(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 April 2021 (22.04.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/074772 A1

(51) International Patent Classification:
A61K 31/713 (2006.01) *C12N 15/00* (2006.01)

(21) International Application Number:
PCT/IB2020/059566

(22) International Filing Date:
12 October 2020 (12.10.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/914,660 14 October 2019 (14.10.2019) US

(71) Applicant: **ASTRAZENECA AB** [SE/SE]; SE-151-85 Södertälje (SE).

(72) Inventor: **LINDEN, Daniel**; AstraZeneca AB, SE-151-85 Södertälje (SE).

(74) Agent: **HOPKIN, Mark**; AstraZeneca Intellectual Property, Milstein Building, Granta Park, Cambridge, Cambridgeshire CB21 6GH (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** MODULATORS OF PNPLA3 EXPRESSION

(57) **Abstract:** The present embodiments provide methods, compounds, and compositions useful for inhibiting PNPLA3 expression, which may be useful for treating, preventing, or ameliorating a disease associated with PNPLA3. In some embodiments, the methods, compounds, and compositions are useful for treating, preventing, or ameliorating a disease associated with PNPLA3 having an I148M mutation.

WO 2021/074772 A1

MODULATORS OF PNPLA3 EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0317USLSEQ_ST25.txt created September 13, 2018, which is 480 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

The present embodiments provide methods, compounds, and compositions useful for inhibiting PNPLA3 (patatin like phospholipase domain containing 3; hypothetical protein dJ796I17.1; adiponutrin; DJ796I17.1) expression, and in certain instances, reducing the amount of PNPLA3 protein in a cell or animal, which can be useful for treating, preventing, or ameliorating a disease associated with PNPLA3. In some embodiments, the methods, compounds, and compositions are useful for treating, preventing, or ameliorating a disease associated with PNPLA3 having an I148M mutation.

Background

Non-alcoholic fatty liver disease (NAFLD) covers a spectrum of liver disease from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. NAFLD is defined as fat accumulation in the liver exceeding 5% by weight, in the absence of significant alcohol consumption, steatogenic medication, or hereditary disorders (Kotronen et al, Arterioscler Thromb. Vasc. Biol. 2008, 28: 27-38).

Non-alcoholic steatohepatitis (NASH) is an aggressive variant of NAFLD with signs of inflammation and hepatic injury. NASH is defined histologically by macrovesicular steatosis, hepatocellular ballooning, and lobular inflammatory infiltrates (Sanyal, Hepatol. Res. 2011. 41: 670-4). NASH is estimated to affect 2-3% of the general population. In the presence of other pathologies, such as obesity or diabetes, the estimated prevalence increases to 7% and 62% respectively (Hashimoto et al, J. Gastroenterol. 2011. 46(1): 63-69).

PNPLA3 is a 481 amino acid member of the patatin-like phospholipase domain-containing family that is expressed in the ER and on lipid droplets. In humans, PNPLA3 is highly expressed in the liver, whereas adipose tissue expression is five-fold less (Huang et al, Proc. Natl. Acad. Sci. USA 2010. 107: 7892-7).

Summary

Certain embodiments provided herein are compounds and methods for reducing the amount or activity of PNPLA3 mRNA, and in certain embodiments, reducing the amount of PNPLA3 protein in a cell or animal. In certain embodiments, the animal has a liver disease. In certain embodiments, the disease is NASH. In certain embodiments, the disease is NAFLD. In certain embodiments, the disease is hepatic steatosis. In certain embodiments, the disease is liver cirrhosis. In certain embodiments, the disease is hepatocellular carcinoma. In certain embodiments, the disease is alcoholic liver disease. In certain embodiments, the disease is alcoholic steatohepatitis (ASH). In certain embodiments, the disease is HCV hepatitis. In certain embodiments, the disease is chronic hepatitis. In certain embodiments, the disease is hereditary hemochromatosis. In certain embodiments, the disease is primary sclerosing cholangitis. Certain compounds provided herein are directed to compounds and compositions that reduce liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an animal.

Certain embodiments provided herein are directed to potent and tolerable compounds and compositions useful for inhibiting PNPLA3 expression, which can be useful for treating, preventing, ameliorating, or slowing progression of liver diseases. Certain embodiments provided herein are directed to compounds and compositions that are more potent or have greater therapeutic value than compounds publicly disclosed.

In some embodiments, the present disclosure provides a method of treating an individual having or at risk of having liver disease, comprising administering a compound targeted to PNPLA3 to the individual, wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

In some embodiments, the present disclosure provides a method of reducing one or more of liver damage, hepatic steatosis, liver inflammation, liver fibrosis, and hepatic lipogenesis in an individual, comprising administering a compound targeted to PNPLA3 to the individual, wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

In some embodiments, the present disclosure provides a method of reducing protein levels of one or more of haptoglobin, MCP1, and TIMP2 in an individual, comprising administering a compound targeted to PNPLA3 to the individual, wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

In some embodiments, the liver disease is selected from non-alcoholic fatty liver disease (NAFLD), hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In some embodiments, the liver disease is hepatic steatosis.

In some embodiments, the method reduces or inhibits liver inflammation or liver fibrosis. In some embodiments, reducing or inhibiting liver inflammation comprises reducing liver macrophage levels. In some embodiments, the liver macrophage levels are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunohistochemical staining of a liver section of the individual.

In some embodiments, the protein levels of haptoglobin are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by a colorimetric assay of serum or plasma of the individual. In some embodiments, the protein levels of MCP1 are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunoblotting of a liver sample of the individual. In some embodiments, the protein levels of TIMP2 are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunoblotting of a liver sample of the individual.

In some embodiments, the individual has a homozygous I148M mutation in PNPLA3. In some embodiments, the individual is a human individual.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is an antisense compound targeted to PNPLA3. In some embodiments, the antisense compound targeted to PNPLA3 is a short interfering RNA (siRNA). In some embodiments, the antisense compound targeted to PNPLA3 is an antisense oligonucleotide (ASO).

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In some embodiments, the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In some embodiments, the compound targeted to PNPLA3 comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 17-2169.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein the nucleobase sequence of the modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2. In some embodiments, the compound targeted to PNPLA3, in an individual

having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence complementary within nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein said modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of a PNPLA3 nucleic acid having the nucleobase sequence of SEQ ID NO: 2, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 2. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a 16 nucleobase portion complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899.

In some embodiments, the modified oligonucleotide has a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% complementary to SEQ ID NO: 2 over the entire length of the nucleobase sequence.

In some embodiments, the modified nucleotide comprises at least one modification selected from at least one modified internucleoside linkage, at least one modified sugar, and at least one modified nucleobase. In some embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

In some embodiments, the modified sugar is a bicyclic sugar. In some embodiments, the bicyclic sugar is selected from the group consisting of: 4'-(CH2)-O-2' (LNA); 4'-(CH2)2-O-2' (ENA); and 4'-CH(CH3)-O-2' (cEt). In some embodiments, the modified sugar is 2'-O-methoxyethyl. In some embodiments, the modified nucleobase is a 5-methylcytosine.

In some embodiments, the modified oligonucleotide comprises: a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is single-stranded. In some embodiments, the compound targeted to PNPLA3 is double-stranded. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises ribonucleotides. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises deoxyribonucleotides.

In some embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In some embodiments, the modified oligonucleotide consists of 12 to 30 linked nucleosides. In some embodiments, the modified oligonucleotide consists of 15 to 30 linked nucleosides.

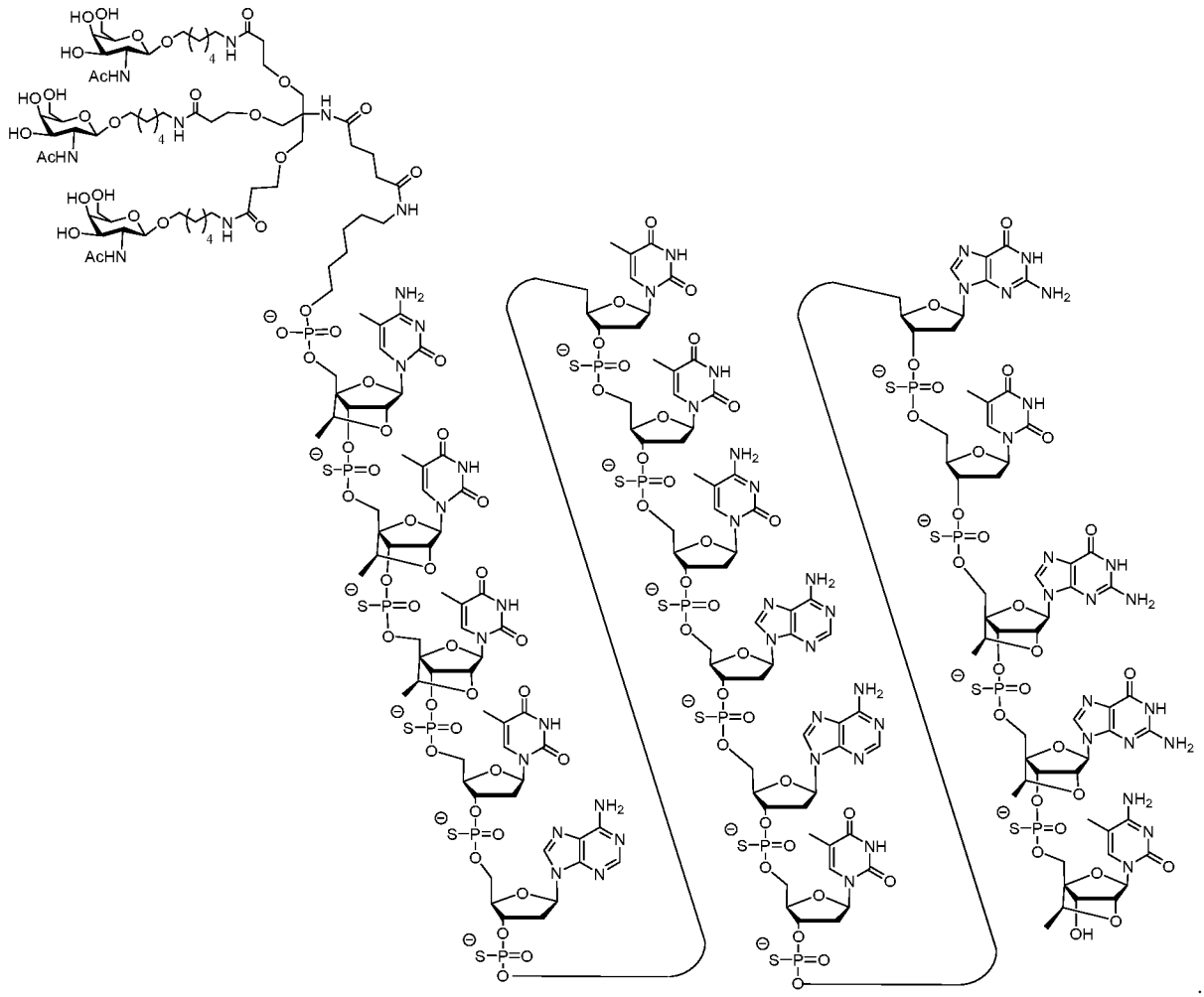
In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 16 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises: a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 16 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises: a gap segment consisting of ten linked deoxynucleosides; a 5' wing segment consisting of three linked nucleosides; and a 3' wing segment consisting of three linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment comprise cEt sugars; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a conjugated moiety and a conjugate linker. In some embodiments, the conjugate group comprises a GalNAc cluster comprising 1 to 3 GalNAc ligands.

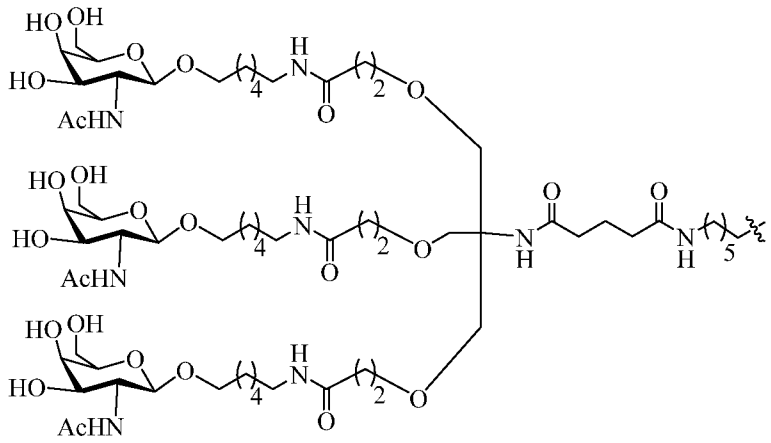
In some embodiments, the conjugate linker consists of a single bond. In some embodiments, the conjugate linker is cleavable. In some embodiments, the conjugate linker comprises 1 to 3 linker-nucleosides. In some embodiments, the conjugate group is attached to the modified oligonucleotide at the 5'-end of the modified oligonucleotide. In some embodiments, the conjugate group is attached to the modified oligonucleotide at the 3'-end of the modified oligonucleotide.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises the following formula or salt thereof:

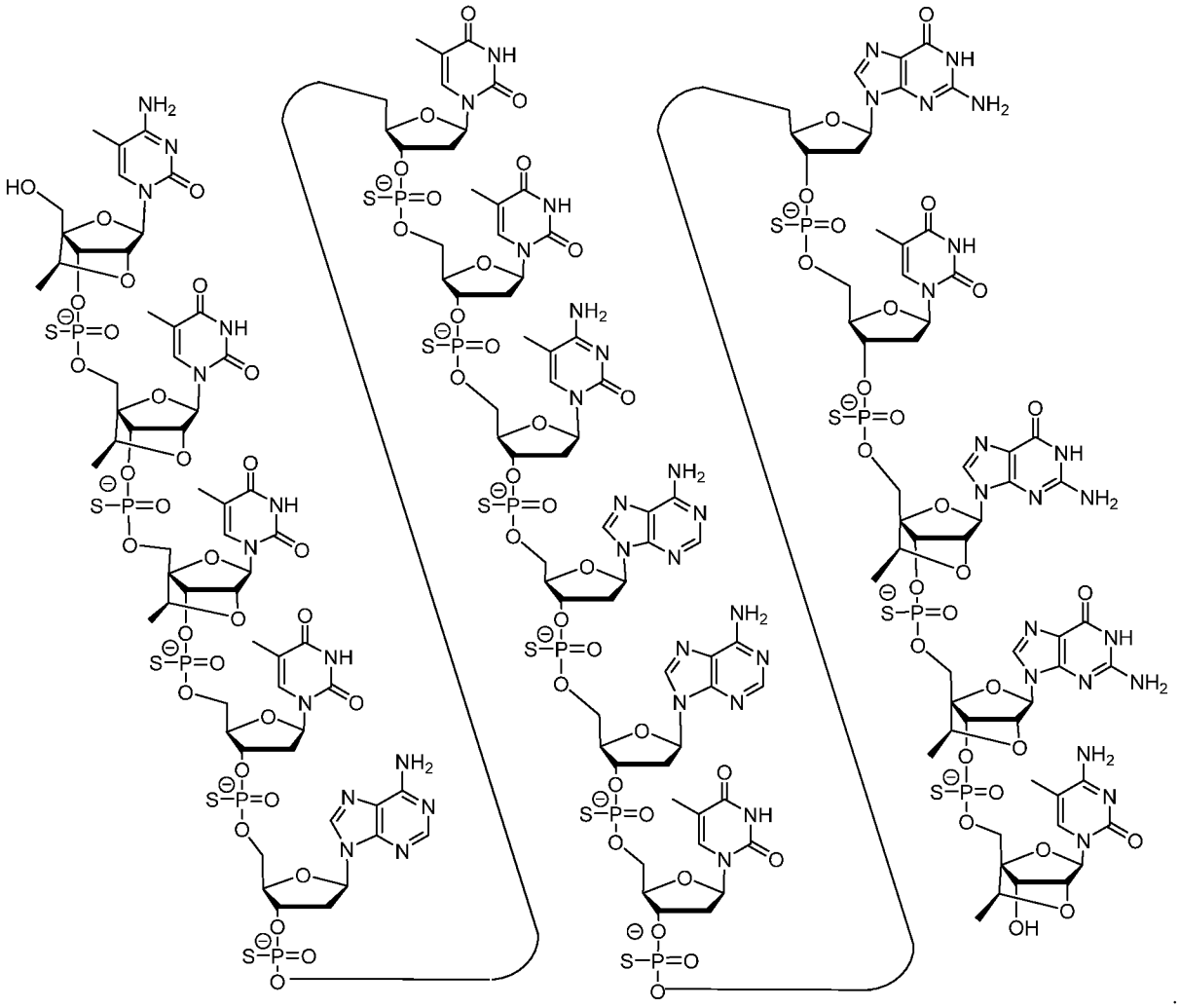


In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide is 16 linked nucleosides in length and consists of the sequence of SEQ ID NO: 1089, wherein the modified oligonucleotide comprises: a gap segment consisting of ten linked deoxynucleosides; a 5' wing segment consisting of three linked nucleosides; and a 3' wing segment consisting of three linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside

of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; wherein each cytosine is a 5-methylcytosine; and wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, has the following formula or salt thereof:



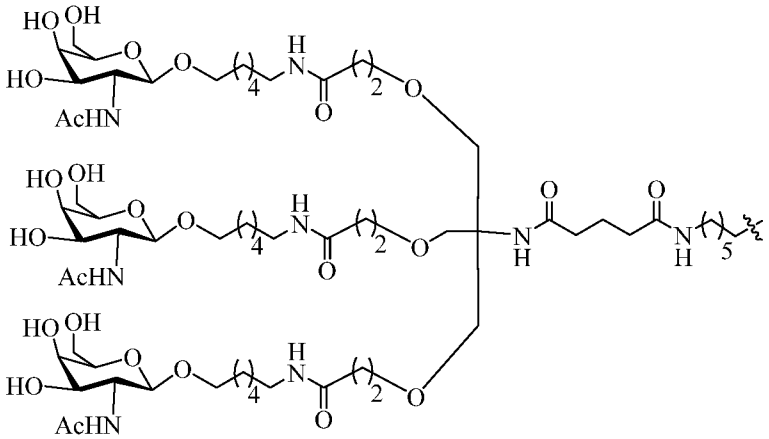
In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is a modified oligonucleotide in a pharmaceutically acceptable salt form. In some embodiments, the pharmaceutically acceptable salt is a sodium salt. In some embodiments, the pharmaceutically acceptable salt is a potassium salt.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is administered to the individual as a composition comprising the compound targeted to PNPLA3 and a pharmaceutically acceptable carrier. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is administered parenterally to the individual.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to SEQ ID NO: 115. In some embodiments, the compound targeted to PNPLA3,

in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to any one of SEQ ID NOs: 2170-2172.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, consists of the sequence of SEQ ID NO: 115, wherein the modified oligonucleotide comprises: a gap segment consisting of ten linked deoxynucleosides; a 5' wing segment consisting of three linked nucleosides; and a 3' wing segment consisting of three linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, further comprises a conjugate group, wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



Brief Description of the Figures

Figs. 1A-1F relate to Example 11. The results in Figs. 1A-1C relate to human HepG2 cells subjected to either a control ASO or PNPLA3 ASO described in embodiments herein. Fig. 1A shows PNPLA3 mRNA levels. Fig. 1B shows the Oil Red O (ORO) staining area. Fig. 1C shows an image of the ORO staining. The results in Figs. 1D-1F relate to human HepG2 cells subjected to either a control siRNA or PNPLA3 siRNA described in embodiments herein. Fig. 1D shows PNPLA3 mRNA levels. Fig. 1E shows the Oil Red O (ORO) staining area. Fig. 1F shows an image of the ORO staining.

Figs. 2A-2J relate to Example 13. The results in Figs. 2A-2J relate to wild-type and PNPLA3 I148M mutant knock-in mice subjected to either a control ASO or PNPLA3 ASO described in embodiments herein. Fig. 2A shows body weight gain before and after ASO treatment. Fig. 2B shows caloric intake before and after ASO treatment. Fig. 2C shows liver *Pnpla3* mRNA levels measured by qPCR and normalized to the *ribosomal*

protein large PO (RplpO). Fig. 2D shows levels of the Pnpla3 protein in hepatic lipid droplets as measured by Western blotting. Figs 2E and 2H show representative images of ORO-stained liver sections after 8 weeks of ASO treatment (black scale bar represents 100 μm). Figs. 2F and 2I show liver lipid levels in PNPLA3 I148M mutant mice and wild-type mice, respectively, as assessed by MRI after 6 weeks of ASO treatment. Figs. 2G and 2J show liver and plasma triglyceride levels in PNPLA3 I148M mutant mice and wild-type mice, respectively, as measured by biochemical assays.

Figs. 3A-3F and 4A-4B relate to Example 14. The results in Figs. 3A-3F and 4A-4B relate to wild-type and PNPLA3 I148M mutant knock-in mice subjected to either a control ASO or PNPLA3 ASO described in embodiments herein. Fig. 3A shows body weight as measured throughout the experiment. Fig. 3B shows caloric intake measured before and after ASO treatment. Fig. 3C shows liver *Pnpla3* mRNA levels measured by qPCR and normalized to the *ribosomal protein large PO (RplpO)*. Fig. 3D shows levels of the Pnpla3 protein in hepatic lipid droplets as measured by Western blotting. Figs. 3E and 3F show levels of plasma ALT, AST, and triglycerides and liver triglyceride content in PNPLA3 I148M mutant mice and wild-type mice, respectively.

Figs. 4A and 4B show liver steatosis score, lobular inflammation score, NAFLD activity score (NAS), and fibrosis stage in PNPLA3 I148M mutant mice and wild-type mice, respectively.

Figs. 5A-5E and Fig. 6 relate to Example 15. The results in Figs. 5A-3E and 6 relate to wild-type and PNPLA3 I148M mutant knock-in mice subjected to either a control ASO or PNPLA3 ASO described in embodiments herein. Fig. 5A shows representative images of Oil Red O-stained liver sections (black scale bar represents 100 μm). Figs. 5B and 5C show liver mRNA expression levels of *Acc1* and *Scd1* in PNPLA3 I148M mutant mice and wild-type mice, respectively. Figs. 5D and 5E show liver lipid droplet fatty acid composition in PNPLA3 I148M mutant mice and wild-type mice, respectively.

Fig. 6 shows additional liver lipid droplet fatty acid composition in PNPLA3 I148M mutant mice and wild-type mice, including monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA).

Figs. 7A-7H, 8A-8E and 9A-9D relate to Example 16. The results in Figs. 7A-7H, 8A-8E, and 9A-9D relate to wild-type and PNPLA3 I148M mutant knock-in mice subjected to either a control ASO or PNPLA3 ASO described in embodiments herein. Figs. 7A and 7B show plasma haptoglobin levels and liver macrophage contents (as determined by Mac2 staining) in PNPLA3 I148M mutant mice and wild-type mice, respectively. Fig. 7C shows representative images of Mac2-stained liver sections (black scale bar represents 100 μm). Figs. 7D-7H show liver protein levels of Mcp1 (Fig. 7D), Il1β (Fig. 7E), Il6 (Fig. 7F), Tnfα (Fig. 7G), and αSma (Fig. 7H) in PNPLA3 I148M mutant mice and wild-type mice.

Figs. 8A and 8B show liver Col1a1 mRNA and protein (immunohistochemistry) levels in PNPLA3 I148M mutant mice and wild-type mice, respectively. Fig. 8C shows representative images of collagen

immunohistochemistry in liver sections (black scale bar represents 100 μm). Figs. 8D and 8E show liver hydroxyproline levels in in PNPLA3 I148M mutant mice and wild-type mice, respectively.

Figs. 9A-9D show liver proteins of Timp2 (Fig. 9A), Mmp2 (Fig. 9B), Timp1 (Fig. 9C), and Tgfβr2 (Fig. 9D) measured with Western blot analyses in in PNPLA3 I148M mutant mice and wild-type mice.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the embodiments, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included," is not limiting.

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

It is understood that the sequence set forth in each SEQ ID NO in the examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Compounds described by ION number indicate a combination of nucleobase sequence, chemical modification, and motif.

Definitions

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

"2'-deoxynucleoside" means a nucleoside comprising 2'-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

"2'-O-methoxyethyl" (also 2'-MOE) refers to a 2'-O(CH_2)$_2$-OCH_3) in the place of the 2'-OH group of a ribosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE modified sugar moiety.

"2'-substituted nucleoside" or "2-modified nucleoside" means a nucleoside comprising a 2'-substituted or 2'-modified sugar moiety. As used herein, "2'-substituted" or "2-modified" in reference to a sugar moiety means a sugar moiety comprising at least one 2'-substituent group other than H or OH.

"3' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 3'-most nucleotide of a particular compound.

"5' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 5'-most nucleotide of a particular compound.

"5-methylcytosine" means a cytosine with a methyl group attached to the 5 position.

"About" means within ±10% of a value. For example, if it is stated, "the compounds affected about 70% inhibition of PNPLA3," it is implied that PNPLA3 levels are inhibited within a range of 60% and 80%.

"Administration" or "administering" refers to routes of introducing a compound or composition provided herein to an individual to perform its intended function. An example of a route of administration that can be used includes, but is not limited to parenteral administration, such as subcutaneous, intravenous, or intramuscular injection or infusion.

"Administered concomitantly" or "co-administration" means administration of two or more compounds in any manner in which the pharmacological effects of both are manifest in the patient. Concomitant administration does not require that both compounds be administered in a single pharmaceutical composition, in the same dosage form, by the same route of administration, or at the same time. The effects of both compounds need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive. Concomitant administration or co-administration encompasses administration in parallel or sequentially.

"Amelioration" refers to an improvement or lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression or severity of one or more indicators of a condition or disease. The progression or severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

"Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

"Antisense activity" means any detectable and/or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound to the target.

"Antisense compound" means a compound comprising an oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, oligonucleotides, ribozymes, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

"Antisense inhibition" means reduction of target nucleic acid levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels in the absence of the antisense compound.

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

"Antisense oligonucleotide" means an oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid or region or segment thereof. In certain embodiments, an antisense oligonucleotide is specifically hybridizable to a target nucleic acid or region or segment thereof.

"Bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety. "Bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

"Branching group" means a group of atoms having at least 3 positions that are capable of forming covalent linkages to at least 3 groups. In certain embodiments, a branching group provides a plurality of reactive sites for connecting tethered ligands to an oligonucleotide via a conjugate linker and/or a cleavable moiety.

"Cell-targeting moiety" means a conjugate group or portion of a conjugate group that is capable of binding to a particular cell type or particular cell types.

"cEt" or "constrained ethyl" means a ribosyl bicyclic sugar moiety wherein the second ring of the bicyclic sugar is formed via a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2', and wherein the methyl group of the bridge is in the *S* configuration.

"cEt nucleoside" means a nucleoside comprising a cEt modified sugar moiety.

"Chemical modification" in a compound describes the substitutions or changes through chemical reaction, of any of the units in the compound relative to the original state of such unit. "Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase. "Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

"Chemically distinct region" refers to a region of a compound that is in some way chemically different than another region of the same compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

"Cleavable bond" means any chemical bond capable of being split. In certain embodiments, a cleavable bond is selected from among: an amide, a polyamide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, a di-sulfide, or a peptide.

"Cleavable moiety" means a bond or group of atoms that is cleaved under physiological conditions, for example, inside a cell, an animal, or a human.

"Complementary" in reference to an oligonucleotide means the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to the following pairs: adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^{m}C) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. By contrast, "fully complementary" or "100% complementary" in reference to oligonucleotides means that such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

"Conjugate group" means a group of atoms that is attached to an oligonucleotide. Conjugate groups include a conjugate moiety and a conjugate linker that attaches the conjugate moiety to the oligonucleotide.

"Conjugate linker" means a group of atoms comprising at least one bond that connects a conjugate moiety to an oligonucleotide.

"Conjugate moiety" means a group of atoms that is attached to an oligonucleotide via a conjugate linker.

"Contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other in a sequence.

"Designing" or "Designed to" refer to the process of designing a compound that specifically hybridizes with a selected nucleic acid molecule.

"Diluent" means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition can be a liquid, e.g. saline solution.

"Differently modified" means chemical modifications or chemical substituents that are different from one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are "differently modified," even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are "differently modified," even though both are naturally-occurring unmodified nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified. For example, a nucleoside comprising a 2'-OMe modified sugar and an unmodified adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and an unmodified thymine nucleobase are not differently modified.

"Dose" means a specified quantity of a compound or pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose may require a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In other embodiments, the compound or pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week or month.

"Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

"Double-stranded antisense compound" means an antisense compound comprising two oligomeric compounds that are complementary to each other and form a duplex, and wherein one of the two said oligomeric compounds comprises an oligonucleotide.

"Effective amount" means the amount of compound sufficient to effectuate a desired physiological outcome in an individual in need of the compound. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

"Efficacy" means the ability to produce a desired effect.

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to, the products of transcription and translation.

"Gapmer" means an oligonucleotide comprising an internal region having a plurality of nucleosides that support RNase H cleavage positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings."

"Hybridization" means the annealing of oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an oligonucleotide and a nucleic acid target.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements of the same kind (e.g. no intervening nucleobases between the immediately adjacent nucleobases).

"Individual" means a human or non-human animal selected for treatment or therapy.

"Inhibiting the expression or activity" refers to a reduction or blockade of the expression or activity relative to the expression of activity in an untreated or control sample and does not necessarily indicate a total elimination of expression or activity.

"Internucleoside linkage" means a group or bond that forms a covalent linkage between adjacent nucleosides in an oligonucleotide. "Modified internucleoside linkage" means any internucleoside linkage other than a naturally occurring, phosphate internucleoside linkage. Non-phosphate linkages are referred to herein as modified internucleoside linkages.

"Lengthened oligonucleotides" are those that have one or more additional nucleosides relative to an oligonucleotide disclosed herein, e.g. a parent oligonucleotide.

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"Linker-nucleoside" means a nucleoside that links an oligonucleotide to a conjugate moiety. Linker-nucleosides are located within the conjugate linker of a compound. Linker-nucleosides are not considered part of the oligonucleotide portion of a compound even if they are contiguous with the oligonucleotide.

"Mismatch" or "non-complementary" means a nucleobase of a first oligonucleotide that is not complementary to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned. For example, nucleobases including but not limited to a universal nucleobase, inosine, and hypoxanthine, are capable of hybridizing with at least one nucleobase but are still mismatched or non-complementary with respect to nucleobase to which it hybridized. As another example, a

nucleobase of a first oligonucleotide that is not capable of hybridizing to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned is a mismatch or non-complementary nucleobase.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating PNPLA3 RNA can mean to increase or decrease the level of PNPLA3 RNA and/or PNPLA3 protein in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a PNPLA3 compound can be a modulator that decreases the amount of PNPLA3 RNA and/or PNPLA3 protein in a cell, tissue, organ or organism.

"MOE" means methoxyethyl.

"Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides.

"Motif" means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

"Natural" or "naturally occurring" means found in nature.

"Non-bicyclic modified sugar" or "non-bicyclic modified sugar moiety" means a modified sugar moiety that comprises a modification, such as a substituent, that does not form a bridge between two atoms of the sugar to form a second ring.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, and double-stranded nucleic acids.

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid. As used herein a "naturally occurring nucleobase" is adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G). A "modified nucleobase" is a naturally occurring nucleobase that is chemically modified. A "universal base" or "universal nucleobase" is a nucleobase other than a naturally occurring nucleobase and modified nucleobase, and is capable of pairing with any nucleobase.

"Nucleobase sequence" means the order of contiguous nucleobases in a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage.

"Nucleoside" means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. "Modified nucleoside" means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides include abasic nucleosides, which lack a nucleobase.

"Oligomeric compound" means a compound comprising a single oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another. Unless otherwise indicated, oligonucleotides consist of 8-80 linked nucleosides. "Modified oligonucleotide" means an oligonucleotide, wherein at least one sugar, nucleobase, or internucleoside linkage is modified. "Unmodified oligonucleotide" means an oligonucleotide that does not comprise any sugar, nucleobase, or internucleoside modification.

"Parent oligonucleotide" means an oligonucleotide whose sequence is used as the basis of design for more oligonucleotides of similar sequence but with different lengths, motifs, and/or chemistries. The newly designed oligonucleotides may have the same or overlapping sequence as the parent oligonucleotide.

"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

"Patatin like phospholipase domain containing 3," abbreviated as PNPLA3 and also referred to as adiponutrin (ADPN), acylglycerol O-acyltransferase, calcium-independent phospholipase A2-epsilon (iPLA2-epsilon), hypothetical protein dJ796I17.1, or DJ796I17.1, is a 481-amino acid protein encoded by the *Pnpla3* gene. PNPLA3 has hydrolase activity towards triglycerides and retinyl esters, promoting lipid droplet remodeling in hepatocytes and hepatic stellate cells. As described herein, PNPLA3 is a member of the patatin-like phospholipase domain-containing family that is expressed on the ER and on lipid droplets. In humans, PNPLA3 is highly expressed in the liver. As used herein, "PNPLA3" can refer to any nucleic acid or protein of PNPLA3. "PNPLA3 nucleic acid" means any nucleic acid encoding PNPLA3. For example, in certain embodiments, a PNPLA3 nucleic acid includes a DNA sequence encoding PNPLA3, an RNA sequence transcribed from DNA encoding PNPLA3 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding PNPLA3. "PNPLA3 mRNA" means an mRNA encoding a PNPLA3 protein. The target may be referred to in either upper or lower case.

"PNPLA3 specific inhibitor" refers to any agent capable of specifically inhibiting PNPLA3 RNA and/or PNPLA3 protein expression or activity at the molecular level. For example, PNPLA3 specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, and other agents capable of inhibiting the expression of PNPLA3 RNA and/or PNPLA3 protein.

"Pharmaceutically acceptable carrier or diluent" means any substance suitable for use in administering to an individual. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution, such as PBS or water-for-injection.

"Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of compounds, such as oligomeric compounds or oligonucleotides, i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

"Pharmaceutical agent" means a compound that provides a therapeutic benefit when administered to an individual.

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more compounds or salt thereof and a sterile aqueous solution.

"Phosphorothioate linkage" means a modified phosphate linkage in which one of the non-bridging oxygen atoms is replaced with a sulfur atom. A phosphorothioate internucleoside linkage is a modified internucleoside linkage.

"Phosphorus moiety" means a group of atoms comprising a phosphorus atom. In certain embodiments, a phosphorus moiety comprises a mono-, di-, or tri-phosphate, or phosphorothioate.

"Portion" means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an oligomeric compound.

"Prevent" refers to delaying or forestalling the onset, development or progression of a disease, disorder, or condition for a period of time from minutes to indefinitely.

"Prodrug" means a compound in a form outside the body which, when administered to an individual, is metabolized to another form within the body or cells thereof. In certain embodiments, the metabolized form is the active, or more active, form of the compound (e.g., drug). Typically conversion of a prodrug within the body is facilitated by the action of an enzyme(s) (e.g., endogenous or viral enzyme) or chemical(s) present in cells or tissues, and/or by physiologic conditions.

"Reduce" means to bring down to a smaller extent, size, amount, or number.

"RefSeq No." is a unique combination of letters and numbers assigned to a sequence to indicate the sequence is for a particular target transcript (e.g., target gene). Such sequence and information about the target gene (collectively, the gene record) can be found in a genetic sequence database. Genetic sequence databases include the NCBI Reference Sequence database, GenBank, the European Nucleotide Archive, and the DNA Data Bank of Japan (the latter three forming the International Nucleotide Sequence Database Collaboration or INSDC).

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"RNAi compound" means an antisense compound that acts, at least in part, through RISC or Ago2, but not through RNase H, to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi compounds include, but are not limited to double-stranded siRNA, single-stranded RNA (ssRNA), and microRNA, including microRNA mimics.

"Segments" are defined as smaller or sub-portions of regions within a nucleic acid.

"Side effects" means physiological disease and/or conditions attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

"Single-stranded" in reference to a compound means the compound has only one oligonucleotide. "Self-complementary" means an oligonucleotide that at least partially hybridizes to itself. A compound consisting of one oligonucleotide, wherein the oligonucleotide of the compound is self-complementary, is a single-stranded compound. A single-stranded compound may be capable of binding to a complementary compound to form a duplex.

"Sites" are defined as unique nucleobase positions within a target nucleic acid.

"Specifically hybridizable" refers to an oligonucleotide having a sufficient degree of complementarity between the oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids. In certain embodiments, specific hybridization occurs under physiological conditions.

"Specifically inhibit" with reference to a target nucleic acid means to reduce or block expression of the target nucleic acid while exhibiting fewer, minimal, or no effects on non-target nucleic acids. Reduction does not necessarily indicate a total elimination of the target nucleic acid's expression.

"Standard cell assay" means assay(s) described in the Examples and reasonable variations thereof.

"Standard in vivo experiment" means the procedure(s) described in the Example(s) and reasonable variations thereof.

"Stereorandom chiral center" in the context of a population of molecules of identical molecular formula means a chiral center having a random stereochemical configuration. For example, in a population of molecules comprising a stereorandom chiral center, the number of molecules having the (*S*) configuration of the stereorandom chiral center may be but is not necessarily the same as the number of molecules having the (*R*) configuration of the stereorandom chiral center. The stereochemical configuration of a chiral center is

considered random when it is the result of a synthetic method that is not designed to control the stereochemical configuration. In certain embodiments, a stereorandom chiral center is a stereorandom phosphorothioate internucleoside linkage.

"Sugar moiety" means an unmodified sugar moiety or a modified sugar moiety. "Unmodified sugar moiety" or "unmodified sugar" means a 2'-OH(H) ribosyl moiety, as found in RNA (an "unmodified RNA sugar moiety"), or a 2'-H(H) moiety, as found in DNA (an "unmodified DNA sugar moiety"). "Modified sugar moiety" or "modified sugar" means a modified furanosyl sugar moiety or a sugar surrogate. "Modified furanosyl sugar moiety" means a furanosyl sugar comprising a non-hydrogen substituent in place of at least one hydrogen or hydroxyl of an unmodified sugar moiety. In certain embodiments, a modified furanosyl sugar moiety is a 2'-substituted sugar moiety. Such modified furanosyl sugar moieties include bicyclic sugars and non-bicyclic sugars.

"Sugar surrogate" means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group in an oligonucleotide. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide and such oligonucleotides are capable of hybridizing to complementary compounds or nucleic acids.

"Synergy" or "synergize" refers to an effect of a combination that is greater than additive of the effects of each component alone at the same doses.

"Target gene" refers to a gene encoding a target.

"Targeting" means the specific hybridization of a compound to a target nucleic acid in order to induce a desired effect.

"Target nucleic acid," "target RNA," "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by compounds described herein.

"Target region" means a portion of a target nucleic acid to which one or more compounds is targeted.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which a compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

"Terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

"Therapeutically effective amount" means an amount of a compound, pharmaceutical agent, or composition that provides a therapeutic benefit to an individual.

"Treat" refers to administering a compound or pharmaceutical composition to an animal in order to effect an alteration or improvement of a disease, disorder, or condition in the animal.

Certain Embodiments

Certain embodiments provide methods, compounds and compositions for inhibiting PNPLA3 (PNPLA3) expression.

Certain embodiments provide compounds targeted to a PNPLA3 nucleic acid. In certain embodiments, the PNPLA3 nucleic acid has the sequence set forth in RefSeq or GENBANK Accession No. NM_025225.2 (incorporated by reference, disclosed herein as SEQ ID NO: 1); NC_000022.11 truncated from nucleotides 43921001 to 43954500 (incorporated by reference, disclosed herein as SEQ ID NO: 2); AK123806.1(incorporated by reference, disclosed herein as SEQ ID NO: 3); BQ686328.1 (incorporated by reference, disclosed herein as SEQ ID NO: 4); BF762711.1 (incorporated by reference, disclosed herein as SEQ ID NO: 5); DA290491.1 (incorporated by reference, disclosed herein as SEQ ID NO: 6); and the sequences listed as SEQ ID Nos 7, 8, 9, and 10. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single-stranded. In certain embodiments, the compound is double-stranded.

In certain embodiments, the compound comprises a modified oligonucleotide 16 linked nucleosides in length. In certain embodiments, the compound is an antisense compound or oligomeric compound.

Certain embodiments provide a compound comprising a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single-stranded. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide is 16 to 30 linked nucleosides in length.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single-stranded. In certain embodiments, the compound is double-stranded.

Certain embodiments provide a compound comprising a modified oligonucleotide 12 to 30 linked nucleosides in length and complementary within nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, and 25844-25912 of SEQ ID NO: 2, wherein said modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain

embodiments, the compound is single-stranded. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide is 16 to 30 linked nucleosides in length.

In certain embodiments, compounds target nucleotides 5567-5620 of a PNPLA3 nucleic acid. In certain embodiments, compounds target within nucleotides 5567-5642, 5644-5731, 5567-5731, 5567-5620 of a PNPLA3 nucleic acid having the nucleobase sequence of SEQ ID NO: 2. In certain embodiments, compounds have at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion within nucleotides 5567-5642, 5644-5731, 5567-5731, 5567-5620 of a PNPLA3 nucleic acid having the nucleobase sequence of SEQ ID NO: 2. In certain embodiments, these compounds are antisense compounds, oligomeric compounds, or oligonucleotides.

In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the modified oligonucleotide is 16 to 30 linked nucleosides in length.

In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the modified oligonucleotide is 16 to 30 linked nucleosides in length.

In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899.

In certain embodiments, compounds targeted to PNPLA3 is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. Out of over 2,384 compounds that were screened as described in the Examples section below, ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, and 975612 emerged as the top lead compounds.

In certain embodiments, any of the foregoing modified oligonucleotides comprises at least one modified internucleoside linkage, at least one modified sugar, and/or at least one modified nucleobase.

In certain embodiments, any of the foregoing modified oligonucleotides comprises at least one modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group. In certain embodiments, at least one modified sugar is a bicyclic sugar, such as a 4'-CH(CH3)-O-2' group, a 4'-CH2-O-2' group, or a 4'-(CH2)2-O-2'group.

In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage, such as a phosphorothioate internucleoside linkage.

In certain embodiments, any of the foregoing modified oligonucleotides comprises at least one modified nucleobase, such as 5-methylcytosine.

In certain embodiments, any of the foregoing modified oligonucleotides comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, the modified oligonucleotide is 12 to 30 linked nucleosides in length having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the modified oligonucleotide is 16 to 30 linked nucleosides in length having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the modified oligonucleotide is 16 linked nucleosides in length having a nucleobase sequence consisting of the sequence recited in any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide 12-30 linked nucleobases in length having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide, wherein the modified oligonucleotide is 16 linked nucleosides in length and consists of the sequence of SEQ ID NO: 1089, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

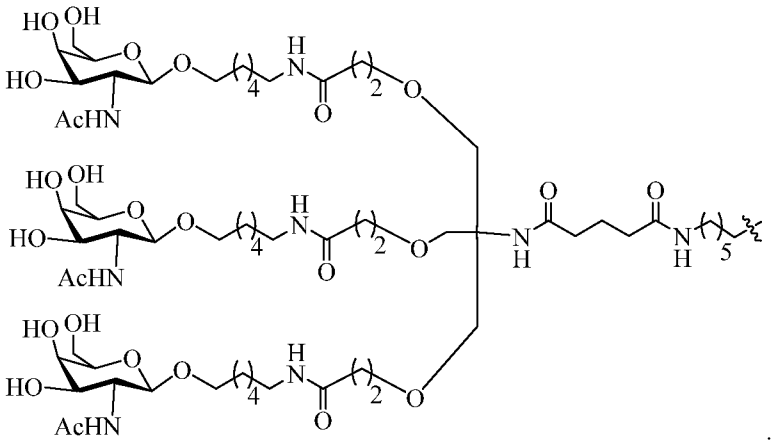
In certain embodiments, a compound consists of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide is 16 linked nucleosides in length and consists of the sequence of SEQ ID NO: 1089, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; wherein each cytosine is a 5-methylcytosine; and wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to SEQ ID NO: 115. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to any one of SEQ ID NOs: 2170-2172.

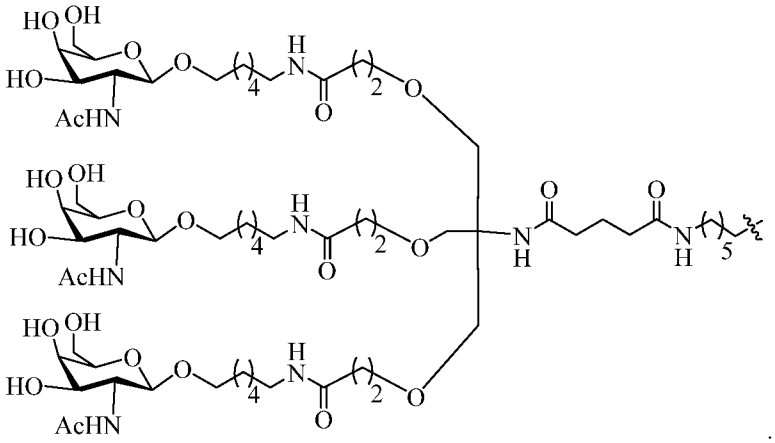
In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, consists of the sequence of SEQ ID NO: 115, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

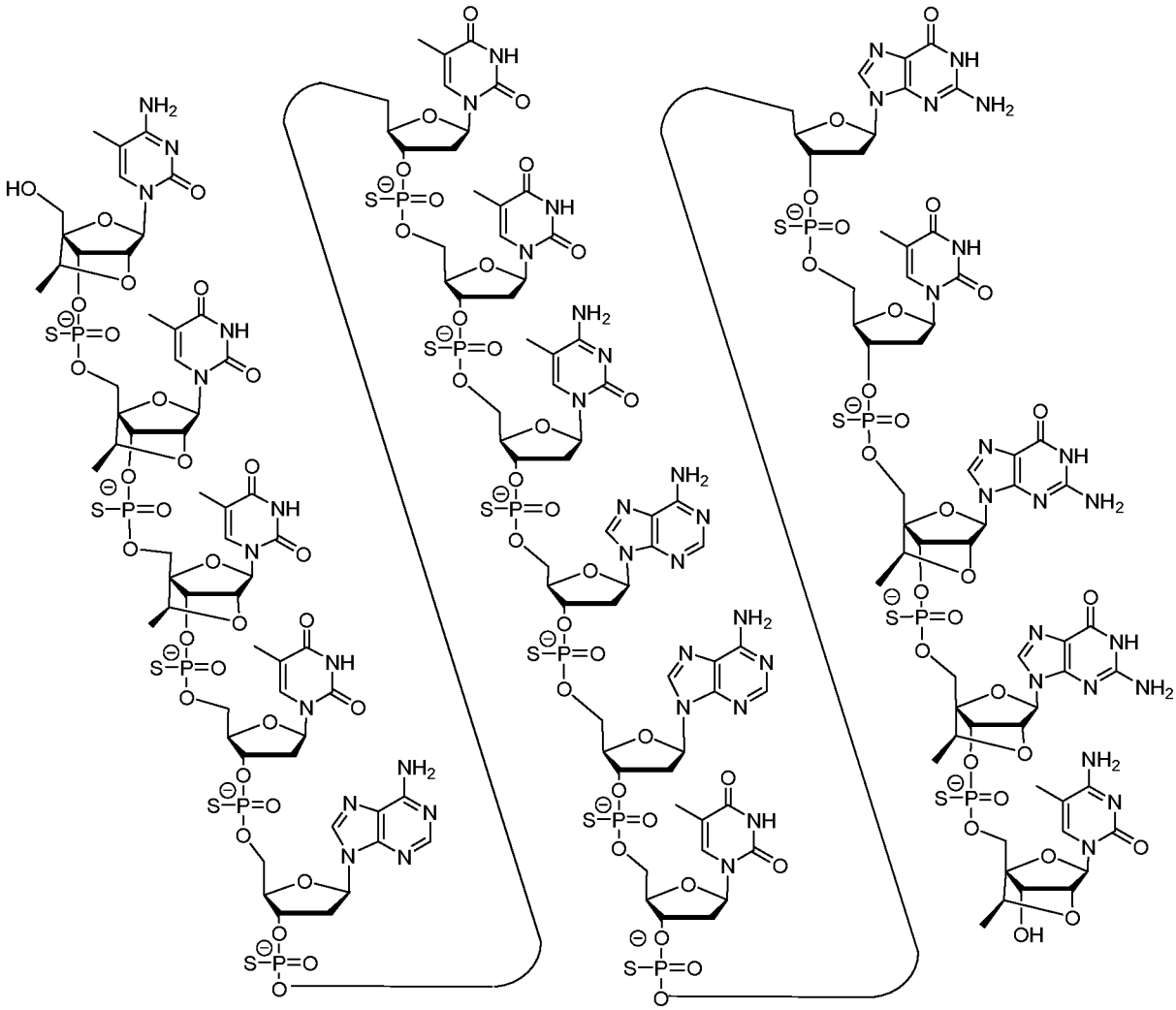
a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

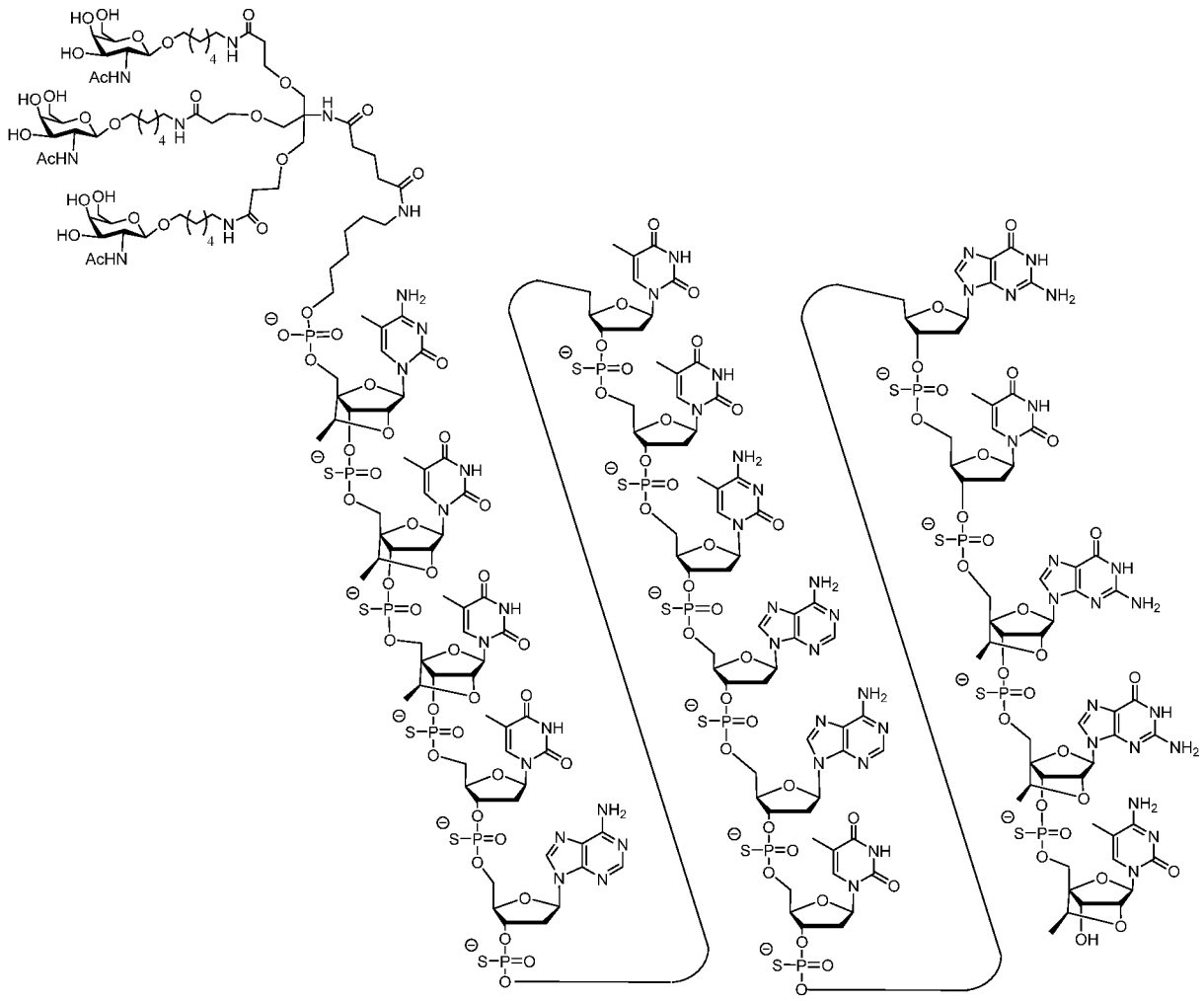
wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, further comprises a conjugate group, wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



