SEQ ID NO:218;
 - f. SEQ ID NO:219; and/or
 - g. SEQ ID NO:220.
125. The antisense oligonucleotide of any of claims 115-124, wherein the antisense
15 oligonucleotide comprises a non-natural sugar moiety, a non-natural internucleotide linkage, or a non-natural sugar moiety and a non-natural internucleotide linkage.
126. The antisense oligonucleotide of claim 125, wherein the antisense oligonucleotide comprises modified sugar moieties.
127. The antisense oligonucleotide of claim 126, wherein the modified sugar moieties
20 comprise 2'-O-methoxyethyl ribose (2'-O-MOE).
128. The antisense oligonucleotide of claim 127, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs.
129. The antisense oligonucleotide of any of claims 124-128, wherein the antisense
25 oligonucleotide comprises non-natural internucleotide linkages.
130. The antisense oligonucleotide of claim 129, wherein the non-natural internucleotide linkages are stereopure.
131. The antisense oligonucleotide of claim 1230, wherein the non-natural internucleotide linkages are all Sp.
- 30 132. The antisense oligonucleotide of claim 1230, wherein the non-natural internucleotide linkages are all Rp.
133. The antisense oligonucleotide of claim 1230, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
134. The antisense oligonucleotide of claim 129, wherein the non-natural internucleotide
35 linkages are stereorandom.
135. The antisense oligonucleotide of claim 126, wherein the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs).

136. The antisense oligonucleotide of claim 135, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs.
137. The antisense oligonucleotide of claim 135 or 136, wherein the antisense oligonucleotide
5 comprises non-natural internucleotide linkages.
138. The antisense oligonucleotide of claim 137, wherein the non-natural internucleotide linkages are stereopure.
139. The antisense oligonucleotide of claim 138, wherein the non-natural internucleotide linkages are all Sp.
- 10 140. The antisense oligonucleotide of claim 138, wherein the non-natural internucleotide linkages are all Rp.
141. The antisense oligonucleotide of claim 138, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
142. The antisense oligonucleotide of claim 137, wherein the non-natural internucleotide
15 linkages are stereorandom.
143. The antisense oligonucleotide of any of claims 115-142, wherein the antisense oligonucleotide comprises modified nucleobases.
144. An antisense oligonucleotide according to claim 31 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell
20 the antisense oligonucleotide of claim 31,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.
145. An antisense oligonucleotide according to claim 32 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell
25 the antisense oligonucleotide of claim 32,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.
146. An antisense oligonucleotide according to claim 33 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell
30 the antisense oligonucleotide of claim 33,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene .
147. An antisense oligonucleotide according to claim 34 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell
35 the antisense oligonucleotide of claim 34,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.

148. An antisense oligonucleotide according to claim 35 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell the antisense oligonucleotide of claim 35,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene
5 and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.
149. An antisense oligonucleotide according to claim 36 for use in a method of inducing Exon-2 skipping in the *CD33* gene during pre-mRNA splicing, comprising introducing into a cell the antisense oligonucleotide of claim 36,
wherein the antisense oligonucleotide hybridizes to a target region of the *CD33* gene
10 and induces Exon-2 skipping during pre-mRNA splicing of the *CD33* gene.
150. The antisense oligonucleotide of claim 144 or 147, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs when the antisense oligonucleotide comprises phosphorodiamidate morpholino oligomers or according to a Standard Exon-Skipping Efficiency
15 Assay for MOE ASOs when the antisense oligonucleotide comprises methoxyethyl ribose oligomers.
151. The antisense oligonucleotide of any of claims 115-150, wherein the cell is an animal cell.
152. The antisense oligonucleotide of claim 151, wherein the animal cell is a human cell.
- 20 153. An antisense oligonucleotide according to claim 1 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 1.
154. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 18-30 nucleotides in length.
- 25 155. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 21-30 nucleotides in length.
156. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 21-25 nucleotides in length.
157. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 18-
30 21 nucleotides in length.
158. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 18-25 nucleotides in length.
159. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 25-30 nucleotides in length.
- 35 160. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 21 nucleotides in length.
161. The antisense oligonucleotide of claim 153, wherein the antisense oligonucleotide is 25 nucleotides in length.