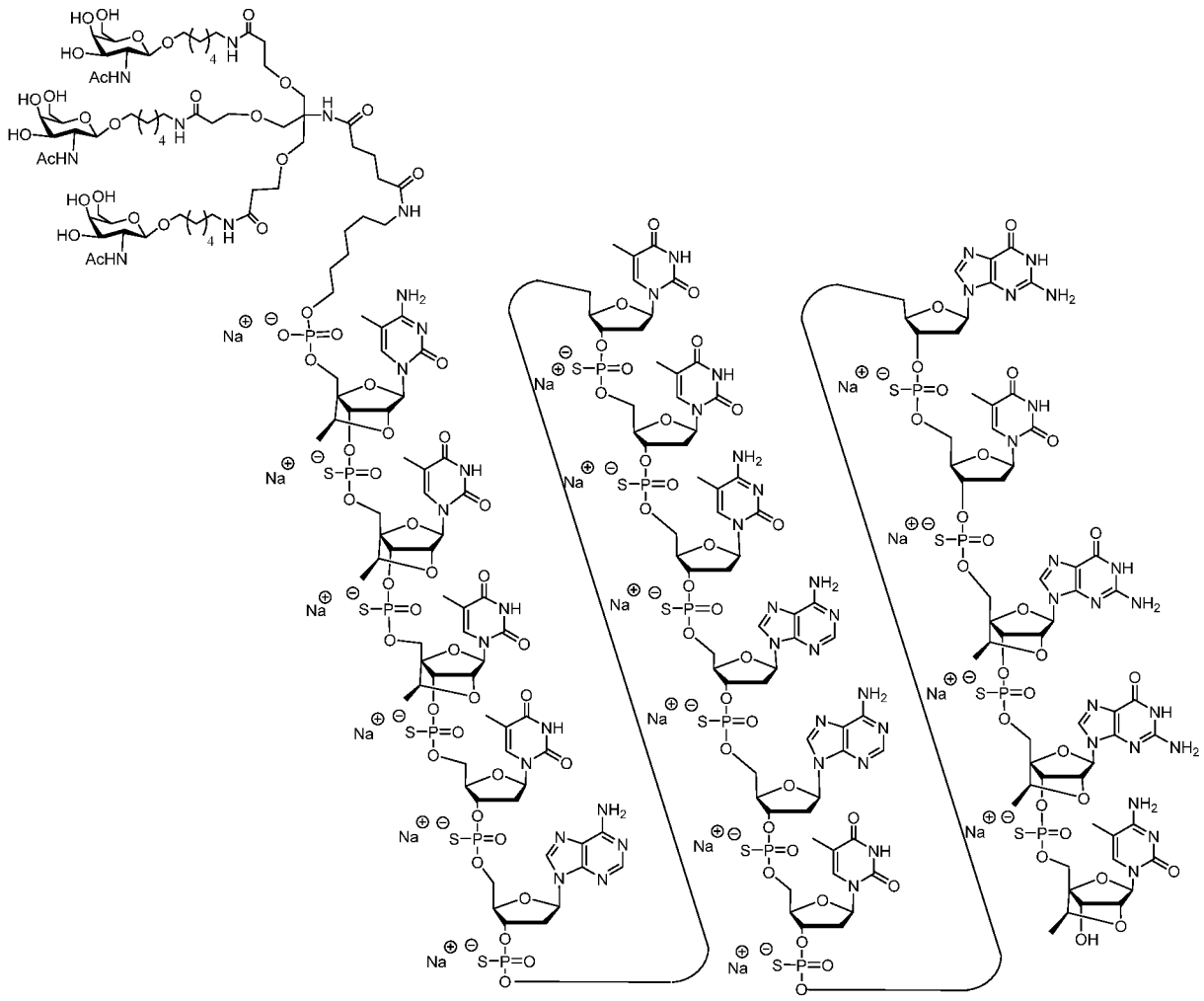
In certain embodiments, a compound comprises or consists of ION 916333 or salt thereof, having the following chemical structure:



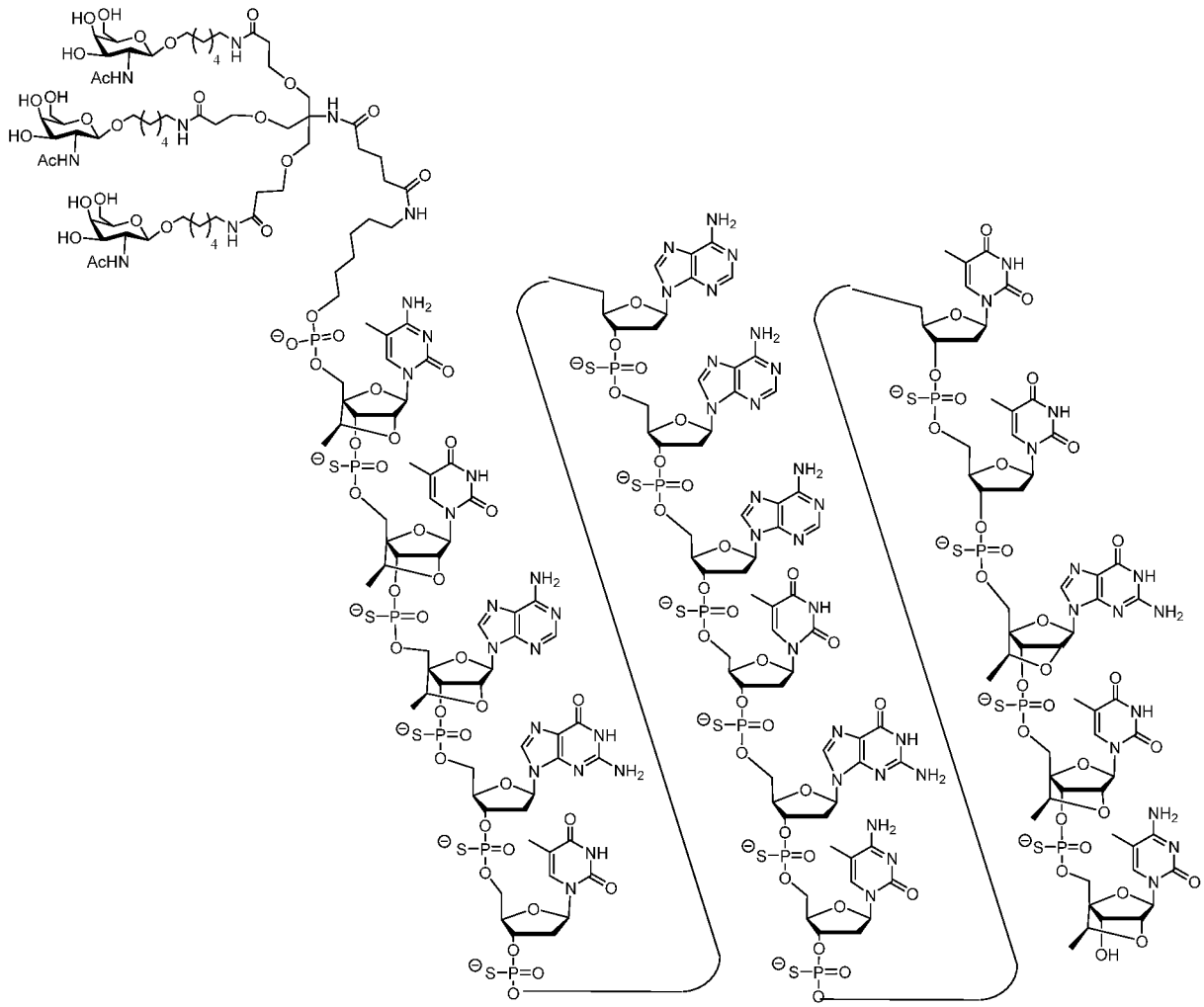
In certain embodiments, a compound comprises or consists of ION 975616 or salt thereof, having the following chemical structure:



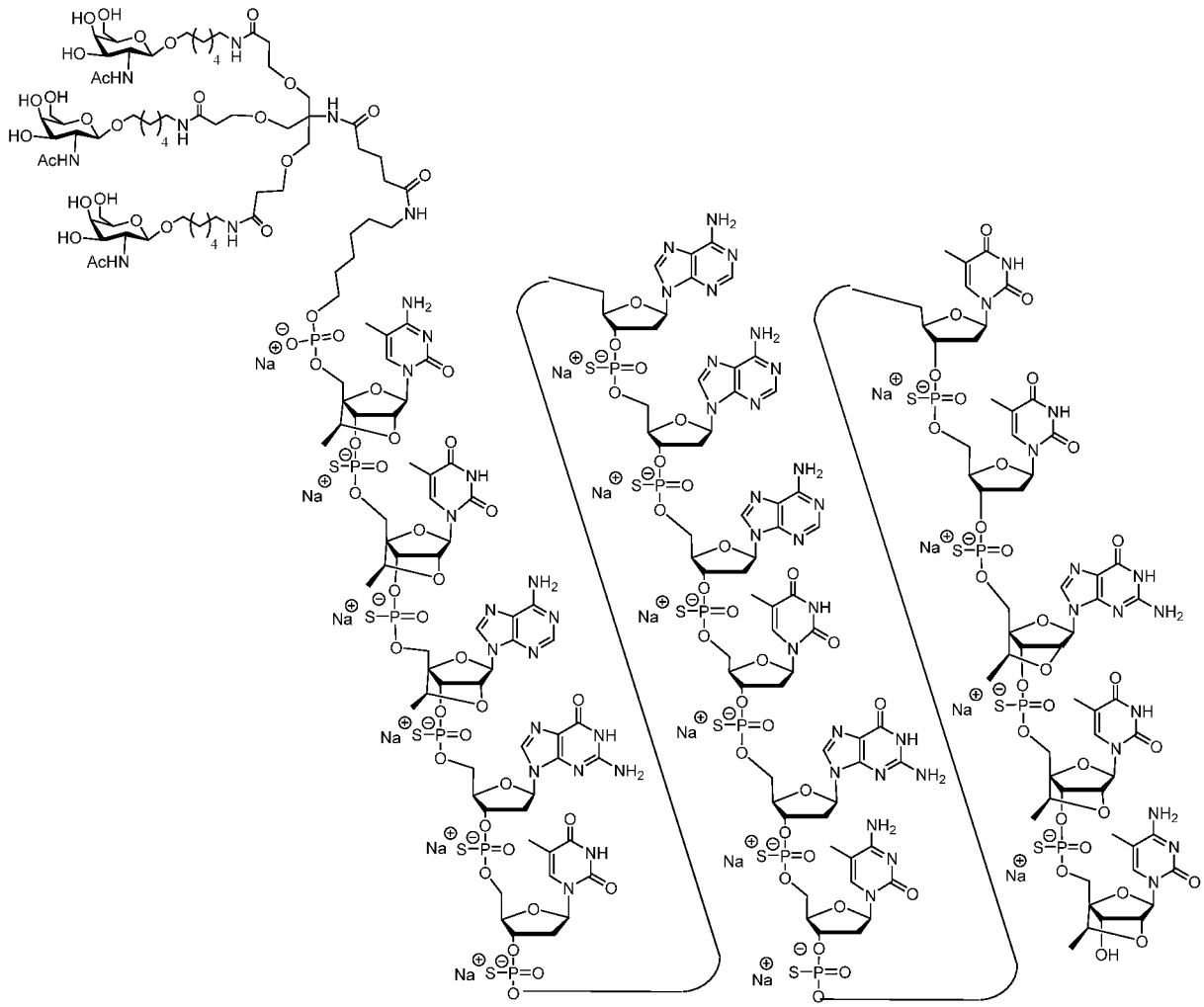
In certain embodiments, a compound comprises or consists of the sodium salt of 975616, having the following chemical structure:



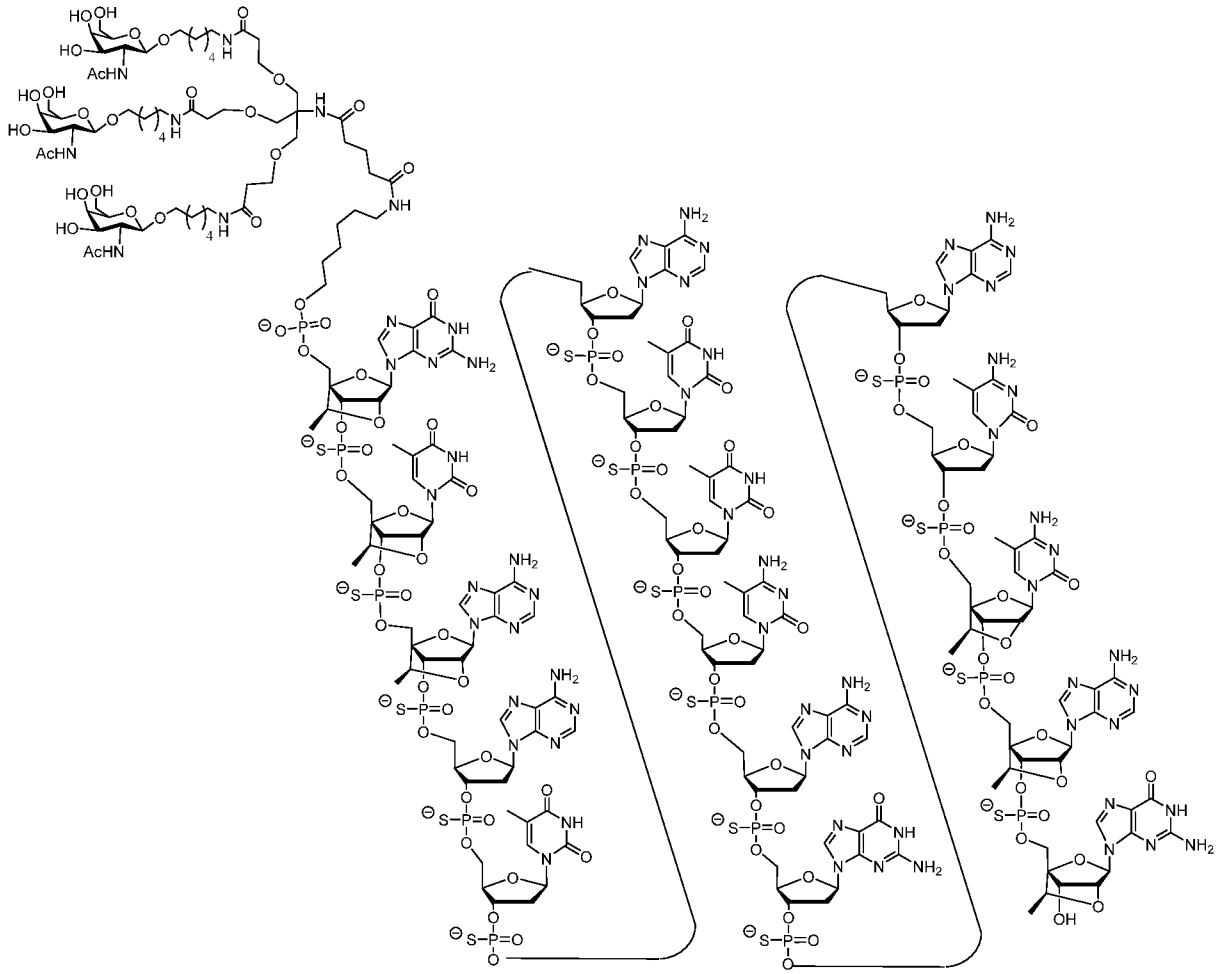
In certain embodiments, a compound comprises or consists of ION 975613 or salt thereof, having the following chemical structure:



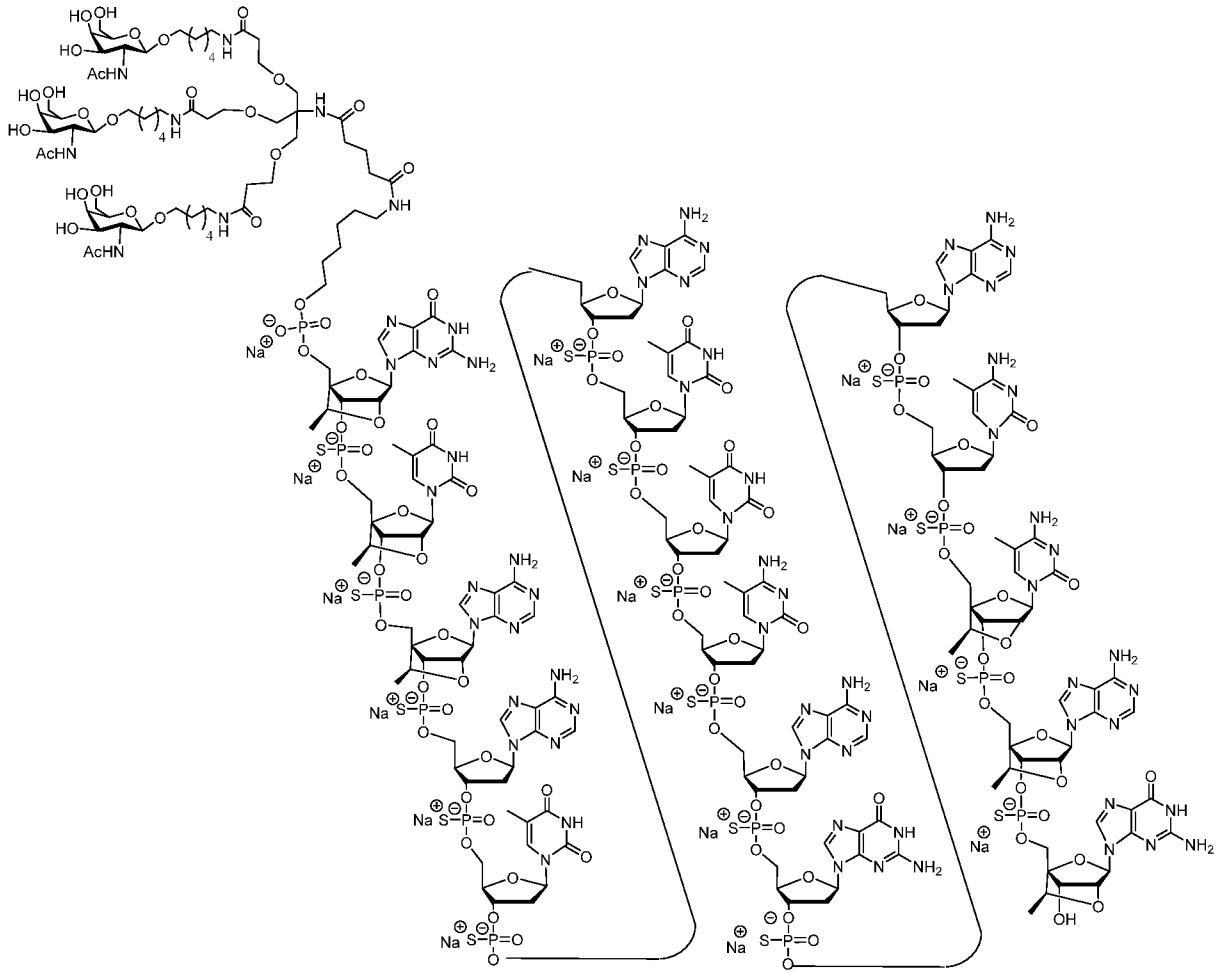
In certain embodiments, a compound comprises or consists of the sodium salt of ION 975613, having the following chemical structure:



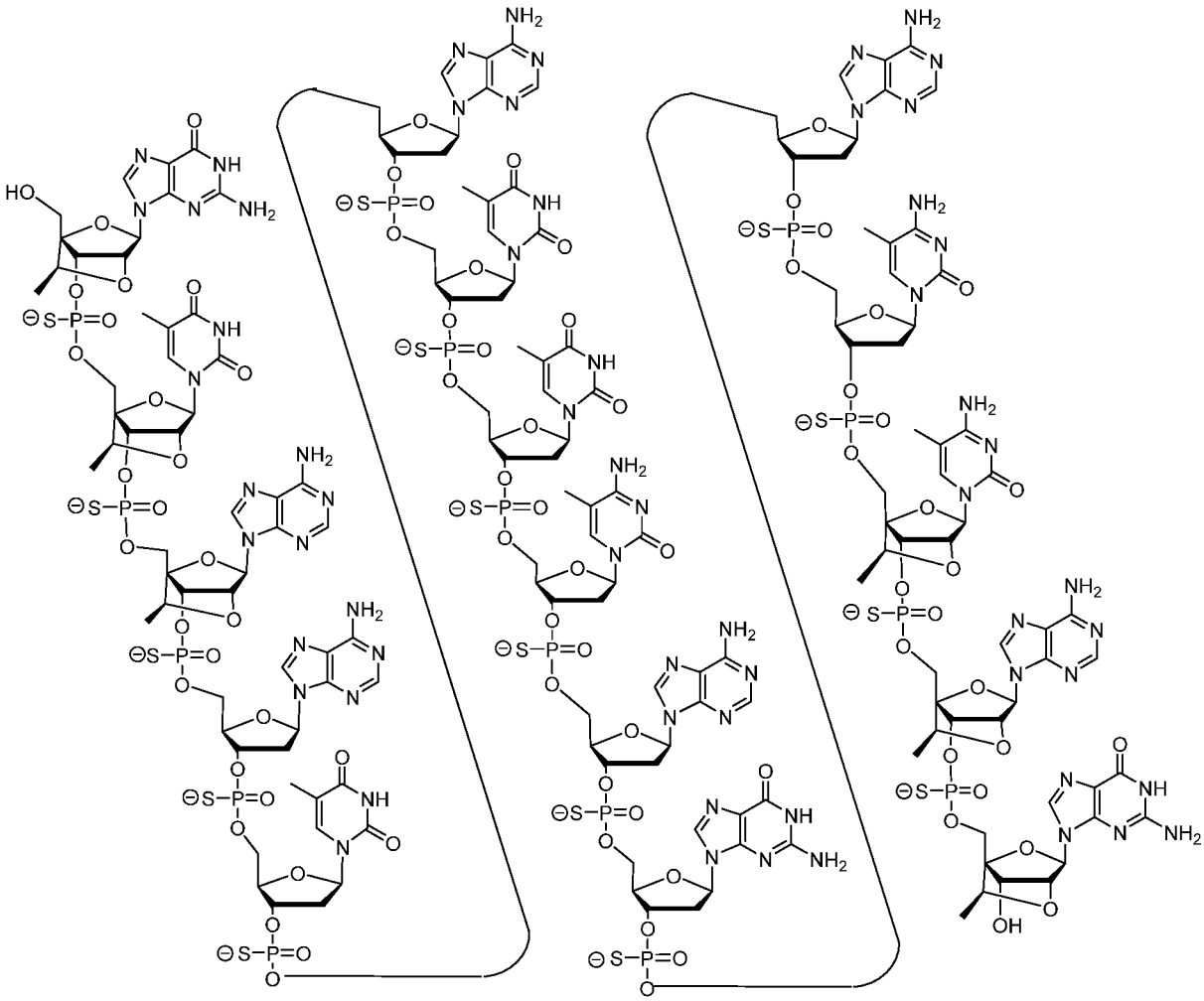
In certain embodiments, a compound comprises or consists of ION 975612 or salt thereof, having the following chemical structure:



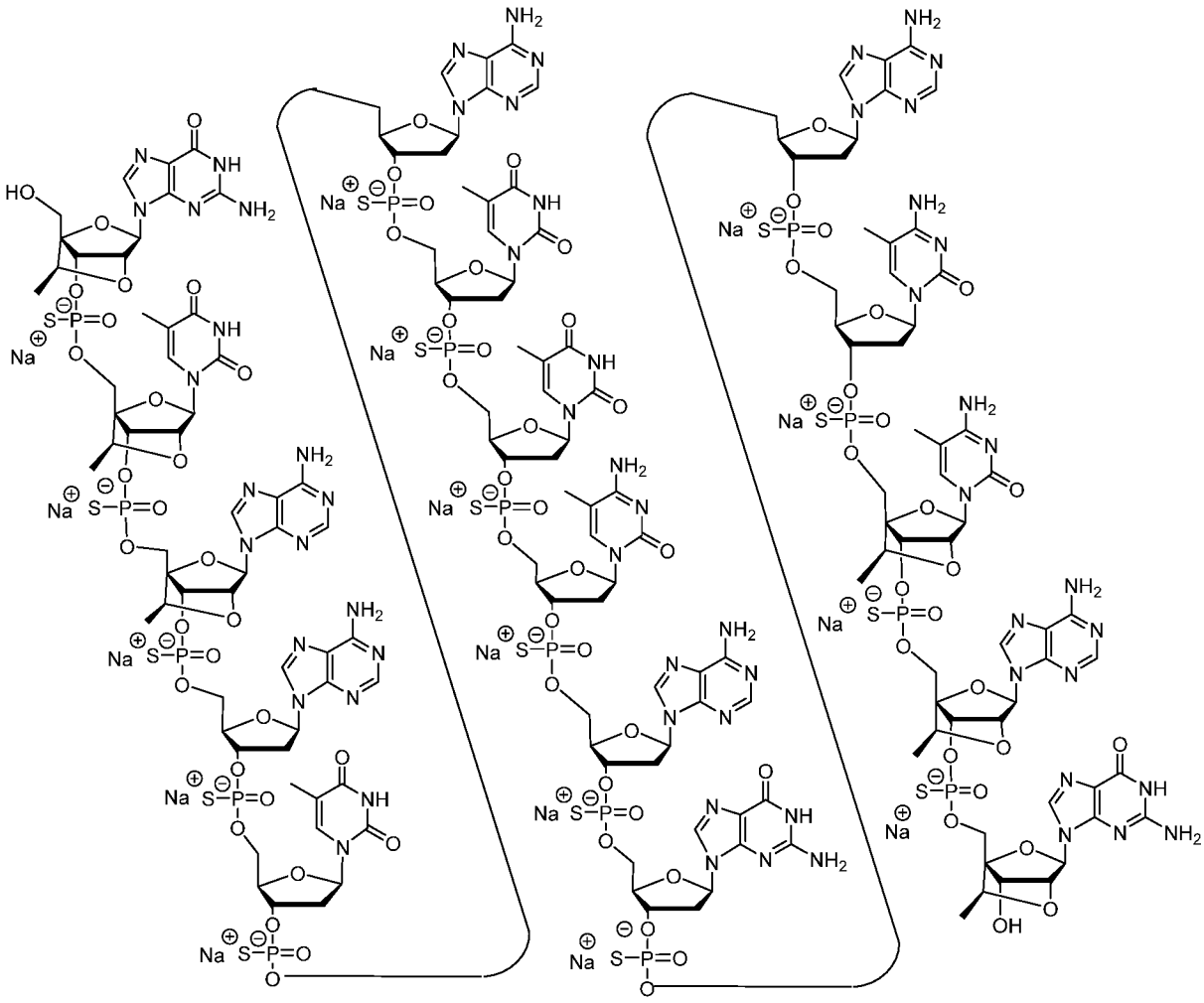
In certain embodiments, a compound comprises or consists of the sodium salt of ION 975612, having the following chemical structure:



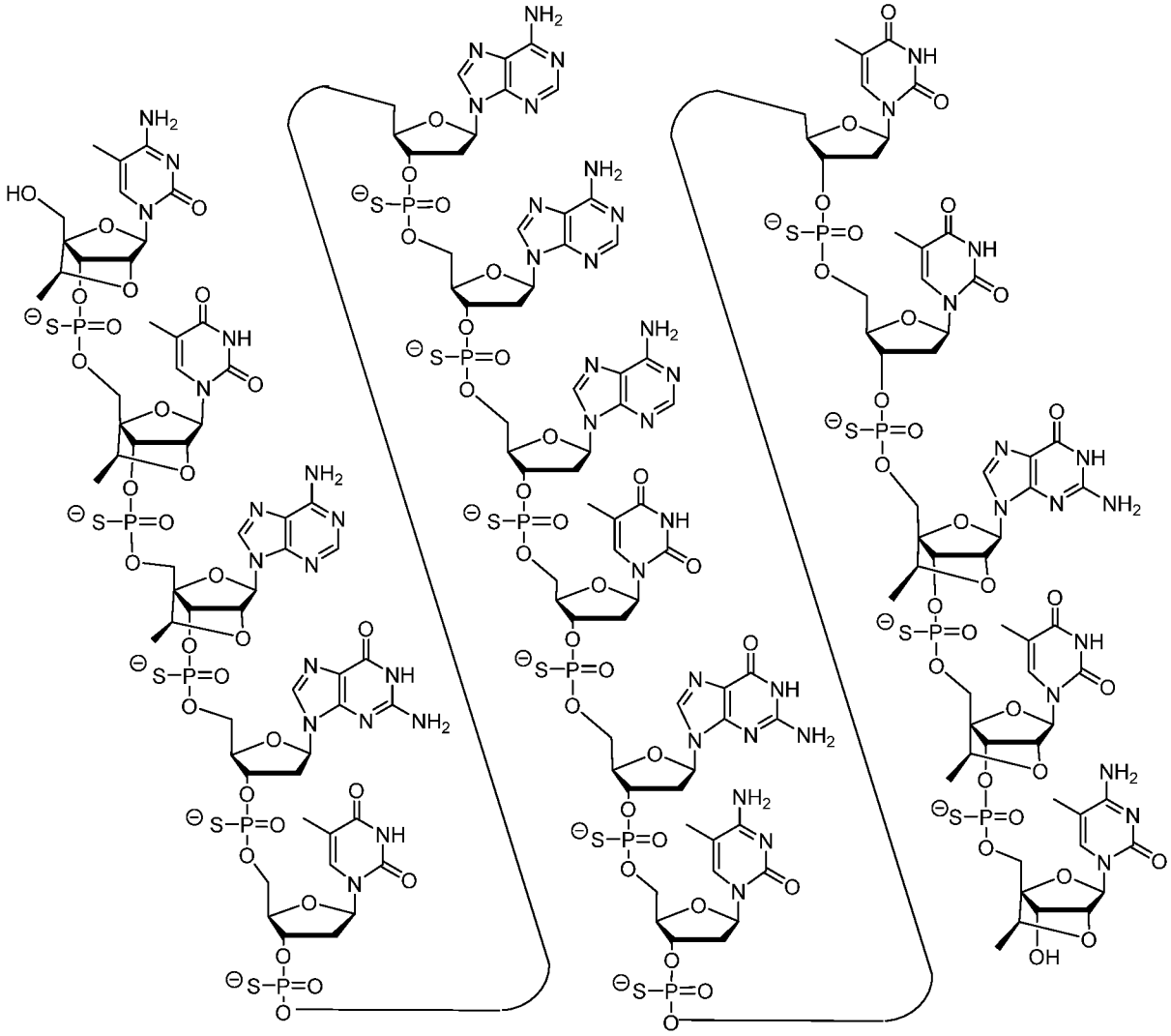
In certain embodiments, a compound comprises or consists of ION 916789 or salt thereof, having the following chemical structure:



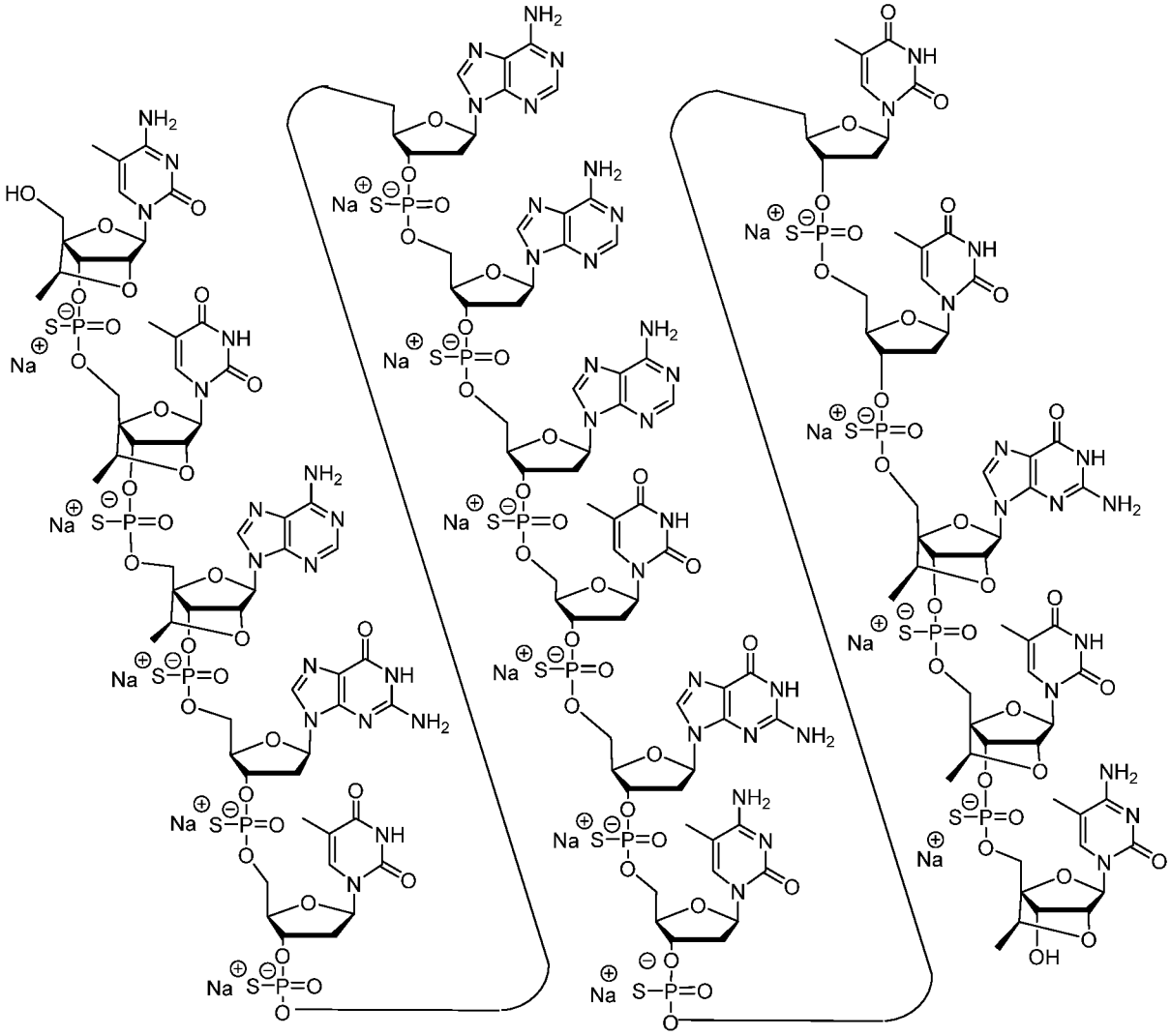
In certain embodiments, a compound comprises or consists of the sodium salt of ION 916789, having the following chemical structure:



In certain embodiments, a compound comprises or consists of ION 916602 or salt thereof, having the following chemical structure:



In certain embodiments, a compound comprises or consists of the sodium salt of ION 916602, having the following chemical structure:



In any of the foregoing embodiments, the compound or oligonucleotide can be at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a nucleic acid encoding PNPLA3.

In any of the foregoing embodiments, the compound can be single-stranded. In certain embodiments, the compound comprises deoxyribonucleotides. In certain embodiments, the compound is double-stranded. In certain embodiments, the compound is double-stranded and comprises ribonucleotides. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound.

In any of the foregoing embodiments, the compound can be 8 to 80, 10 to 30, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked nucleosides in length. In certain embodiments, the compound comprises or consists of an oligonucleotide.

In certain embodiments, a compound comprises a modified oligonucleotide described herein and a conjugate group. In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 5' end of the modified oligonucleotide. In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 3' end of the modified oligonucleotide. In certain embodiments, the conjugate group comprises at least one N- Acetylgalactosamine (GalNAc), at least two N- Acetylgalactosamines (GalNAcs), or at least three N- Acetylgalactosamines (GalNAcs).

In certain embodiments, compounds or compositions provided herein comprise a pharmaceutically acceptable salt of the modified oligonucleotide. In certain embodiments, the salt is a sodium salt. In certain embodiments, the salt is a potassium salt.

In certain embodiments, the compounds or compositions as described herein are active by virtue of having at least one of an *in vitro* IC_{50} of less than 2 μM, less than 1.5 μM, less than 1 μM, less than 0.9 μM, less than 0.8 μM, less than 0.7 μM, less than 0.6 μM, less than 0.5 μM, less than 0.4 μM, less than 0.3 μM, less than 0.2 μM, less than 0.1 μM, less than 0.05 μM, less than 0.04 μM, less than 0.03 μM, less than 0.02 μM, or less than 0.01 μM.

In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having at least one of an increase in alanine transaminase (ALT) or aspartate transaminase (AST) value of no more than 4 fold, 3 fold, or 2 fold over control animals, or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2% compared to control animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase of ALT or AST over control animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over control animals.

Certain embodiments provide a composition comprising the compound of any of the aforementioned embodiments or any pharmaceutically acceptable salt thereof and at least one of a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition has a viscosity less than about 40 centipoise (cP), less than about 30 centipose (cP), less than about 20 centipose (cP), less than about 15 centipose (cP), or less than about 10 centipose (cP). In certain embodiments, the composition having any of the aforementioned viscosities comprises a compound provided herein at a concentration of about 100 mg/mL, about 125 mg/mL, about 150 mg/mL, about 175 mg/mL, about 200 mg/mL, about 225 mg/mL, about 250 mg/mL, about 275 mg/mL, or about 300 mg/mL. In certain embodiments, the composition having any of the aforementioned viscosities and/or compound concentrations has a temperature of room temperature, or about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, or about 30°C.

Certain Indications

Certain embodiments provided herein relate to methods of inhibiting PNPLA3 expression, which can be useful for treating, preventing, or ameliorating a disease associated with PNPLA3 in an individual, by administration of a compound that targets PNPLA3. In certain embodiments, the compound can be a PNPLA3 specific inhibitor. In certain embodiments, the compound can be an antisense compound, an oligomeric compound, or an oligonucleotide targeted to PNPLA3.

Examples of diseases associated with PNPLA3 treatable, preventable, and/or ameliorable with the methods provided herein include liver disease, NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. Certain compounds provided herein are directed to compounds and compositions that reduce liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an animal.

In certain embodiments, a method of treating, preventing, or ameliorating a disease associated with PNPLA3 in an individual comprises administering to the individual a compound comprising a PNPLA3 specific inhibitor, thereby treating, preventing, or ameliorating the disease. In certain embodiments, the individual is identified as having, or at risk of having, a disease associated with PNPLA3. In certain embodiments, the disease is a liver disease. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound. In certain embodiments, the compound is administered to the individual parenterally. In certain

embodiments, administering the compound improves, preserves, or prevents liver damage, steatosis, liver fibrosis, cirrhosis, elevated transaminases, or hepatic fat accumulation in an animal.

In certain embodiments, a method of treating, preventing, or ameliorating liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an animal comprises administering to the individual a compound comprising a PNPLA3 specific inhibitor, thereby treating, preventing, or ameliorating liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound. In certain embodiments, the compound is administered to the individual parenterally. In certain embodiments, administering the compound improves, preserves, or prevents liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation. In certain embodiments, the individual is identified as having, or at risk of having, a disease associated with PNPLA3.

In certain embodiments, a method of inhibiting expression of PNPLA3 in an individual having, or at risk of having, a disease associated with PNPLA3 comprises administering to the individual a compound comprising a PNPLA3 specific inhibitor, thereby inhibiting expression of PNPLA3 in the individual. In certain embodiments, administering the compound inhibits expression of PNPLA3 in the liver. In certain embodiments, the disease is a liver disease. In certain embodiments, the individual has, or is at risk of having, NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary

hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the individual has, or is at risk of having, liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound. In certain embodiments, the compound is administered to the individual parenterally. In certain embodiments, administering the compound improves, preserves, or prevents liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation.

In certain embodiments, a method of inhibiting expression of PNPLA3 in a cell comprises contacting the cell with a compound comprising a PNPLA3 specific inhibitor, thereby inhibiting expression of PNPLA3 in the cell. In certain embodiments, the cell is a hepatocyte. In certain embodiments, the cell is in the liver. In certain embodiments, the cell is in the liver of an individual who has, or is at risk of having, liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length

having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound.

In certain embodiments, a method of reducing or inhibiting liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an individual having, or at risk of having, a disease associated with PNPLA3 comprises administering to the individual a compound comprising a PNPLA3 specific inhibitor, thereby reducing or inhibiting liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in the individual. In certain embodiments, the individual has, or is at risk of having, NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound. In certain embodiments, the compound is administered to the individual parenterally. In certain embodiments, the individual is identified as having, or at risk of having, a disease associated with PNPLA3.

Certain embodiments are drawn to a compound comprising a PNPLA3 specific inhibitor for use in treating a disease associated with PNPLA3. In certain embodiments, the disease is NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound. In certain embodiments, the compound is administered to the individual parenterally.

Certain embodiments are drawn to a compound comprising a PNPLA3 specific inhibitor for use in reducing or inhibiting liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an individual having, or at risk of having, NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408,

830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound.

Certain embodiments are drawn to the use of a compound comprising a PNPLA3 specific inhibitor for the manufacture or preparation of a medicament for treating a disease associated with PNPLA3. Certain embodiments are drawn to the use of a compound comprising a PNPLA3 specific inhibitor for the preparation of a medicament for treating a disease associated with PNPLA3. In certain embodiments, the disease is a liver disease. In certain embodiments, the disease is NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or an oligomeric compound.

Certain embodiments are drawn to the use of a compound comprising a PNPLA3 specific inhibitor for the manufacture or preparation of a medicament for reducing or inhibiting liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in an individual having, or at risk of having, a liver disease associated with PNPLA3. In certain embodiments, the liver disease is NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV

hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. Certain embodiments are drawn to use of a compound comprising a PNPLA3 specific inhibitor for the preparation of a medicament for treating a disease associated with PNPLA3. In certain embodiments, the disease is NAFLD, hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis. In certain embodiments, the compound comprises an antisense compound targeted to PNPLA3. In certain embodiments, the compound comprises an oligonucleotide targeted to PNPLA3. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, a compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, a compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In certain embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In any of the foregoing embodiments, the compound can be single-stranded or double-stranded. In any of the foregoing embodiments, the compound can be an antisense compound or an oligomeric compound.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to SEQ ID NO: 115. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to any one of SEQ ID NOs: 2170-2172.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, consists of the sequence of SEQ ID NO: 115, wherein the modified oligonucleotide comprises:

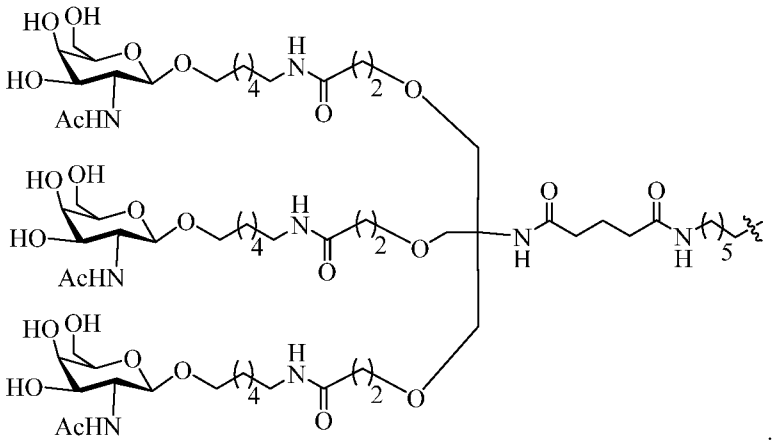
a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a

phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, further comprises a conjugate group, wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



Certain PNPLA3 Variants

Further embodiments of the present disclosure relate to individuals with certain variants in patatin-like phospholipase domain-containing protein 3 (PNPLA3). An isoleucine-to-methionine mutation at position 148 of the PNPLA3 protein (referred to herein as "PNPLA3 I148M," "I148M," "148I allelic variant," or "PNPLA3 rs738409 polymorphism"; amino acid residue numbering relative to human PNPLA3) can be a strong genetic determinant of non-alcoholic steatohepatitis (NASH). The PNPLA3 I148M mutant protein exhibits reduced enzymatic activity. It was discovered that certain treatments, e.g., compounds described herein, can have an unexpectedly effective treatment of liver disease in individuals, e.g., human patients, with the PNPLA3 I148M mutation. As used herein, an individual "having" or "with" an I148M mutation in PNPLA3 means that the individual has a mutation in the nucleotide sequence of the gene encoding PNPLA3 corresponding to an isoleucine-to-methionine substitution at position 148 of the PNPLA3 protein.

In some embodiments, the present disclosure provides a method of treating an individual having or at risk of having liver disease, comprising administering a compound targeted to PNPLA3 in the individual, wherein the individual has an I148M mutation in PNPLA3.

In some embodiments, treating an individual having liver disease means slowing or stopping the progression of the disease. In some embodiments, treating an individual having liver disease means the liver of the individual returns to a normal, healthy state from a diseased state, for example, as measured by the amount of liver lipids and/or scar tissue, and/or by the amount of liver function as compared to a healthy individual. In some embodiments, when an individual with the PNPLA3 I148M mutation and having liver disease is treated

with the method, the individual's liver lipids do not substantially increase. In some embodiments, the method reduces the individual's liver lipids by about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100%. Methods of determining the amount of liver lipid are known to one of ordinary skill in the art and include, e.g., Oil Red O staining of a liver biopsy, magnetic resonance spectroscopy (MRS), and lipoprotein subfraction assays.

In some embodiments, when an individual with the PNPLA3 I148M mutation and having liver disease is treated with the method, the individual's liver scar tissue does not substantially increase. Methods of determining the amount of liver scar tissue are known to one of ordinary skill in the art. In some embodiments, the method reduces the individual's liver scar tissue by about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100%. Methods of determining the amount of scar tissue are known to one of ordinary skill in the art and include, for example, imaging tests such as, e.g., ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound elastography, magnetic resonance elastography, and/or acoustic radiation force impulse imaging; blood tests; and liver biopsy.

In some embodiments, when an individual with the PNPLA3 I148M mutation and having liver disease is treated with the method, the individual's liver function does not substantially decrease. In some embodiments, after being treated with the method, the individual's liver function increases. In some embodiments, after being treated with the method, the individual's liver function increases by about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100%. In some embodiments, after being treated with the method, the individual's liver function is about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or greater than 99% of the liver function of a healthy individual. Methods of measuring liver function are known to one of ordinary skill in the art and include, for example, measuring levels of one or more of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin.

In some embodiments, treating an individual at risk of having liver disease means preventing or reducing the likelihood that the individual develops the disease, for example, by reducing liver lipids and/or scar tissue, or any other compounds, e.g., proteins, polynucleotides, that can cause or exacerbate the development of liver disease.

Examples of liver diseases including, e.g., diseases associated with PNPLA3, are described herein. In some embodiments, the liver disease is selected from non-alcoholic fatty liver disease (NAFLD), hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or

primary sclerosing cholangitis. In some embodiments, the liver disease is hepatic steatosis. In some embodiments, the method provides a highly effective treatment for the liver disease, e.g., hepatic steatosis, when administered to an individual having an I148M mutation in PNPLA3.

In some embodiments, the present disclosure provides a method of reducing one or more of liver damage, hepatic steatosis, liver inflammation, liver fibrosis, and hepatic lipogenesis in an individual, comprising administering a compound targeted to PNPLA3 to the individual, wherein the individual has an I148M mutation in PNPLA3. In some embodiments, the method reduces or inhibits liver inflammation. In some embodiments, the method reduces or inhibits liver fibrosis.

In some embodiments, treating liver disease in an individual comprises reducing one or more of liver damage, hepatic steatosis, liver inflammation, liver fibrosis, and hepatic lipogenesis. Examples of liver diseases are described herein. In some embodiments, the method is highly effective at reducing one or more of liver damage, hepatic steatosis, liver inflammation, liver fibrosis, and hepatic lipogenesis in an individual having an I148M mutation in PNPLA3. In some embodiments, the method is highly effective at reducing one or more of hepatic steatosis, liver inflammation, and liver fibrosis in an individual having an I148M mutation in PNPLA3.

In some embodiments, reducing or inhibiting liver inflammation comprises reducing liver macrophage levels. Liver macrophage levels can be quantified, e.g., by immunohistochemical staining of macrophage antigen 2 (Mac2), which is expressed on the surface of inflammatory macrophages. In some embodiments, the liver macrophage levels are reduced by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% relative to an individual not administered the compound targeted to PNPLA3, as measured by an immunohistochemical staining, e.g., of Mac2, of a liver section of the individual.

In some embodiments, the reducing liver macrophage levels comprises reducing the amount of monocyte chemoattractant protein (MCP1) in the liver, e.g., in a liver cell. MCP1, also known as chemokine (C-C- motif) ligand 2 (CCL2) and small inducible cytokine A2, and its receptor C-C chemokine receptor-2 (CCR2) play a role in recruiting monocytes, dendritic cells, and macrophages to sites of inflammation in the liver. In some embodiments, reducing expression of MCP1 in the liver reduces liver macrophage levels. In some embodiments, reducing expression of MCP1 in the liver reduces liver inflammation. In some embodiments, reducing liver macrophage levels comprises reducing the amount of haptoglobin in the liver, e.g., in a liver cell. Haptoglobin is an acute phase protein produced in the liver and adipose tissue, typically in response to inflammation, infection, and/or tissue injury. Haptoglobin can attract monocytes and macrophages in part through interaction with CCR2, described herein. In some embodiments, reducing expression of haptoglobin in the liver reduces liver inflammation.

In some embodiments, the present disclosure provides a method of reducing protein levels of one or more of haptoglobin, MCP1, and TIMP2 in an individual, comprising administering a compound targeted to PNPLA3 to the individual, wherein the individual has an I148M mutation in PNPLA3.

Haptoglobin and its role in liver inflammation are described herein. In some embodiments, the method reduces protein levels of haptoglobin in an individual with an I148M mutation in PNPLA3. In some embodiments, the method reduces expression of haptoglobin in an individual with an I148M mutation in PNPLA3. In some embodiments, protein levels of haptoglobin are reduced by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% relative to an individual not administered the compound targeted to PNPLA3. Methods of measuring haptoglobin levels in a sample are known to one of ordinary skill in the art and can include, e.g., spectrophotometry, immunoassay, electrophoresis and the like. In some embodiments, haptoglobin levels in a sample from an individual, e.g., an individual with PNPLA3 I148M, are measured by a turbidimetric assay, e.g., using an ABX Pentra instrument. In some embodiments, haptoglobin protein levels in a sample from an individual, e.g., an individual with PNPLA3 I148M, are measured by a colorimetric assay, e.g., the PHASE™ Range Haptoglobin Colorimetric Assay.

MCP1 and its role in liver inflammation are described herein. In some embodiments, the method reduces protein levels of MCP1 in an individual with an I148M mutation in PNPLA3. In some embodiments, the method reduces expression of MCP1 in an individual with an I148M mutation in PNPLA3. In some embodiments, protein levels of MCP1 are reduced by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% relative to an individual not administered the compound targeted to PNPLA3. Methods of measuring MCP1 levels in a sample are known to one of ordinary skill in the art and can include, e.g., immunoassay (e.g., ELISA), immunoblotting, electrophoresis, chromatography and the like. In some embodiments, MCP1 protein levels in a sample from an individual, e.g., an individual with PNPLA I148M, are measured by immunoblotting of a liver sample of the individual.