162. The antisense oligonucleotide of any of claims 153-161, wherein the antisense oligonucleotide is complementary to a portion of:
- SEQ ID NO:213;
 - SEQ ID NO:214;
 - 5 SEQ ID NO:215;
 - SEQ ID NO:217;
 - SEQ ID NO:218;
 - SEQ ID NO:219; and/or
 - SEQ ID NO:220.
- 10 163. The antisense oligonucleotide of any of claims 153-162, wherein the antisense oligonucleotide comprises a non-natural sugar moiety, a non-natural internucleotide linkage, or a non-natural sugar moiety and a non-natural internucleotide linkage.
164. The antisense oligonucleotide of claim 163, wherein the antisense oligonucleotide comprises modified sugar moieties.
- 15 165. The antisense oligonucleotide of claim 164, wherein the modified sugar moieties comprise 2'-O-methoxyethyl ribose (2'-O-MOE).
166. The antisense oligonucleotide of claim 165, wherein the antisense oligonucleotide has a *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for MOE ASOs.
- 20 167. The antisense oligonucleotide of any of claims 162-166, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.
168. The antisense oligonucleotide of claim 167, wherein the non-natural internucleotide linkages are stereopure.
169. The antisense oligonucleotide of claim 168, wherein the non-natural internucleotide
- 25 linkages are all Sp.
170. The antisense oligonucleotide of claim 168, wherein the non-natural internucleotide linkages are all Rp.
171. The antisense oligonucleotide of claim 168, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
- 30 172. The antisense oligonucleotide of claim 167, wherein the non-natural internucleotide linkages are stereorandom.
173. The antisense oligonucleotide of claim 164, wherein the modified sugar moieties comprise phosphorodiamidate morpholino oligomers (PMOs).
174. The antisense oligonucleotide of claim 173, wherein the antisense oligonucleotide has a
- 35 *CD33* Exon-2 skipping efficiency of 30% or greater according to a Standard Exon-Skipping Efficiency Assay for PMO ASOs.
175. The antisense oligonucleotide of claim 173 or 174, wherein the antisense oligonucleotide comprises non-natural internucleotide linkages.

176. The antisense oligonucleotide of claim 175, wherein the non-natural internucleotide linkages are stereopure.
177. The antisense oligonucleotide of claim 176, wherein the non-natural internucleotide linkages are all Sp.
- 5 178. The antisense oligonucleotide of claim 176, wherein the non-natural internucleotide linkages are all Rp.
179. The antisense oligonucleotide of claim 176, wherein the non-natural internucleotide linkages are independently selected from Sp and Rp.
180. The antisense oligonucleotide of claim 175, wherein the non-natural internucleotide
10 linkages are stereorandom.
181. The antisense oligonucleotide of any of claims 153-179, wherein the antisense oligonucleotide comprises modified nucleobases.
182. An antisense oligonucleotide according to claim 31 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a
15 therapeutically effective amount of the antisense oligonucleotide of claim 31.
183. An antisense oligonucleotide according to claim 32 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 32.
184. An antisense oligonucleotide according to claim 33 for use in a method of treating a
20 subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 33.
185. An antisense oligonucleotide according to claim 34 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 34.
- 25 186. An antisense oligonucleotide according to claim 35 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a therapeutically effective amount of the antisense oligonucleotide of claim 35.
187. An antisense oligonucleotide according to claim 36 for use in a method of treating a subject having a neurodegenerative disease comprising administering to said subject a
30 therapeutically effective amount of the antisense oligonucleotide of claim 36.
188. The antisense oligonucleotide of any of claims 153-187, wherein the neurodegenerative disease is Alzheimer's Disease.