In some embodiments, the method reduces protein levels of TIMP2 in an individual with an I148M mutation in PNPLA3. In some embodiments, the method reduces expression of TIMP2 in an individual with an I148M mutation in PNPLA3. Tissue inhibitor of metalloproteases 2 (TIMP2) is a member of the TIMP family, which are generally natural inhibitors of the matrix metalloproteinase (MMP) group of peptidases involved in degradation of the extracellular matrix. TIMP2 expression was shown to be elevated in activated human hepatic stellate cells and in fibrotic rat livers (see, e.g., Xu et al., *Gut* 54(1):142-151, 2005; and Peng et al., *Exp Biol Med* 238(6):668-677, 2013). Moreover, TIMP2 can inhibit the collagenolytic activity of matrix metalloproteinase 2 (MMP2), which is increased in experimental models of liver fibrosis and in humans with chronic liver disease (see, e.g., Lindén et al., *Mol Metab* 22:49-61, 2019). In some embodiments, TIMP2

inhibition of MMP2 reduces liver fibrosis. In some embodiments, protein levels of TIMP2 are reduced by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% relative to an individual not administered the compound targeted to PNPLA3. Methods of measuring TIMP2 levels in a sample are known to one of ordinary skill in the art and can include, e.g., immunoassay (e.g., ELISA), immunoblotting, electrophoresis, chromatography and the like. In some embodiments, TIMP2 protein levels in a sample from an individual, e.g., an individual with PNPLA I148M, are measured by immunoblotting of a liver sample of the individual.

In some embodiments, the individual has a heterozygous I148M mutation in PNPLA3. As used herein, a "heterozygous" mutation means a mutation of one allele (with the other allele being non-mutated, i.e., wild type). In some embodiments, the individual has a homozygous I148M mutation in PNPLA3. As used herein, a "homozygous" mutation means an identical mutation of two alleles. In some embodiments, the individual has a compound heterozygous mutation at position 148 of PNPLA3. As used herein, a "compound heterozygous" mutation means a different mutation at each of the two alleles. For example, a compound heterozygous mutation at position 148 of PNPLA3 can comprise an I148M mutation at one allele, and a different mutation at the other allele. In some embodiments, the compound heterozygous mutation at position 148 of PNPLA3, wherein one allele is I148M, has the same phenotype as the homozygous I148M mutation in PNPLA3. In some embodiments, the compound heterozygous mutation at position 148 of PNPLA3, wherein one allele is I148M, has a different phenotype from either the homozygous I148M mutation or the heterozygous I148M mutation in PNPLA3. In some embodiments, an individual having a PNPLA3 I148M mutation in at least one allele has elevated risk of liver disease. In some embodiments, an individual having a homozygous PNPLA3 I148M mutation has an elevated risk of liver disease. In some embodiments, the methods provided herein unexpectedly provide a highly effective treatment of liver disease in an individual having a PNPLA3 I148M mutation in at least one allele. In some embodiments, the methods provided herein unexpectedly provide a highly effective treatment of liver disease in an individual having a homozygous PNPLA3 I148M mutation.

In some embodiments, the individual is a human individual. In some embodiments, the individual is an animal, e.g., a cow, horse, dog, cat, rat, or mouse. In embodiments where the individual is non-human, it will be understood by one of ordinary skill in the art that the amino acid residue numbers for PNPLA3 may not be the same as the human PNPLA3. The skilled artisan can determine the residue corresponding to residue 148 in human PNPLA3 using sequence alignment methods known in the field, e.g., BLAST, Clustal, HMMER, and the like.

In some embodiments, the methods herein comprise administering a compound targeted to PNPLA3 to an individual having an I148M mutation in PNPLA3. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is an antisense compound targeted to PNPLA3.

Antisense compounds are described herein. In some embodiments, the antisense compound targeted to PNPLA3, in an individual having an I148M mutation, is a short interfering RNA (siRNA). In some embodiments, the antisense compound targeted to PNPLA3, in an individual having an I148M mutation, comprises any of

5′-GGUCCUCUCAGAUCUUGUGtt-3′ (SEQ ID NO: 2170),

5′-GGAGUGAGUGACAACGUACtt-3′ (SEQ ID NO: 2171), or

5′-GGUUCUUGGAAGAGAAGGGtt-3′ (SEQ ID NO: 2172).

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to any of SEQ ID NOs: 2170-2172.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, is an antisense oligonucleotide (ASO). ASOs are described herein. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide 8 to 80 linked nucleosides in length having a having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In some embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169. In some embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In some embodiments, the compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound comprises a modified oligonucleotide 16 to 30 linked nucleosides in length having a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In some embodiments, the compound comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899. In some embodiments, the compound is ION 975616, 994284, 975605, 994282, 975613, 975617, 975735, 975736, or 975612. In some embodiments, the compound comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to SEQ ID NO: 115.

In some embodiments, the compound comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8

contiguous nucleobases 100% complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein the nucleobase sequence of the modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2. In some embodiments, the compound comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence complementary within nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein said modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2.

In some embodiments, the compound comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of a PNPLA3 nucleic acid having the nucleobase sequence of SEQ ID NO: 2, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 2. In some embodiments, the compound comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a 16 nucleobase portion complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2. In some embodiments, the modified oligonucleotide has a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% complementary to SEQ ID NO: 2 over the entire length of the nucleobase sequence.

In any of the foregoing methods or uses, the compound can be targeted to PNPLA3. In certain embodiments, the compound comprises or consists of a modified oligonucleotide, for example, a modified oligonucleotide 8 to 80 linked nucleosides in length, 10 to 30 linked nucleosides in length, 12 to 30 linked nucleosides in length, or 20 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is at least 80%, at least 85%, at least 90%, at least 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-10. In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage, the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl modified sugar, and the modified nucleobase is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide comprises a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between

the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In any of the foregoing embodiments, the modified oligonucleotide is 12 to 30, 15 to 30, 15 to 25, 15 to 24, 16 to 24, 17 to 24, 18 to 24, 19 to 24, 20 to 24, 19 to 22, 20 to 22, 16 to 20, or 16 or 20 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is at least 80%, at least 85%, at least 90%, at least 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-10.

In any of the foregoing methods or uses, the compound comprises or consists of a modified oligonucleotide 16 to 30 linked nucleosides in length and having a nucleobase sequence comprising any one of SEQ ID NOs: 17-2169, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked 2'-deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In any of the foregoing methods or uses, the compound comprises or consists a modified oligonucleotide 16 linked nucleosides in length having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide is 16-30 linked nucleosides in length.

In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to SEQ ID NO: 115. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to any one of SEQ ID NOs: 2170-2172.

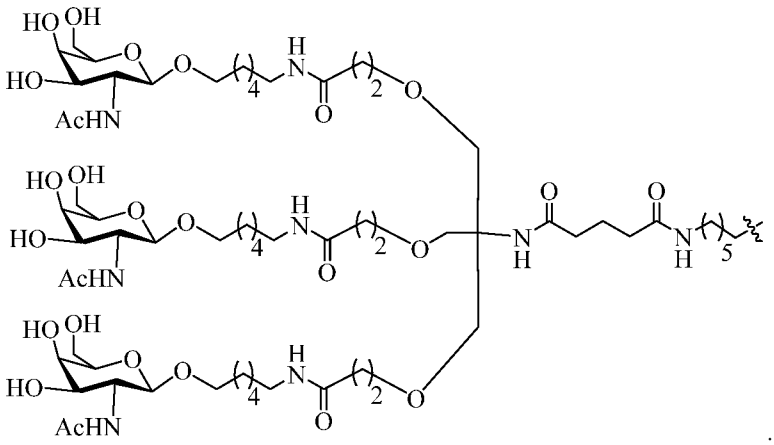
In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, consists of the sequence of SEQ ID NO: 115, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

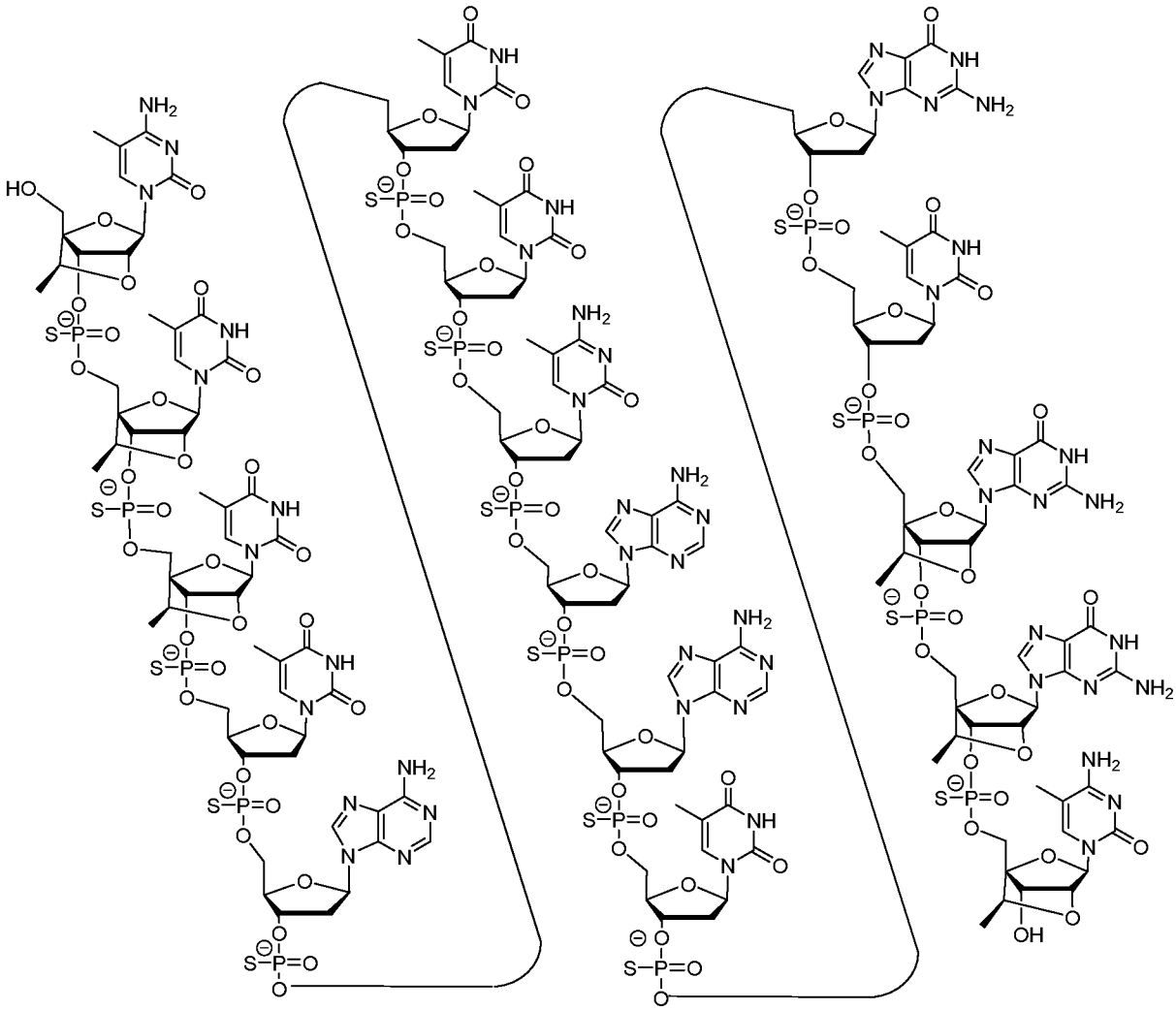
a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

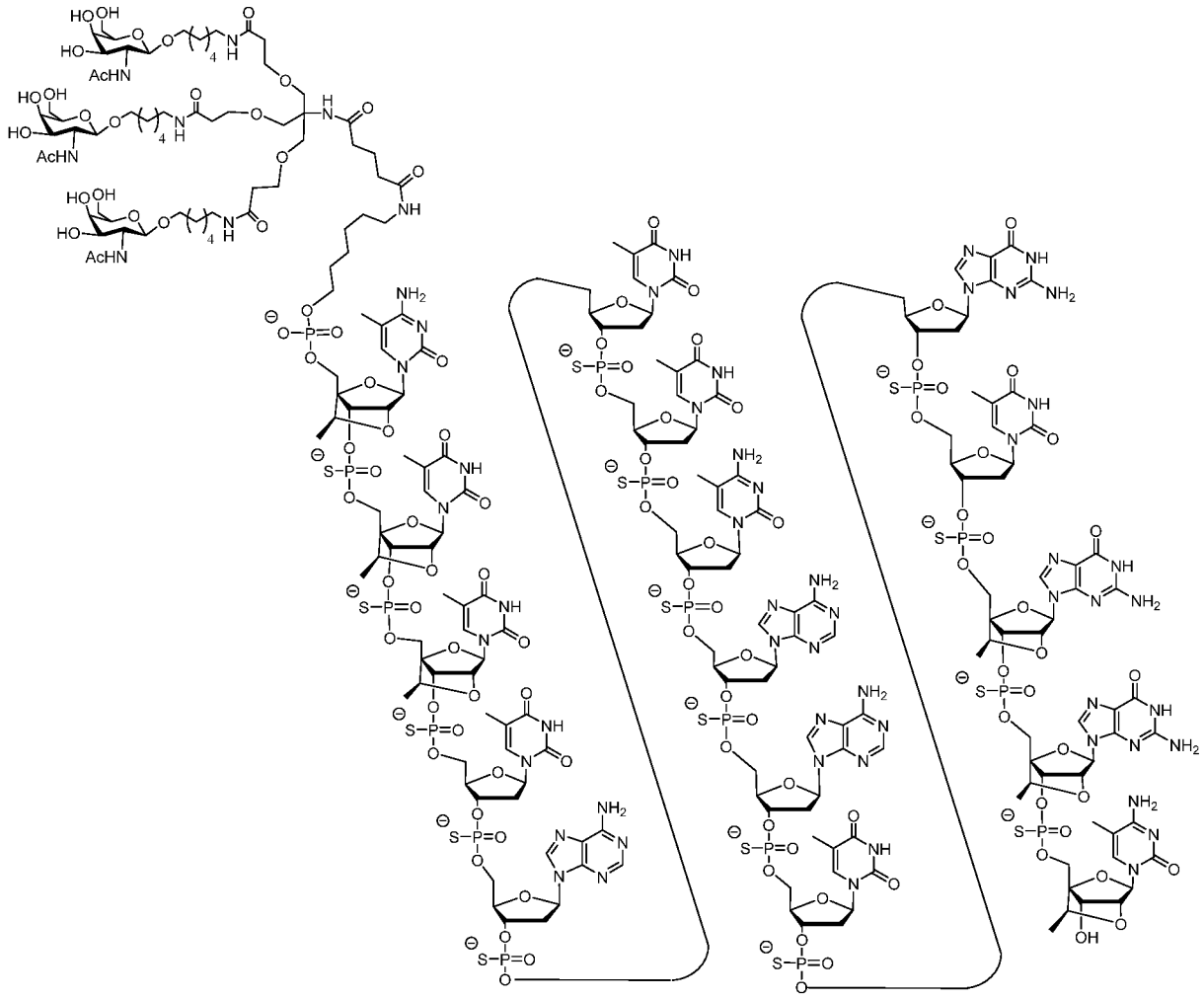
wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In some embodiments, the compound targeted to PNPLA3, in an individual having an I148M mutation, further comprises a conjugate group, wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



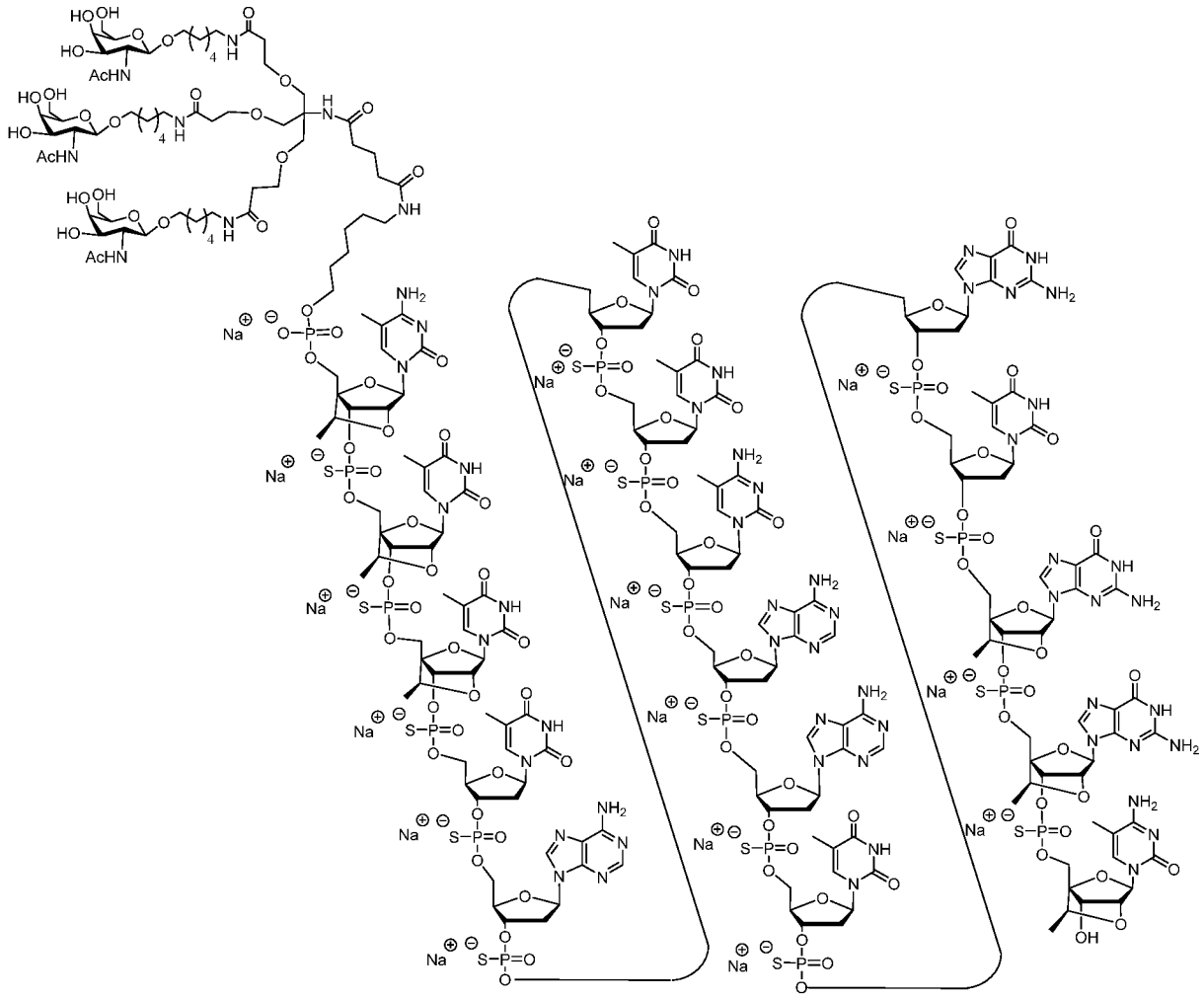
In certain embodiments, a compound comprises or consists of ION 916333 or salt thereof, having the following chemical structure:



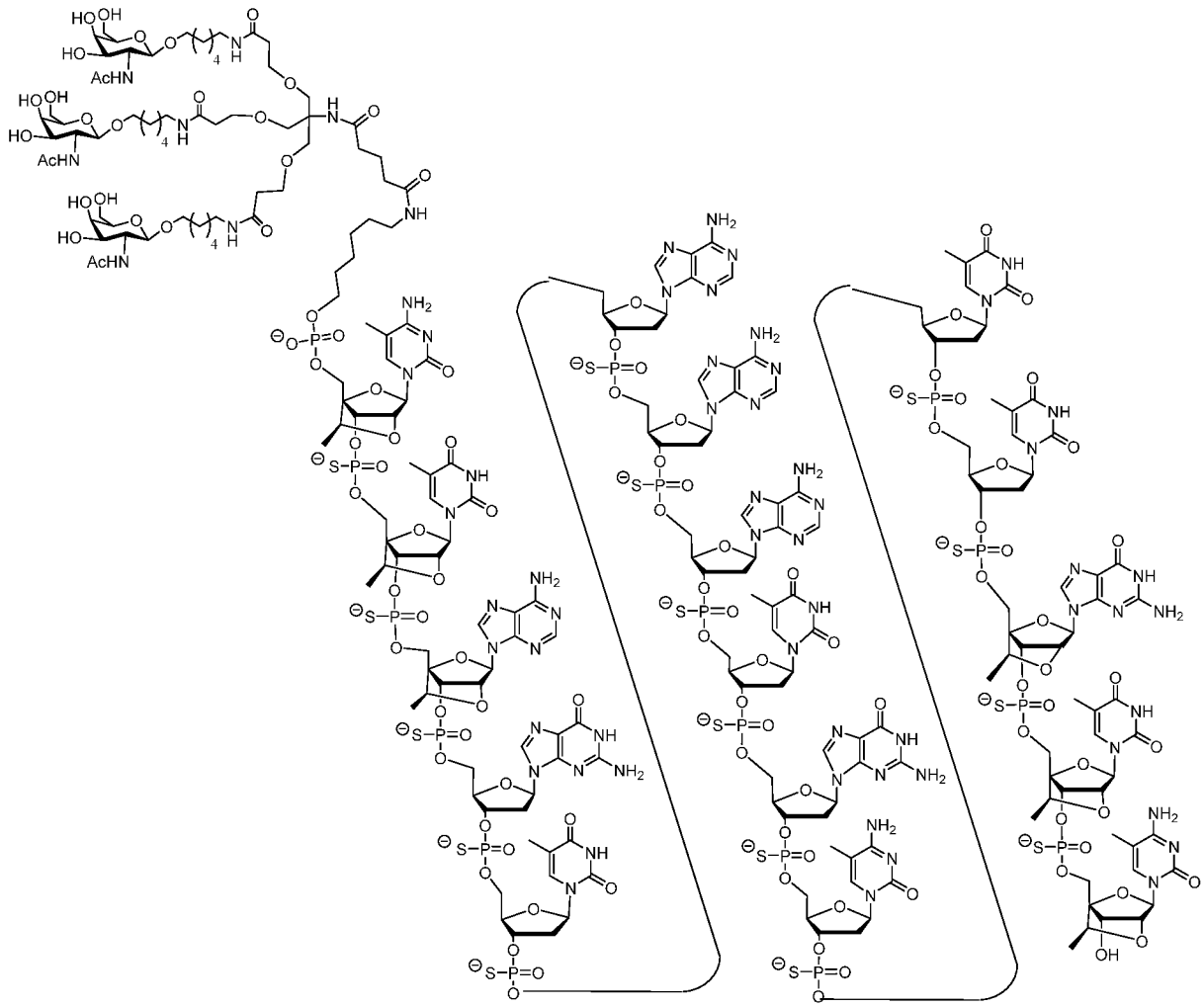
In certain embodiments, a compound comprises or consists of ION 975616 or salt thereof, having the following chemical structure:



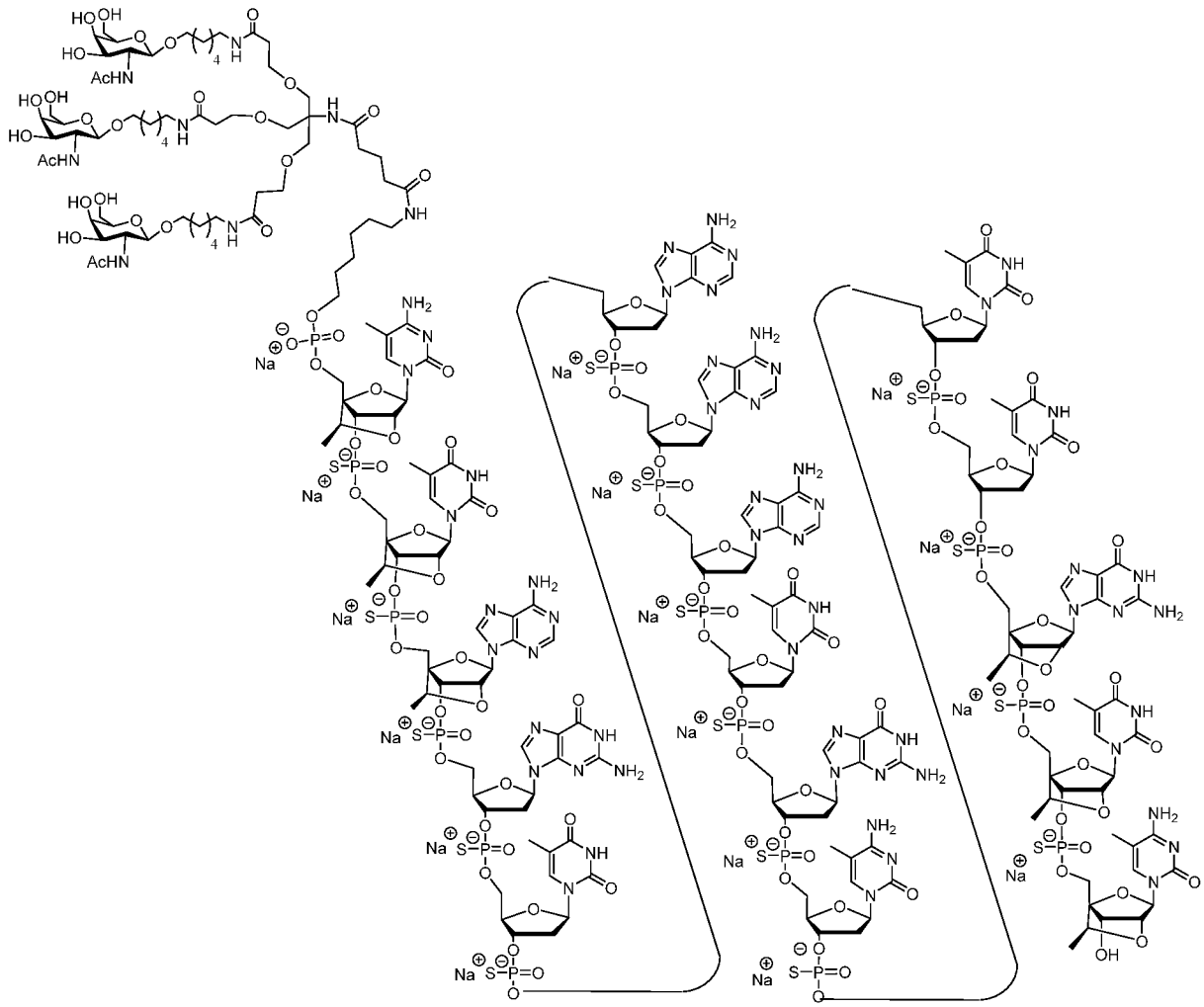
In certain embodiments, a compound comprises or consists of the sodium salt of ION 975616, having the following chemical structure:



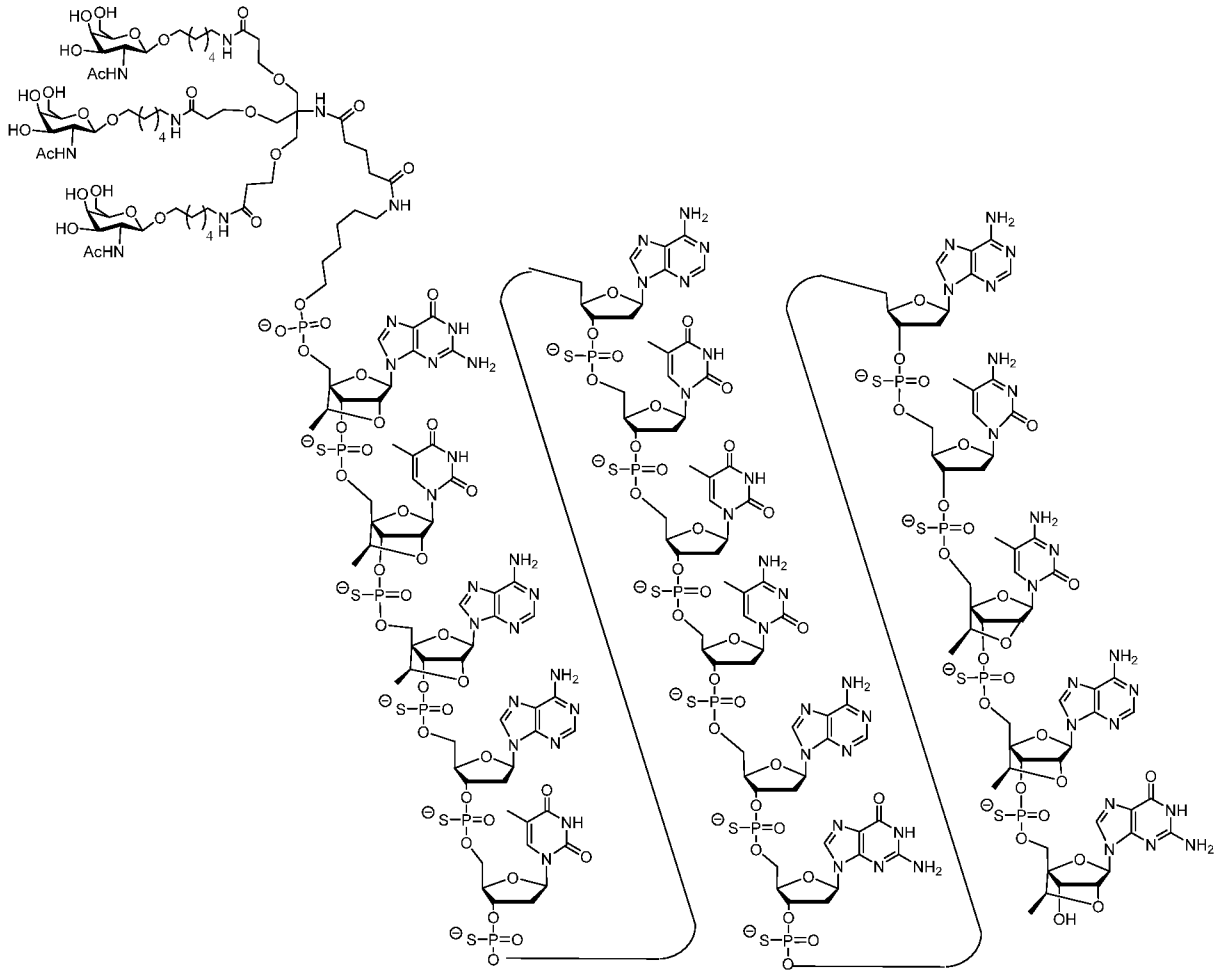
In certain embodiments, a compound comprises or consists of ION 975613 or salt thereof, having the following chemical structure:



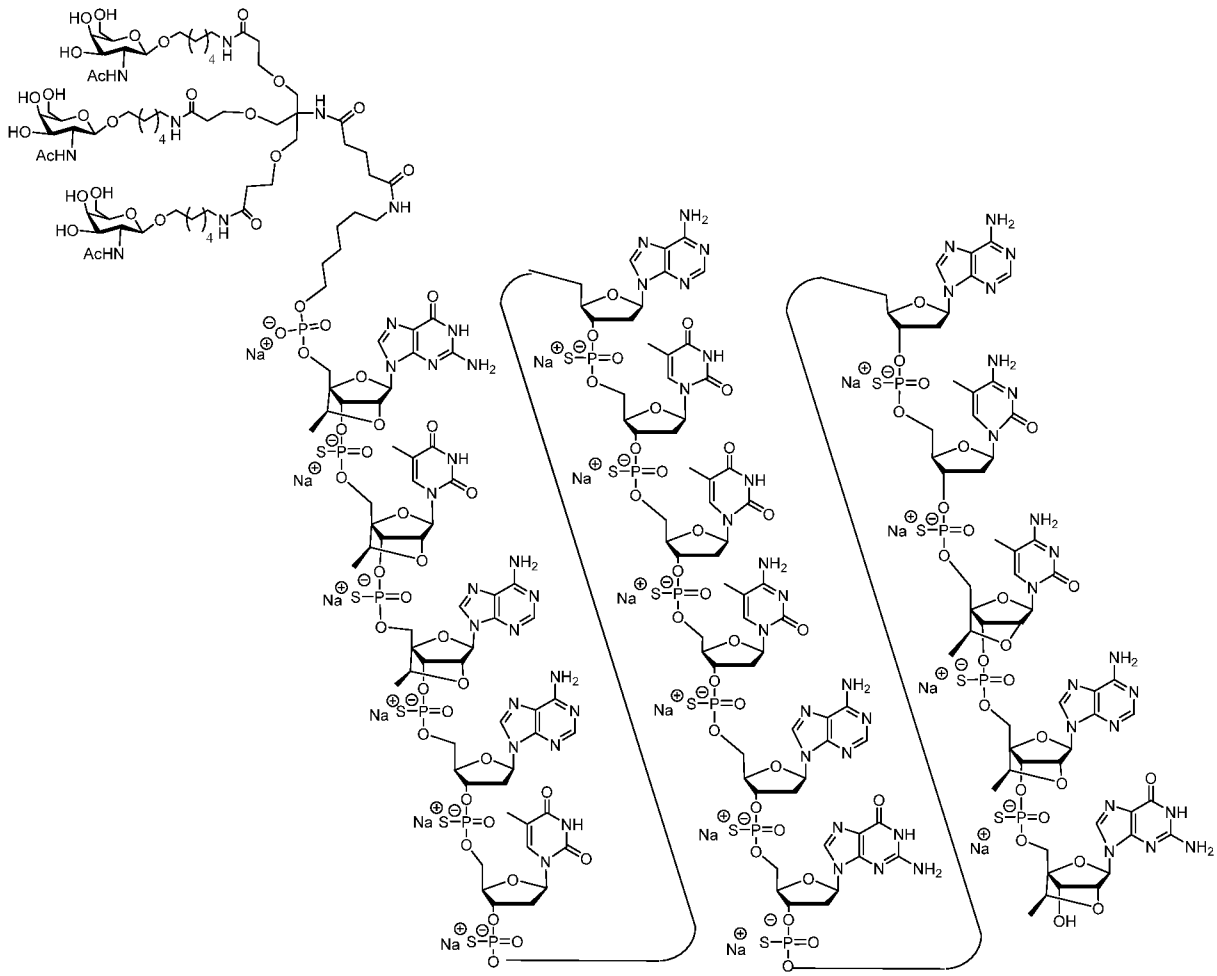
In certain embodiments, a compound comprises or consists of the sodium salt of ION 975613, having the following chemical structure:



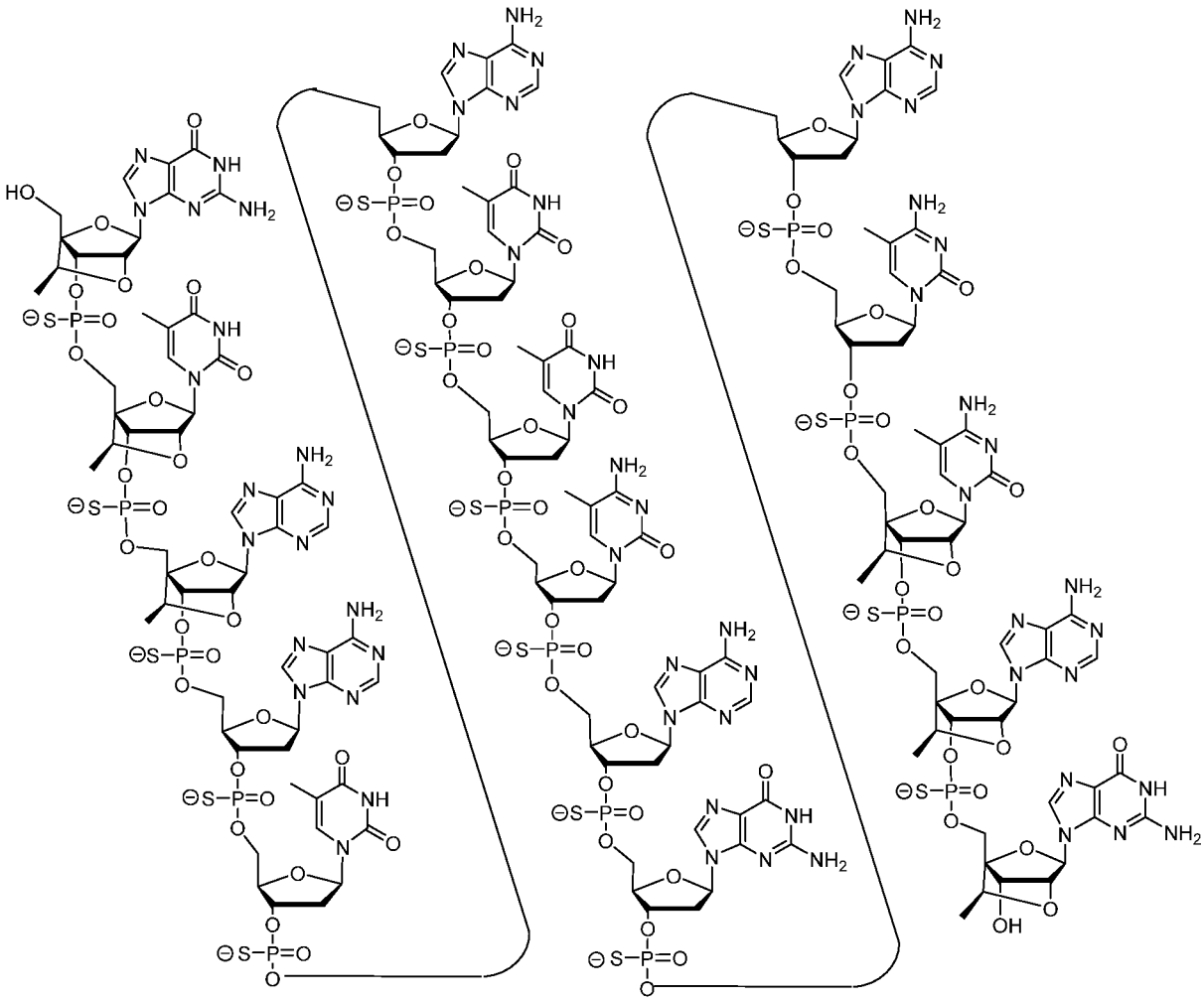
In certain embodiments, a compound comprises or consists of ION 975612 or salt thereof, having the following chemical structure:



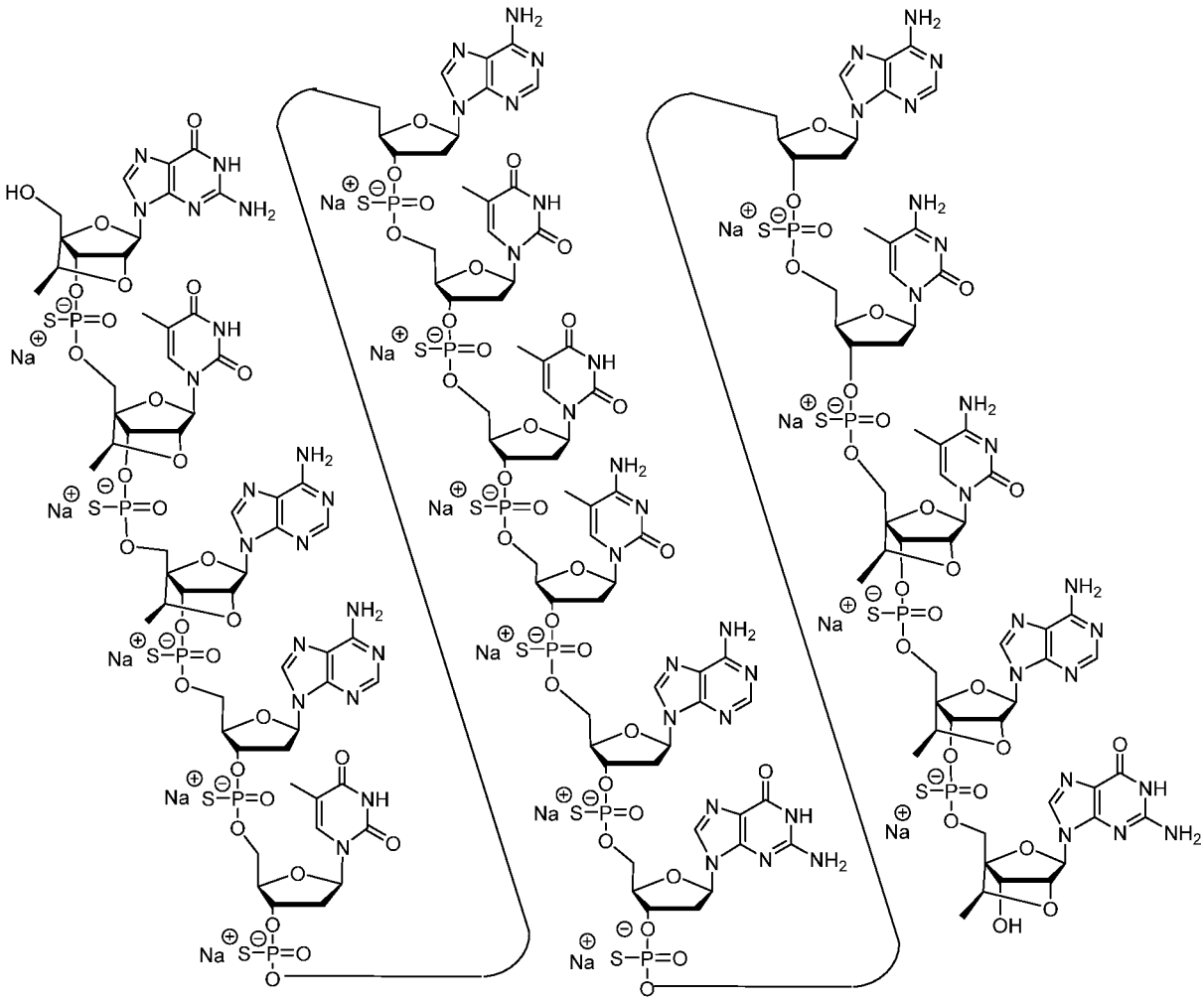
In certain embodiments, a compound comprises or consists of the sodium salt of ION 975612, having the following chemical structure:



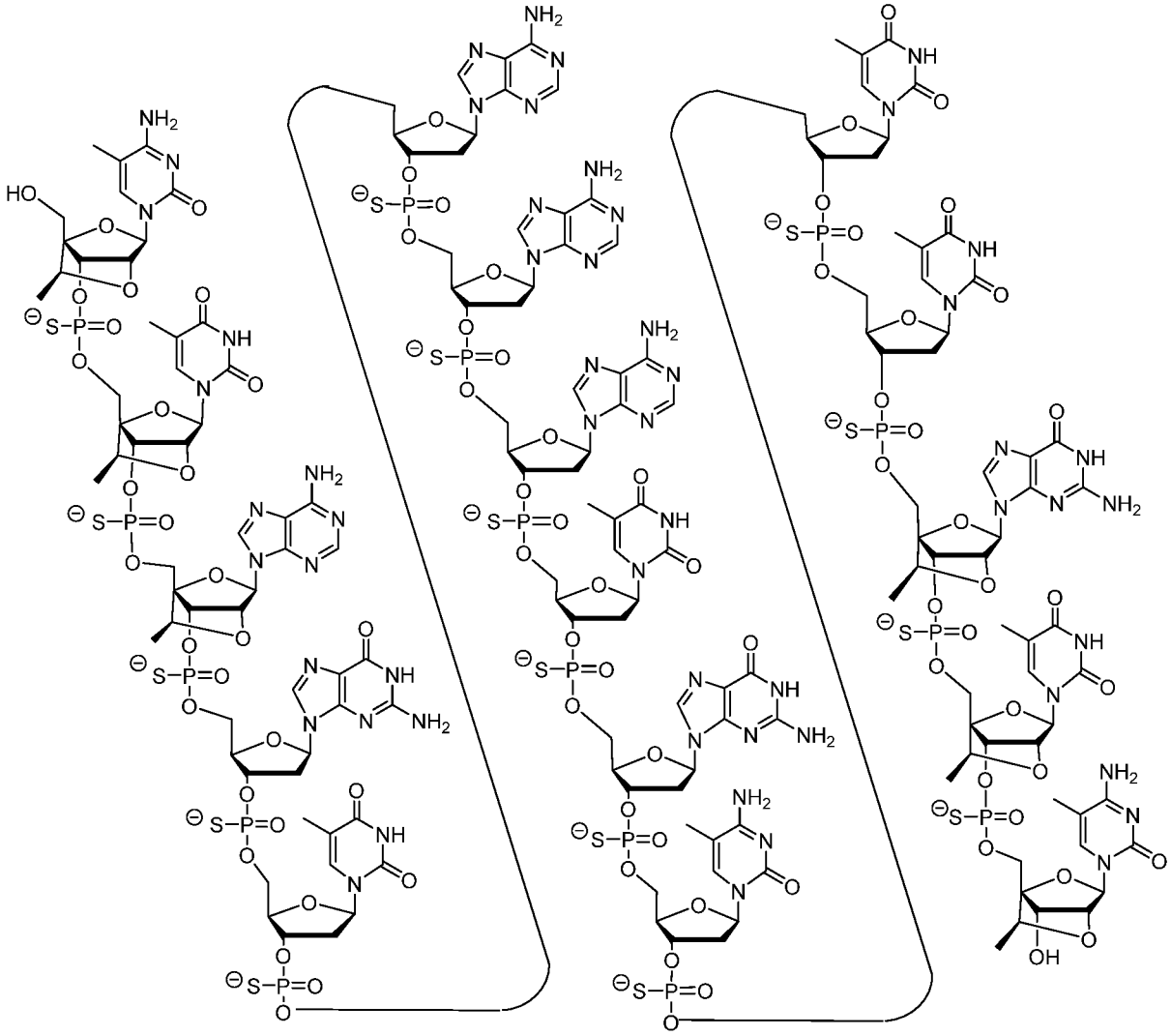
In certain embodiments, a compound comprises or consists of ION 916789 or salt thereof, having the following chemical structure:



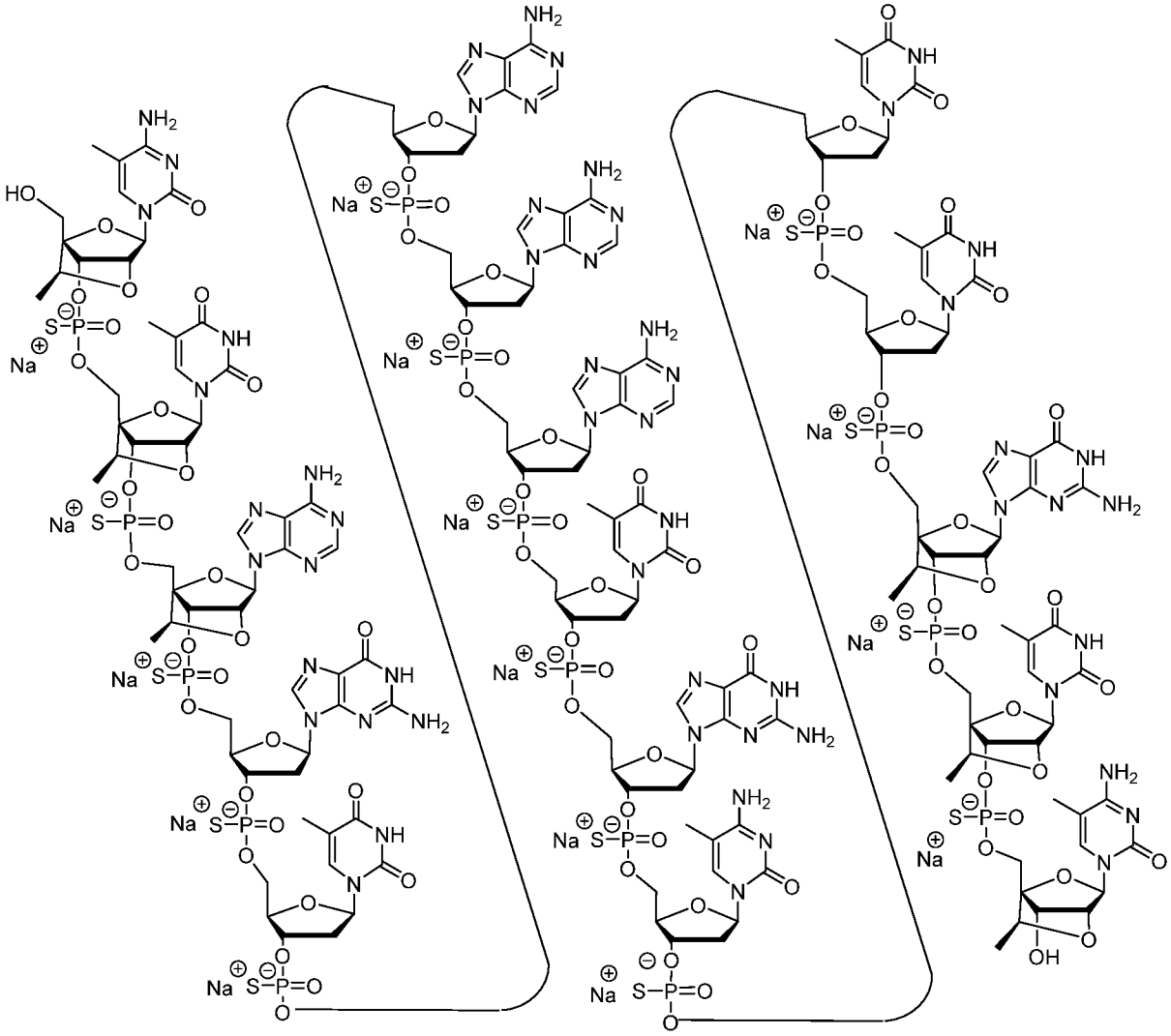
In certain embodiments, a compound comprises or consists of the sodium salt of ION 916789, having the following chemical structure:



In certain embodiments, a compound comprises or consists of ION 916602 or salt thereof, having the following chemical structure:



In certain embodiments, a compound comprises or consists of the sodium salt of ION 916602, having the following chemical structure:



In any of the foregoing methods or uses, the compound can be administered parenterally. For example, in certain embodiments the compound can be administered through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

Certain Compounds

In certain embodiments, compounds described herein can be antisense compounds. In certain embodiments, the antisense compound comprises or consists of an oligomeric compound. In certain embodiments, the oligomeric compound comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound described herein comprises or consists of a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound or antisense compound is single-stranded. Such a single-stranded compound or antisense compound comprises or consists of an oligomeric compound. In certain embodiments, such an oligomeric compound comprises or consists of an oligonucleotide and optionally a conjugate group. In certain embodiments, the oligonucleotide is an antisense oligonucleotide. In certain embodiments, the oligonucleotide is modified. In certain embodiments, the oligonucleotide of a single-stranded antisense compound or oligomeric compound comprises a self-complementary nucleobase sequence.

In certain embodiments, compounds are double-stranded. Such double-stranded compounds comprise a first modified oligonucleotide having a region complementary to a target nucleic acid and a second modified oligonucleotide having a region complementary to the first modified oligonucleotide. In certain embodiments, the modified oligonucleotide is an RNA oligonucleotide. In such embodiments, the thymine nucleobase in the modified oligonucleotide is replaced by a uracil nucleobase. In certain embodiments, compound comprises a conjugate group. In certain embodiments, one of the modified oligonucleotides is conjugated. In certain embodiments, both the modified oligonucleotides are conjugated. In certain embodiments, the first modified oligonucleotide is conjugated. In certain embodiments, the second modified oligonucleotide is conjugated. In certain embodiments, the first modified oligonucleotide is 16-30 linked nucleosides in length and the second modified oligonucleotide is 16-30 linked nucleosides in length. In certain embodiments, one of the modified oligonucleotides has a nucleobase sequence comprising at least 8 contiguous nucleobases of any of SEQ ID NOs: 17-2169.

In certain embodiments, antisense compounds are double-stranded. Such double-stranded antisense compounds comprise a first oligomeric compound having a region complementary to a target nucleic acid and a second oligomeric compound having a region complementary to the first oligomeric compound. The first oligomeric compound of such double stranded antisense compounds typically comprises or consists of a modified oligonucleotide and optionally a conjugate group. The oligonucleotide of the second oligomeric compound of such a double-stranded antisense compound may be modified or unmodified. Either or both oligomeric compounds of a double-stranded antisense compound may comprise a conjugate group. The oligomeric compounds of double-stranded antisense compounds may include non-complementary overhanging nucleosides.

Examples of single-stranded and double-stranded compounds include, but are not limited to, oligonucleotides, siRNAs, microRNA targeting oligonucleotides, and single-stranded RNAi compounds, such as small hairpin RNAs (shRNAs), single-stranded siRNAs (ssRNAs), and microRNA mimics.

In certain embodiments, a compound described herein has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, a compound described herein comprises an oligonucleotide 12 to 30 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 12 to 22 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide 14 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide 14 to 20 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 15 to 30 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 15 to 20 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 16 to 30 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 16 to 20 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 17 to 30 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 17 to 20 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 18 to 30 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 18 to 20 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 20 to 30 linked subunits in length. In other words, such oligonucleotides are 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, or 20 to 30 subunits in length, respectively. In certain embodiments, a compound described herein comprises an oligonucleotide 14 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 16 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 17 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide 18 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 19 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 20 linked subunits in length. In other embodiments, a compound described herein comprises an oligonucleotide 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the compound described herein comprises an oligonucleotide 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 linked subunits in length, or a range defined by any two of the above values. In some embodiments the linked subunits are nucleotides, nucleosides, or nucleobases.

In certain embodiments, the compound may further comprise additional features or elements, such as a conjugate group, that are attached to the oligonucleotide. In certain embodiments, such compounds are antisense compounds. In certain embodiments, such compounds are oligomeric compounds. In embodiments where a conjugate group comprises a nucleoside (*i.e.* a nucleoside that links the conjugate group to the oligonucleotide), the nucleoside of the conjugate group is not counted in the length of the oligonucleotide.

In certain embodiments, compounds may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated compound targeted to a PNPLA3 nucleic acid may have two subunits deleted from the 5' end, or alternatively, may have two subunits deleted from the 3' end of the compound. Alternatively, the deleted nucleosides may be dispersed throughout the compound.

When a single additional subunit is present in a lengthened compound, the additional subunit may be located at the 5' or 3' end of the compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in a compound having two subunits added to the 5' end (5' addition), or alternatively, to the 3' end (3' addition) of the compound. Alternatively, the added subunits may be dispersed throughout the compound.

It is possible to increase or decrease the length of a compound, such as an oligonucleotide, and/or introduce mismatch bases without eliminating activity (Woolf et al. *Proc. Natl. Acad. Sci. USA* 1992, 89:7305-7309; Gautschi *et al. J. Natl. Cancer Inst.* March 2001, 93:463-471; Maher and Dolnick *Nuc. Acid. Res.* 1998, 16:3341-3358). However, seemingly small changes in oligonucleotide sequence, chemistry and motif can make large differences in one or more of the many properties required for clinical development (Seth et al. *J. Med. Chem.* 2009, 52, 10; Egli et al. *J. Am. Chem. Soc.* 2011, 133, 16642).

In certain embodiments, compounds described herein are interfering RNA compounds (RNAi), which include double-stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence-specific RNAi, for example, short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term "RNAi" is meant to be equivalent to other terms used to describe sequence-specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

In certain embodiments, a compound described herein can comprise any of the oligonucleotide sequences targeted to PNPLA3 described herein. In certain embodiments, the compound can be double-stranded. In certain embodiments, the compound comprises a first strand comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion of any one of SEQ ID NOs: 17-2169 and a second strand. In certain embodiments, the compound comprises a first strand comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169 and a second strand. In certain embodiments, the compound comprises ribonucleotides in which the first strand has uracil (U) in place of thymine (T) in any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound comprises (i) a first strand comprising a nucleobase sequence complementary to the site on PNPLA3 to which any of SEQ ID NOs: 17-2169 is targeted, and (ii) a second strand. In certain embodiments, the compound comprises one or more modified nucleotides in which the 2' position of the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, the compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the dsRNA compound. In certain embodiments, the compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the compound contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000.

In certain embodiments, the first strand of the compound is an siRNA guide strand and the second strand of the compound is an siRNA passenger strand. In certain embodiments, the second strand of the compound is complementary to the first strand. In certain embodiments, each strand of the compound is 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides in length. In certain embodiments, the first or second strand of the compound can comprise a conjugate group.

In certain embodiments, a compound described herein can comprise any of the oligonucleotide sequences targeted to PNPLA3 described herein. In certain embodiments, the compound is single stranded. In certain embodiments, such a compound is a single-stranded RNAi (ssRNAi) compound. In certain embodiments, the compound comprises at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion of any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound comprises the nucleobase sequence of any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound comprises ribonucleotides in which uracil (U) is in place of thymine (T) in any one of SEQ ID NOs: 17-2169. In certain embodiments, the compound comprises a nucleobase sequence complementary to the site on PNPLA3 to which any of SEQ ID NOs: 17-2169 is targeted. In certain embodiments, the compound comprises one or more

modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, the compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the compound. In certain embodiments, the compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the compound contains a capped strand, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the compound consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, the compound can comprise a conjugate group.

Certain Mechanisms

In certain embodiments, compounds described herein comprise or consist of modified oligonucleotides. In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, compounds comprise oligomeric compounds. In certain embodiments, compounds described herein are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity. In certain embodiments, compounds described herein selectively affect one or more target nucleic acid. Such compounds comprise a nucleobase sequence that hybridizes to one or more target nucleic acid, resulting in one or more desired antisense activity and does not hybridize to one or more non-target nucleic acid or does not hybridize to one or more non-target nucleic acid in such a way that results in a significant undesired antisense activity.

In certain antisense activities, hybridization of a compound described herein to a target nucleic acid results in recruitment of a protein that cleaves the target nucleic acid. For example, certain compounds described herein result in RNase H mediated cleavage of the target nucleic acid. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. The DNA in such an RNA:DNA duplex need not be unmodified DNA. In certain embodiments, compounds described herein are sufficiently "DNA-like" to elicit RNase H activity. Further, in certain embodiments, one or more non-DNA-like nucleoside in the gap of a gapmer is tolerated.

In certain antisense activities, compounds described herein or a portion of the compound is loaded into an RNA-induced silencing complex (RISC), ultimately resulting in cleavage of the target nucleic acid. For example, certain compounds described herein result in cleavage of the target nucleic acid by Argonaute. Compounds that are loaded into RISC are RNAi compounds. RNAi compounds may be double-stranded (siRNA) or single-stranded (ssRNA).

In certain embodiments, hybridization of compounds described herein to a target nucleic acid does not result in recruitment of a protein that cleaves that target nucleic acid. In certain such embodiments, hybridization of the compound to the target nucleic acid results in alteration of splicing of the target nucleic acid. In certain embodiments, hybridization of the compound to a target nucleic acid results in inhibition of a binding interaction between the target nucleic acid and a protein or other nucleic acid. In certain such embodiments, hybridization of the compound to a target nucleic acid results in alteration of translation of the target nucleic acid.

Antisense activities may be observed directly or indirectly. In certain embodiments, observation or detection of an antisense activity involves observation or detection of a change in an amount of a target nucleic acid or protein encoded by such target nucleic acid, a change in the ratio of splice variants of a nucleic acid or protein, and/or a phenotypic change in a cell or animal.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

In certain embodiments, compounds described herein comprise or consist of an oligonucleotide comprising a region that is complementary to a target nucleic acid. In certain embodiments, the target nucleic acid is an endogenous RNA molecule. In certain embodiments, the target nucleic acid encodes a protein. In certain such embodiments, the target nucleic acid is selected from an mRNA and a pre-mRNA, including intronic, exonic and untranslated regions. In certain embodiments, the target RNA is an mRNA. In certain embodiments, the target nucleic acid is a pre-mRNA. In certain such embodiments, the target region is entirely within an intron. In certain embodiments, the target region spans an intron/exon junction. In certain embodiments, the target region is at least 50% within an intron.

Nucleotide sequences that encode PNPLA3 include, without limitation, the following: RefSeq or GENBANK Accession Nos. NM_025225.2 (incorporated by reference, disclosed herein as SEQ ID NO: 1); GENBANK Accession No. NC_000022.11 truncated from nucleotides 43921001 to 43954500 (incorporated by reference, disclosed herein as SEQ ID NO: 2); AK123806.1(incorporated by reference, disclosed herein as SEQ ID NO: 3); BQ686328.1 (incorporated by reference, disclosed herein as SEQ ID NO: 4); BF762711.1 (incorporated by reference, disclosed herein as SEQ ID NO: 5); DA290491.1 (incorporated by reference, disclosed herein as SEQ ID NO: 6); and the sequences listed as SEQ ID Nos. 7, 8, 9, and 10.

Hybridization

In some embodiments, hybridization occurs between a compound disclosed herein and a PNPLA3 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Hybridization conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the compounds provided herein are specifically hybridizable with a PNPLA3 nucleic acid.

Complementarity

An oligonucleotide is said to be complementary to another nucleic acid when the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to the following pairs: adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (mC) and guanine (G), unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. An oligonucleotide is fully complementary or 100% complementary when such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

In certain embodiments, compounds described herein comprise or consist of modified oligonucleotides. In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, compounds comprise oligomeric compounds. Non-complementary nucleobases between a compound and a PNPLA3 nucleic acid may be tolerated provided that the compound remains able to specifically hybridize to a target nucleic acid. Moreover, a compound may hybridize over one or more segments of a PNPLA3 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the compounds provided herein, or a specified portion thereof are at least, or are up to 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a PNPLA3 nucleic acid, a target region, target segment, or specified portion thereof. In certain embodiments, the compounds provided herein, or a specified portion thereof, are 70% to 75%, 75% to 80%, 80% to 85%, 85% to 90%, 90% to 95%, 95% to 100%, or any number in between these ranges, complementary to a PNPLA3 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of a compound with a target nucleic acid can be determined using routine methods.

For example, a compound in which 18 of 20 nucleobases of the compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As

such, a compound which is 18 nucleobases in length having four non-complementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid. Percent complementarity of a compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, compounds described herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, a compound may be fully complementary to a PNPLA3 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of a compound is complementary to the corresponding nucleobase of a target nucleic acid. For example, a 20 nucleobase compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the compound. "Fully complementary" can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase compound is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the compound. At the same time, the entire 30 nucleobase compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the compound are also complementary to the target sequence.

In certain embodiments, compounds described herein comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain such embodiments, antisense activity against the target is reduced by such mismatch, but activity against a non-target is reduced by a greater amount. Thus, in certain such embodiments, selectivity of the compound is improved. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, or 8 from the 5'-end of the gap region. In certain such embodiments, the mismatch is at position 9, 8, 7, 6, 5, 4, 3, 2, 1 from the 3'-end of the gap region. In certain such embodiments, the mismatch is at position 1, 2, 3, or 4 from the 5'-end of the wing region. In certain such embodiments, the mismatch is at position 4, 3, 2, or 1 from the 3'-end of the wing region. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide not having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 5'-end of the oligonucleotide. In certain such

embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 3'-end of the oligonucleotide.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer oligonucleotide.

In certain embodiments, compounds described herein that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a PNPLA3 nucleic acid, or specified portion thereof.

In certain embodiments, compounds described herein that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a PNPLA3 nucleic acid, or specified portion thereof.

In certain embodiments, compounds described herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of a compound. In certain embodiments, the compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 16 nucleobase portion of a target segment. Also contemplated are compounds that are complementary to at least a 9, 10, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific ION number, or portion thereof. In certain

embodiments, compounds described herein are antisense compounds or oligomeric compounds. In certain embodiments, compounds described herein are modified oligonucleotides. As used herein, a compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the compounds described herein as well as compounds having non-identical bases relative to the compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the compound. Percent identity of an compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, compounds described herein, or portions thereof, are, or are at least, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the compounds or SEQ ID NOs, or a portion thereof, disclosed herein. In certain embodiments, compounds described herein are about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, or any percentage between such values, to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific ION number, or portion thereof, in which the compounds comprise an oligonucleotide having one or more mismatched nucleobases. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 5'-end of the oligonucleotide. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 3'-end of the oligonucleotide.

In certain embodiments, compounds described herein comprise or consist of antisense compounds. In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, compounds described herein comprise or consist of oligonucleotides. In certain embodiments, a portion of the oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Certain Modified Compounds

In certain embodiments, compounds described herein comprise or consist of oligonucleotides consisting of linked nucleosides. Oligonucleotides may be unmodified oligonucleotides (RNA or DNA) or may be modified oligonucleotides. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA (i.e., comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase) and/or at least one modified internucleoside linkage).

Modified Nucleosides

Modified nucleosides comprise a modified sugar moiety or a modified nucleobase or both a modifed sugar moiety and a modified nucleobase.

1. Modified Sugar Moieties

In certain embodiments, sugar moieties are non-bicyclic modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of other types of modified sugar moieties.

In certain embodiments, modified sugar moieties are non-bicyclic modified furanosyl sugar moieties comprising one or more acyclic substituent, including, but not limited, to substituents at the 2', 4', and/or 5' positions. In certain embodiments, the furanosyl sugar moiety is a ribosyl sugar moiety. In certain embodiments, one or more acyclic substituent of non-bicyclic modified sugar moieties is branched. Examples of 2'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 2'-F, 2'-OCH_3 ("OMe" or "O-methyl"), and 2'-$O(CH_2)_2OCH_3$ ("MOE"). In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_1-C_{10} alkoxy, O-C_1-C_{10} substituted alkoxy, O-C_1-C_{10} alkyl, O-C_1-C_{10} substituted alkyl, S-alkyl, $N(R_m)$-alkyl, O-alkenyl, S-alkenyl, $N(R_m)$-alkenyl, O-alkynyl, S-alkynyl, $N(R_m)$-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl, and the 2'-substituent groups described in Cook et al., U.S. 6,531,584; Cook et al., U.S. 5,859,221; and Cook et al., U.S. 6,005,087. Certain embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO_2), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 4'-substituent groups suitable for linearly non-bicyclic modified sugar moieties include, but are not limited to, alkoxy (*e.g.*, methoxy), alkyl, and those described in Manoharan et al., WO 2015/106128. Examples of 5'-substituent groups suitable for non-bicyclic modified sugar moieties include, but are not limited to: 5'-methyl (R or S), 5'-vinyl, and 5'-methoxy. In certain embodiments, non-bicyclic modified sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties and the modified sugar moieties and modified nucleosides described in Migawa et al., WO 2008/101157 and Rajeev et al., US2013/0203836.

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$,

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

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

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

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

Certain modifed sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. In certain such embodiments, the furanose ring is a ribose ring. Examples of such 4' to 2' bridging sugar substituents include, but are not limited to: 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2' ("LNA"), 4'-CH_2-S-2', 4'-$(CH_2)_2$-O-2' ("ENA"), 4'-$CH(CH_3)$-O-2' (referred to as "constrained ethyl" or "cEt" when in the *S* configuration), 4'-CH_2-O-CH_2-2', 4'-CH_2-N(R)-2', 4'-$CH(CH_2OCH_3)$-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.,* Seth et al., U.S. 7,399,845, Bhat et al., U.S. 7,569,686, Swayze et al., U.S. 7,741,457, and Swayze et al., U.S. 8,022,193), 4'-$C(CH_3)(CH_3)$-O-2' and analogs thereof (*see, e.g.,* Seth et al., U.S. 8,278,283), 4'-CH_2-$N(OCH_3)$-2' and analogs thereof (*see, e.g.,* Prakash et al., U.S. 8,278,425), 4'-CH_2-O-$N(CH_3)$-2' (*see, e.g.,* Allerson et al., U.S. 7,696,345 and Allerson et al., U.S. 8,124,745), 4'-CH_2-C(H)(CH_3)-2' (*see, e.g.,* Zhou, *et al., J. Org. Chem.,*2009, *74*, 118-134), 4'-CH_2-$C(=CH_2)$-2' and analogs thereof (*see e.g.*,, Seth et al., U.S. 8,278,426), 4'-$C(R_aR_b)$-N(R)-O-2', 4'-$C(R_aR_b)$-O-N(R)-2', 4'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C_1-C_{12} alkyl (*see, e.g.* Imanishi et al., U.S. 7,427,672).

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

wherein:

x is 0, 1, or 2;

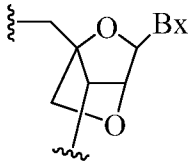
n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12}

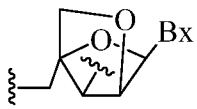
alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl ($S(=O)$-J_1); and each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl, or a protecting group.

Additional bicyclic sugar moieties are known in the art, see, for example: Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443, Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740, Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, 2007, *129,* 8362-8379; Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; Wengel et al.,U.S. 7,053,207, Imanishi et al., U.S. 6,268,490, Imanishi et al. U.S. 6,770,748, Imanishi et al., U.S. RE44,779; Wengel et al., U.S. 6,794,499, Wengel et al., U.S. 6,670,461; Wengel et al., U.S. 7,034,133, Wengel et al., U.S. 8,080,644; Wengel et al., U.S. 8,034,909; Wengel et al., U.S. 8,153,365; Wengel et al., U.S. 7,572,582; and Ramasamy et al., U.S. 6,525,191, Torsten et al., WO 2004/106356, Wengel et al., WO 1999/014226; Seth et al.,WO 2007/134181; Seth et al., U.S. 7,547,684; Seth et al., U.S. 7,666,854; Seth et al., U.S. 8,088,746; Seth et al., U.S. 7,750,131; Seth et al., U.S. 8,030,467; Seth et al., U.S. 8,268,980; Seth et al., U.S. 8,546,556; Seth et al., U.S. 8,530,640; Migawa et al., U.S. 9,012,421; Seth et al., U.S. 8,501,805; Allerson et al., US2008/0039618; and Migawa et al., US2015/0191727.

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



LNA (β-D-configuration)
bridge = 4'-CH_2-O-2'



α-*L*-LNA (α-*L*-configuration)
bridge = 4'-CH_2-O-2'

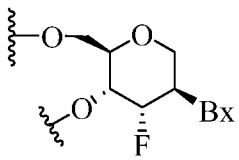
α-L-methyleneoxy (4'-CH_2-O-2') or α-L-LNA bicyclic nucleosides have been incorporated into oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research,* 2003, *21*, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions

of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β-D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars).

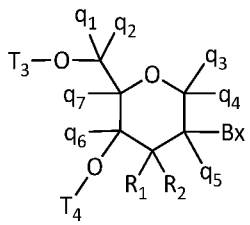
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.,* with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described herein. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (*see*, *e.g.,* Bhat et al., U.S. 7,875,733 and Bhat et al., U.S. 7,939,677) and/or the 5' position.

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



F-HNA

("F-HNA," *see e.g.,* Swayze et al., U.S. 8,088,904; Swayze et al., U.S. 8,440,803; and Swayze et al., U.S. 9,005,906) F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran, and nucleosides comprising additional modified THP compounds having the formula:



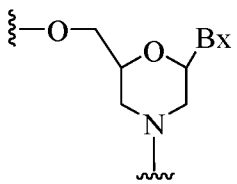
wherein, independently, for each of said modified THP nucleoside:

Bx is a nucleobase moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T_3 and T_4 is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group; q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl; and each of R_1 and R_2 is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN, wherein X is O, S or NJ_1, and each J_1, J_2, and J_3 is, independently, H or C_1-C_6 alkyl.

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

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



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

In certain embodiments, sugar surrogates comprise acyclic moieites. Examples of nucleosides and oligonucleotides comprising such acyclic sugar surrogates include, but are not limited to: peptide nucleic acid ("PNA"), acyclic butyl nucleic acid (*see, e.g.,* Kumar et al., *Org. Biomol. Chem.*, 2013, *11*, 5853-5865), and nucleosides and oligonucleotides described in Manoharan et al., US2013/130378.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides.

2. Modified Nucleobases

Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications can impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds.

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

In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, 5-methylcytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine , 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($C\equiv C\text{-}CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly, 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one, and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in Merigan et al., U.S. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, Antisense Drug Technology, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

Publications that teach the preparation of certain of the above noted modified nucleobases, as well as other modified nucleobases include without limitation, Manoharan et al., US2003/0158403, Manoharan et al., US2003/0175906; Dinh et al., U.S. 4,845,205; Spielvogel et al., U.S. 5,130,302; Rogers et al., U.S. 5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908;

Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; Haralambidis et al., U.S. 5,552,540; Cook et al., U.S. 5,587,469; Froehler et al., U.S. 5,594,121; Switzer et al., U.S. 5,596,091; Cook et al., U.S. 5,614,617; Froehler et al., U.S. 5,645,985; Cook et al., U.S. 5,681,941; Cook et al., U.S. 5,811,534; Cook et al., U.S. 5,750,692; Cook et al., U.S. 5,948,903; Cook et al., U.S. 5,587,470; Cook et al., U.S. 5,457,191; Matteucci et al., U.S. 5,763,588; Froehler et al., U.S. 5,830,653; Cook et al., U.S. 5,808,027; Cook et al., U.S. 6,166,199; and Matteucci et al., U.S. 6,005,096.

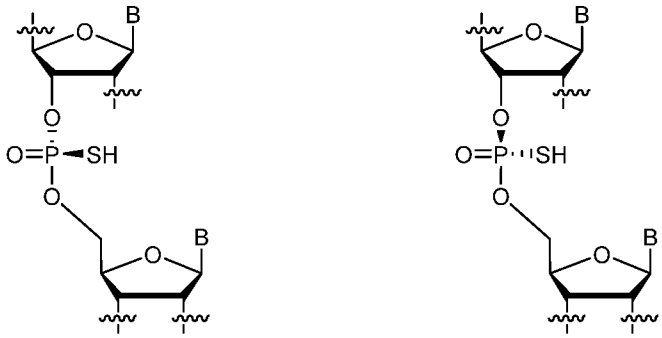
In certain embodiments, compounds targeted to a PNPLA3 nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

3. Modified Internucleoside Linkages

The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. In certain embodiments, compounds described herein having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Representative internucleoside linkages having a chiral center include but are not limited to alkylphosphonates and phosphorothioates. Modified oligonucleotides comprising internucleoside linkages having a chiral center can be prepared as populations of modified oligonucleotides comprising stereorandom internucleoside linkages, or as populations of modified oligonucleotides comprising phosphorothioate linkages in particular stereochemical configurations. In certain embodiments, populations of modified oligonucleotides comprise phosphorothioate internucleoside linkages wherein all of the phosphorothioate internucleoside linkages are stereorandom. Such modified oligonucleotides can be generated using synthetic methods that result in random selection of the stereochemical configuration of each phosphorothioate linkage. Nonetheless, as is well understood by those of skill in the art, each individual phosphorothioate of each individual oligonucleotide molecule has a defined stereoconfiguration. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising one or more particular phosphorothioate internucleoside linkages in a particular, independently selected stereochemical configuration. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 65% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 70% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 80% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 90% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 99% of the molecules in the population. Such

chirally enriched populations of modified oligonucleotides can be generated using synthetic methods known in the art, *e.g.*, methods described in Oka et al., *JACS* 125, 8307 (2003), Wan et al. *Nuc. Acid. Res.* 42, 13456 (2014), and WO 2017/015555. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one indicated phosphorothioate in the (*S*p) configuration. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one phosphorothioate in the (*R*p) configuration. In certain embodiments, modified oligonucleotides comprising (*R*p) and/or (*S*p) phosphorothioates comprise one or more of the following formulas, respectively, wherein "B" indicates a nucleobase:



Unless otherwise indicated, chiral internucleoside linkages of modified oligonucleotides described herein can be stereorandom or in a particular stereochemical configuration.

In certain embodiments, compounds targeted to a PNPLA3 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

In certain embodiments, compounds described herein comprise oligonucleotides. Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include, but are not limited to, phosphates, which contain a phosphodiester bond ("P=O") (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, phosphoramidates, and

phosphorothioates ("P=S"), and phosphorodithioates ("HS-P=S"). Representative non-phosphorus containing internucleoside linking groups include, but are not limited to, methylenemethylimino ($-CH_2-N(CH_3)-O-CH_2$), thiodiester, thionocarbamate (-O-C(=O)(NH)-S-); siloxane ($-O-SiH_2-O-$); and N,N'-dimethylhydrazine ($-CH_2-N(CH_3)-N(CH_3)-$). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral internucleoside linkages include, but are not limited to, alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI ($3'-CH_2-N(CH_3)-O-5'$), amide-3 ($3'-CH_2-C(=O)-N(H)-5'$), amide-4 ($3'-CH_2-N(H)-C(=O)-5'$), formacetal ($3'-O-CH_2-O-5'$), methoxypropyl, and thioformacetal ($3'-S-CH_2-O-5'$). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See, for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH_2 component parts.

In certain embodiments, oligonucleotides comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, internucleoside linkages are arranged in a gapped motif. In such embodiments, the internucleoside linkages in each of two wing regions are different from the internucleoside linkages in the gap region. In certain embodiments, the internucleoside linkages in the wings are phosphodiester and the internucleoside linkages in the gap are phosphorothioate. The nucleoside motif is independently selected, so such oligonucleotides having a gapped internucleoside linkage motif may or may not have a gapped nucleoside motif and, if it does have a gapped nucleoside motif, the wing and gap lengths may or may not be the same.

In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside

linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least block of at least one 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

In certain embodiments, oligonucleotides comprise one or more methylphosphonate linkages. In certain embodiments, oligonucleotides having a gapmer nucleoside motif comprise a linkage motif comprising all phosphorothioate linkages except for one or two methylphosphonate linkages. In certain embodiments, one methylphosphonate linkage is in the central gap of an oligonucleotide having a gapmer nucleoside motif.

In certain embodiments, it is desirable to arrange the number of phosphorothioate internucleoside linkages and phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, it is desirable to arrange the number and position of phosphorothioate internucleoside linkages and the number and position of phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased while still maintaining nuclease resistance. In certain embodiments, it is desirable to decrease the number of phosphorothioate internucleoside linkages while retaining nuclease resistance. In certain embodiments, it is desirable to increase the number of phosphodiester internucleoside linkages while retaining nuclease resistance.

4. Certain Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. Oligonucleotides can have a motif, *e.g.* a pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified sugar. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified internucleoside linkage. In such embodiments, the modified, unmodified, and differently modified sugar moieties, nucleobases, and/or internucleoside linkages of a modified oligonucleotide define a pattern or motif. In certain embodiments, the patterns of sugar moieties, nucleobases, and internucleoside linkages are each independent of one another. Thus, a modified oligonucleotide may be

described by its sugar motif, nucleobase motif and/or internucleoside linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the sequence of nucleobases).

a. Certain Sugar Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified sugar moiety arranged along the oligonucleotide or region thereof in a defined pattern or sugar motif. In certain instances, such sugar motifs include, but are not limited to, any of the sugar modifications discussed herein.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a gapmer motif, which comprises two external regions or "wings" and a central or internal region or "gap." The three regions of a gapmer motif (the 5'-wing, the gap, and the 3'-wing) form a contiguous sequence of nucleosides, wherein at least some of the sugar moieties of the nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the wings and the gap (i.e., the wing/gap junction). In certain embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes one or more nucleosides having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric gapmer). In certain embodiments, the sugar motif of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric gapmer).

In certain embodiments, the wings of a gapmer comprise 1-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 2-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 3-5 nucleosides. In certain embodiments, the nucleosides of a gapmer are all modified nucleosides.

In certain embodiments, the gap of a gapmer comprises 7-12 nucleosides. In certain embodiments, the gap of a gapmer comprises 7-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 8-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 10 nucleosides. In certain embodiments, each nucleoside of the gap of a gapmer is an unmodified 2'-deoxy nucleoside.

In certain embodiments, the gapmer is a deoxy gapmer. In such embodiments, the nucleosides on the gap side of each wing/gap junction are unmodified 2'-deoxy nucleosides and the nucleosides on the wing sides of each wing/gap junction are modified nucleosides. In certain such embodiments, each nucleoside of the gap is an unmodified 2'-deoxy nucleoside. In certain such embodiments, each nucleoside of each wing is a modified nucleoside.

In certain embodiments, a modified oligonucleotide has a fully modified sugar motif wherein each nucleoside of the modified oligonucleotide comprises a modified sugar moiety. In certain embodiments,

modified oligonucleotides comprise or consist of a region having a fully modified sugar motif wherein each nucleoside of the region comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif, wherein each nucleoside within the fully modified region comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified comprises the same 2'-modification.

b. *Certain Nucleobase Motifs*

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise modified and/or unmodified nucleobases arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, each nucleobase is modified. In certain embodiments, none of the nucleobases are modified. In certain embodiments, each purine or each pyrimidine is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each uracil is modified. In certain embodiments, each cytosine is modified. In certain embodiments, some or all of the cytosine nucleobases in a modified oligonucleotide are 5-methylcytosines.

In certain embodiments, modified oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments, the block is within 3 nucleosides of the 3'-end of the oligonucleotide. In certain embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments, the block is within 3 nucleosides of the 5'-end of the oligonucleotide.

In certain embodiments, oligonucleotides having a gapmer motif comprise a nucleoside comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer motif. In certain such embodiments, the sugar moiety of said nucleoside is a 2'-deoxyribosyl moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

c. *Certain Internucleoside Linkage Motifs*

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise modified and/or unmodified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, essentially each internucleoside linking group is a phosphate internucleoside linkage (P=O). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate (P=S). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is independently selected from

a phosphorothioate and phosphate internucleoside linkage. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer and the internucleoside linkages within the gap are all modified. In certain such embodiments, some or all of the internucleoside linkages in the wings are unmodified phosphate linkages. In certain embodiments, the terminal internucleoside linkages are modified. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer, and the internucleoside linkage motif comprises at least one phosphodiester internucleoside linkage in at least one wing, wherein the at least one phosphodiester linkage is not a terminal internucleoside linkage, and the remaining internucleoside linkages are phosphorothioate internucleoside linkages. In certain such embodiments, all of the phosphorothioate linkages are stereorandom. In certain embodiments, all of the phosphorothioate linkages in the wings are (*S*p) phosphorothioates, and the gap comprises at least one *S*p, *S*p, *R*p motif. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising such internucleoside linkage motifs.

5. Certain Modified Oligonucleotides

In certain embodiments, compounds described herein comprise modified oligonucleotides. In certain embodiments, the above modifications (sugar, nucleobase, internucleoside linkage) are incorporated into a modified oligonucleotide. In certain embodiments, modified oligonucleotides are characterized by their modification, motifs, and overall lengths. In certain embodiments, such parameters are each independent of one another. Thus, unless otherwise indicated, each internucleoside linkage of an oligonucleotide having a gapmer sugar motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. For example, the internucleoside linkages within the wing regions of a sugar gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region of the sugar motif. Likewise, such gapmer oligonucleotides may comprise one or more modified nucleobases independent of the gapmer pattern of the sugar modifications. Furthermore, in certain instances, an oligonucleotide is described by an overall length or range and by lengths or length ranges of two or more regions (e.g., a region of nucleosides having specified sugar modifications). In such circumstances, it may be possible to select numbers for each range that result in an oligonucleotide having an overall length falling outside the specified range. In such circumstances, both elements must be satisfied. For example, in certain embodiments, a modified oligonucleotide consists of 15-20 linked nucleosides and has a sugar motif consisting of three regions, A, B, and C, wherein region A consists of 2-6 linked nucleosides having a specified sugar motif, region B consists of 6-10 linked nucleosides having a specified sugar motif, and region C consists of 2-6 linked nucleosides having a specified sugar motif. Such embodiments do not include modified oligonucleotides where A and C each consist of 6 linked nucleosides and B consists of 10 linked nucleosides (even though those numbers of nucleosides are permitted within the requirements for A, B, and C) because the overall length of such oligonucleotide will be 22, which exceeds the upper limit of the overall length of the modified oligonucleotide (20). Herein, if a description of an oligonucleotide is silent with respect to one or

more parameters, such parameter is not limited. Thus, a modified oligonucleotide described only as having a gapmer sugar motif without further description may have any length, internucleoside linkage motif, and nucleobase motif. Unless otherwise indicated, all modifications are independent of nucleobase sequence.

Certain Conjugated Compounds

In certain embodiments, the compounds described herein comprise or consist of an oligonucleotide (modified or unmodified) and, optionally, one or more conjugate groups and/or terminal groups. Conjugate groups consist of one or more conjugate moiety and a conjugate linker which links the conjugate moiety to the oligonucleotide. Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

In certain embodiments, the oligonucleotide is modified. In certain embodiments, the oligonucleotide of a compound has a nucleobase sequence that is complementary to a target nucleic acid. In certain embodiments, oligonucleotides are complementary to a messenger RNA (mRNA). In certain embodiments, oligonucleotides are complementary to a pre-mRNA. In certain embodiments, oligonucleotides are complementary to a sense transcript.

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

Certain Conjugate Groups

In certain embodiments, oligonucleotides are covalently attached to one or more conjugate groups. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide, including, but not limited to, pharmacodynamics, pharmacokinetics, stability, binding, absorption, tissue distribution, cellular distribution, cellular uptake, charge and clearance. In certain embodiments, conjugate groups impart a new property on the attached oligonucleotide, *e.g.*, fluorophores or reporter groups that enable detection of the oligonucleotide.

Certain conjugate groups and conjugate moieties have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan

et al., *Bioorg. Med. Chem. Lett.*, 1994, *4*, 1053-1060), a thioether, *e.g.,* hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.,* 1993, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, *20*, 533-538), an aliphatic chain, *e.g.,* do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.,* 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.,* 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic, a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.,* 1996, *i*, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, *4*, e220; doi:10.1038/mtna.2014.72 and Nishina et al., *Molecular Therapy,* 2008, 16, 734-740), or a GalNAc cluster (*e.g.*, WO2014/179620).

1. Conjugate Moieties

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates (e.g., GalNAc), vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes.

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

2. Conjugate linkers

Conjugate moieties are attached to oligonucleotides through conjugate linkers. In certain embodiments, a conjugate group is a single chemical bond (i.e. conjugate moiety is attached to an oligonucleotide via a conjugate linker through a single bond). In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units, such as ethylene glycol, nucleosides, or amino acid units.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain

embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

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

Examples of conjugate linkers include, but are not limited to, pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other conjugate linkers include, but are not limited to, substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl, or substituted or unsubstituted C_2-C_{10} alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl, and alkynyl.

In certain embodiments, conjugate linkers comprise 1-10 linker-nucleosides. In certain embodiments, such linker-nucleosides are modified nucleosides. In certain embodiments, such linker-nucleosides comprise a modified sugar moiety. In certain embodiments, linker-nucleosides are unmodified. In certain embodiments, linker-nucleosides comprise an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, a cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. It is typically desirable for linker-nucleosides to be cleaved from the compound after it reaches a target tissue. Accordingly, linker-nucleosides are typically linked to one another and to the remainder of the compound through cleavable bonds. In certain embodiments, such cleavable bonds are phosphodiester bonds.

Herein, linker-nucleosides are not considered to be part of the oligonucleotide. Accordingly, in embodiments in which a compound comprises an oligonucleotide consisting of a specified number or range of linked nucleosides and/or a specified percent complementarity to a reference nucleic acid and the compound also comprises a conjugate group comprising a conjugate linker comprising linker-nucleosides, those linker-nucleosides are not counted toward the length of the oligonucleotide and are not used in determining the percent complementarity of the oligonucleotide for the reference nucleic acid. For example, a compound may comprise

(1) a modified oligonucleotide consisting of 8-30 nucleosides and (2) a conjugate group comprising 1-10 linker-nucleosides that are contiguous with the nucleosides of the modified oligonucleotide. The total number of contiguous linked nucleosides in such a compound is more than 30. Alternatively, a compound may comprise a modified oligonucleotide consisting of 8-30 nucleosides and no conjugate group. The total number of contiguous linked nucleosides in such a compound is no more than 30. Unless otherwise indicated, conjugate linkers comprise no more than 10 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 5 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 3 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 2 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 1 linker-nucleoside.

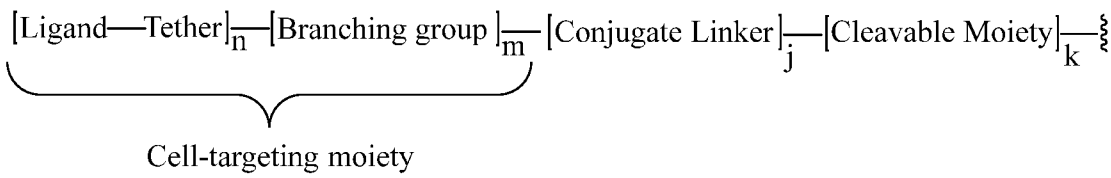
In certain embodiments, it is desirable for a conjugate group to be cleaved from the oligonucleotide. For example, in certain circumstances, compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the compound has been taken up, it is desirable that the conjugate group be cleaved to release the unconjugated or parent oligonucleotide. Thus, certain conjugates may comprise one or more cleavable moieties, typically within the conjugate linker. In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

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

In certain embodiments, a cleavable moiety comprises or consists of one or more linker-nucleosides. In certain such embodiments, one or more linker-nucleosides are linked to one another and/or to the remainder of the compound through cleavable bonds. In certain embodiments, such cleavable bonds are unmodified phosphodiester bonds. In certain embodiments, a cleavable moiety is 2'-deoxy nucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphate internucleoside linkage and covalently attached to the remainder of the conjugate linker or conjugate moiety by a phosphate or phosphorothioate linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

3. Certain Cell-Targeting Conjugate Moieties

In certain embodiments, a conjugate group comprises a cell-targeting conjugate moiety. In certain embodiments, a conjugate group has the general formula:



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

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

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

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

In certain embodiments, each tether of a cell-targeting moiety comprises one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amino, oxo, amide, phosphodiester, and polyethylene

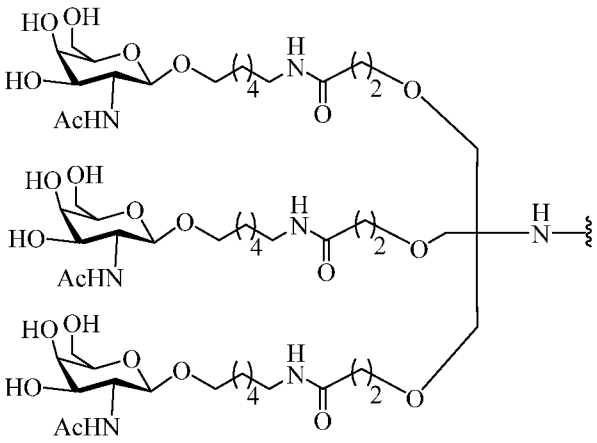
glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amino, oxo, amide, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, phosphodiester, ether, amino, oxo, and amide, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, amino, oxo, and amide, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, amino, and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and phosphodiester, in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group. In certain embodiments, each tether comprises a chain from about 6 to about 20 atoms in length. In certain embodiments, each tether comprises a chain from about 10 to about 18 atoms in length. In certain embodiments, each tether comprises about 10 atoms in chain length.

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

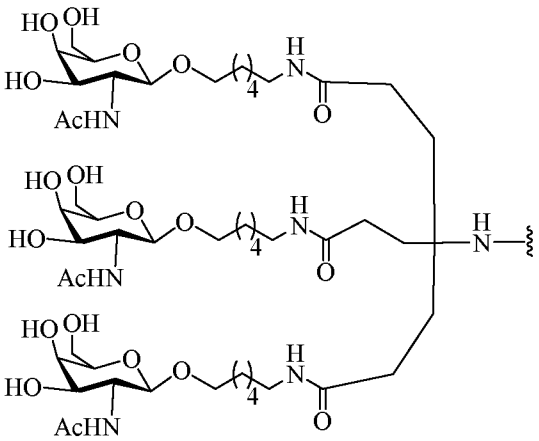
In certain embodiments, each ligand of a cell-targeting moiety is a carbohydrate, carbohydrate derivative, modified carbohydrate, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain such embodiments, the conjugate group comprises a carbohydrate cluster (*see*, e.g., Maier et al., "Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular Targeting," *Bioconjugate Chemistry*, 2003, 14, 18-29, or Rensen et al., "Design and Synthesis of Novel *N*-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asiaglycoprotein Receptor," *J. Med. Chem.* 2004, 47, 5798-5808, which are incorporated herein by reference in their entirety). In certain such embodiments, each ligand is an amino sugar or a thio sugar. For example, amino sugars may be selected from any number of compounds known in the art, such as sialic acid, α-D-galactosamine, β-muramic acid, 2-deoxy-2-methylamino-L-glucopyranose, 4,6-dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-deoxy-2-sulfoamino-D-glucopyranose and *N*-sulfo-D-glucosamine, and *N*-glycoloyl-α-neuraminic acid. For example, thio sugars may be selected from 5-Thio-β-D-glucopyranose, methyl 2,3,4-tri-

O-acetyl-1-thio-6-*O*-trityl-α-D-glucopyranoside, 4-thio-β-D-galactopyranose, and ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio-α-D-*gluco*-heptopyranoside.

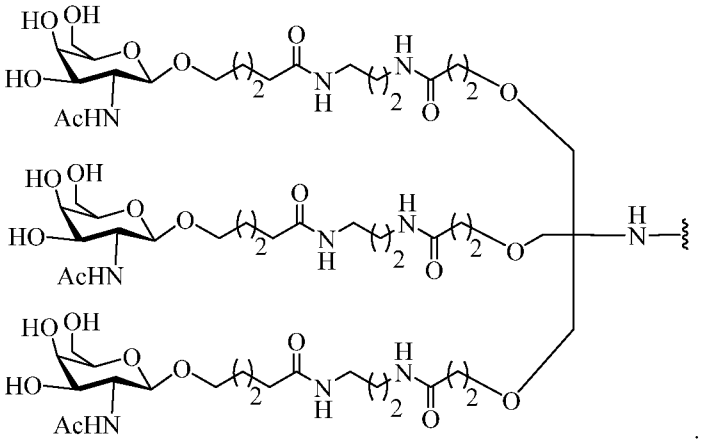
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



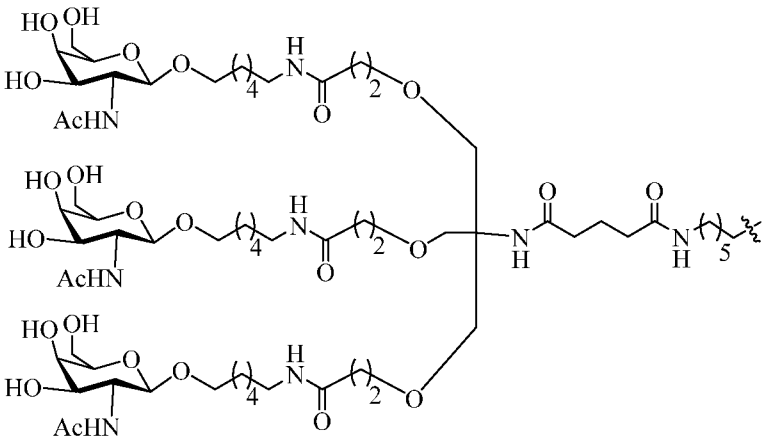
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:

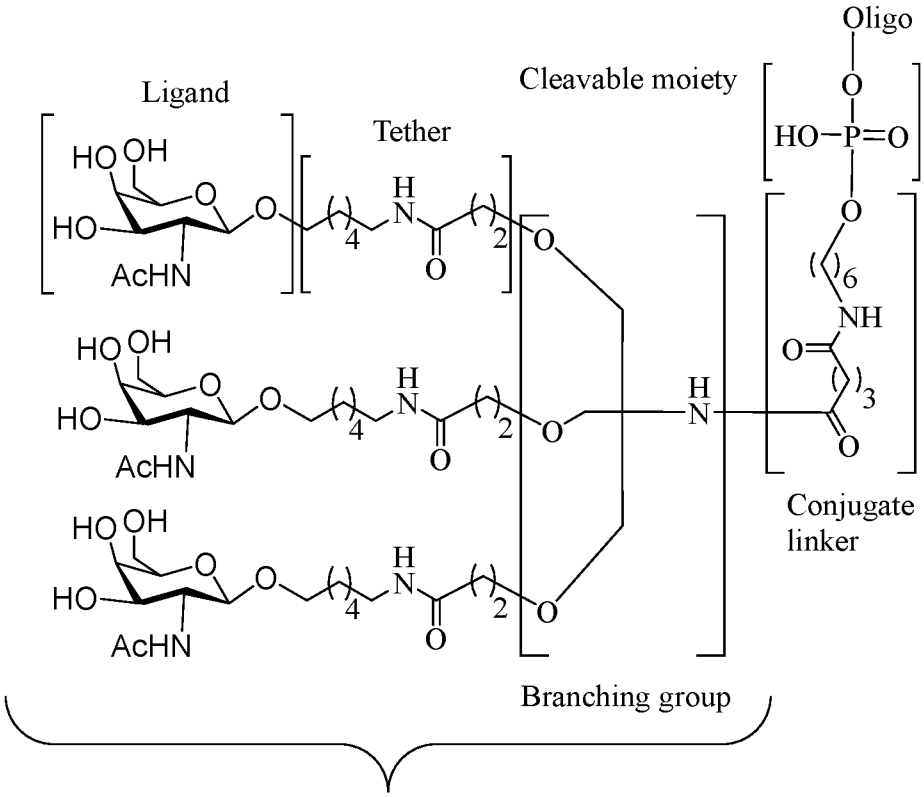


In certain embodiments, compounds described herein comprise a conjugate group described herein as "LICA-1." LICA-1 is shown below without the optional cleavable moiety at the end of the conjugate linker:



In certain embodiments, compounds described herein comprise LICA-1 and a cleavable moiety within

the conjugate linker have the formula:



wherein 'oligo' is an oligonucleotide.

Representative publications that teach the preparation of certain of the above noted conjugate groups and compounds comprising conjugate groups, tethers, conjugate linkers, branching groups, ligands, cleavable moieties as well as other modifications include, without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 9,127,276, US 2006/0148740, US 2011/0123520, WO 2013/033230 and WO 2012/037254, Biessen et al., *J. Med. Chem.* 1995, 38, 1846-1852, Lee et al., *Bioorganic & Medicinal Chemistry* 2011,19, 2494-2500, Rensen et al., *J. Biol. Chem.* 2001, 276, 37577-37584, Rensen et al., *J. Med. Chem.* 2004, 47, 5798-5808, Sliedregt et al., *J. Med. Chem.* 1999, 42, 609-618, and Valentijn et al., *Tetrahedron,* 1997, 53, 759-770, each of which is incorporated by reference herein in its entirety.

In certain embodiments, compounds described herein comprise modified oligonucleotides comprising a gapmer or fully modified motif and a conjugate group comprising at least one, two, or three GalNAc ligands. In certain embodiments, compounds described herein comprise a conjugate group found in any of the following references: Lee, *Carbohydr Res*, 1978, 67, 509-514; Connolly et al., *J Biol Chem*, 1982, 257, 939-945; Pavia et al., *Int J Pep Protein Res*, 1983, 22, 539-548; Lee et al., *Biochem*, 1984, 23, 4255-4261; Lee et al.,

Glycoconjugate J, 1987, 4, 317-328; Toyokuni et al., *Tetrahedron Lett*, 1990, 31, 2673-2676; Biessen et al., *J Med Chem*, 1995, 38, 1538-1546; Valentijn et al., *Tetrahedron*, 1997, 53, 759-770; Kim et al., *Tetrahedron Lett*, 1997, 38, 3487-3490; Lee et al., *Bioconjug Chem*, 1997, 8, 762-765; Kato et al., *Glycobiol*, 2001, 11, 821-829; Rensen et al., *J Biol Chem*, 2001, 276, 37577-37584; Lee et al., *Methods Enzymol*, 2003, 362, 38-43; Westerlind et al., *Glycoconj J*, 2004, 21, 227-241; Lee et al., *Bioorg Med Chem Lett*, 2006, 16(19), 5132-5135; Maierhofer et al., *Bioorg Med Chem*, 2007, 15, 7661-7676; Khorev et al., *Bioorg Med Chem*, 2008, 16, 5216-5231; Lee et al., *Bioorg Med Chem*, 2011, 19, 2494-2500; Kornilova et al., Analyt Biochem, 2012, 425, 43-46; Pujol et al., *Angew Chemie Int Ed Engl*, 2012, 51, 7445-7448; Biessen et al., *J Med Chem*, 1995, 38, 1846-1852; Sliedregt et al., *J Med Chem*, 1999, 42, 609-618; Rensen et al., *J Med Chem*, 2004, 47, 5798-5808; Rensen et al., *Arterioscler Thromb Vasc Biol*, 2006, 26, 169-175; van Rossenberg et al., *Gene Ther*, 2004, 11, 457-464; Sato et al., *J Am Chem Soc*, 2004, 126, 14013-14022; Lee et al., *J Org Chem*, 2012, 77, 7564-7571; Biessen et al., *FASEB J*, 2000, 14, 1784-1792; Rajur et al., *Bioconjug Chem*, 1997, 8, 935-940; Duff et al., *Methods Enzymol*, 2000, 313, 297-321; Maier et al., *Bioconjug Chem*, 2003, 14, 18-29; Jayaprakash et al., *Org Lett*, 2010, 12, 5410-5413; Manoharan, *Antisense Nucleic Acid Drug Dev*, 2002, 12, 103-128; Merwin et al., *Bioconjug Chem*, 1994, 5, 612-620; Tomiya et al., Bioorg Med Chem, 2013, 21, 5275-5281; International applications WO1998/013381; WO2011/038356; WO1997/046098; WO2008/098788; WO2004/101619; WO2012/037254; WO2011/120053; WO2011/100131; WO2011/163121; WO2012/177947; WO2013/033230; WO2013/075035; WO2012/083185; WO2012/083046; WO2009/082607; WO2009/134487; WO2010/144740; WO2010/148013; WO1997/020563; WO2010/088537; WO2002/043771; WO2010/129709; WO2012/068187; WO2009/126933; WO2004/024757; WO2010/054406; WO2012/089352; WO2012/089602; WO2013/166121; WO2013/165816; U.S. Patents 4,751,219; 8,552,163; 6,908,903; 7,262,177; 5,994,517; 6,300,319; 8,106,022; 7,491,805; 7,491,805; 7,582,744; 8,137,695; 6,383,812; 6,525,031; 6,660,720; 7,723,509; 8,541,548; 8,344,125; 8,313,772; 8,349,308; 8,450,467; 8,501,930; 8,158,601; 7,262,177; 6,906,182; 6,620,916; 8,435,491; 8,404,862; 7,851,615; Published U.S. Patent Application Publications US2011/0097264; US2011/0097265; US2013/0004427; US2005/0164235; US2006/0148740; US2008/0281044; US2010/0240730; US2003/0119724; US2006/0183886; US2008/0206869; US2011/0269814; US2009/0286973; US2011/0207799; US2012/0136042; US2012/0165393; US2008/0281041; US2009/0203135; US2012/0035115; US2012/0095075; US2012/0101148; US2012/0128760; US2012/0157509; US2012/0230938; US2013/0109817; US2013/0121954; US2013/0178512; US2013/0236968; US2011/0123520; US2003/0077829; US2008/0108801; and US2009/0203132; each of which is incorporated by reference in its entirety.

Compositions and Methods for Formulating Pharmaceutical Compositions

Compounds described herein may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

Certain embodiments provide pharmaceutical compositions comprising one or more compounds or a salt thereof. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compounds comprise or consist of a modified oligonucleotide. In certain such embodiments, the pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more compound. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one compound and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more compounds and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one or more compound and sterile PBS. In certain embodiments, the sterile PBS is pharmaceutical grade PBS. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

A compound described herein targeted to PNPLA3 nucleic acid can be utilized in pharmaceutical compositions by combining the compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising a compound targeted to PNPLA3 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the compound comprises or consists of a modified oligonucleotide provided herein.

Pharmaceutical compositions comprising compounds provided herein encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compound comprises or consists of a modified oligonucleotide. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of a compound which are cleaved by endogenous nucleases within the body, to form the active compound.

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

Certain Selected Compounds

Approximately 2,384 newly designed compounds of various lengths, chemistries, and motifs were tested for their effect on human PNPLA3 mRNA *in vitro* in several cell types (Example 1). Of 2,384 compounds tested for potency at a single dose *in vitro*, over 400 selected compounds were tested for dose dependent inhibition in A431 cells (Example 2). Of the over 400 compounds tested by dose response assays, the compounds were further screened for high dose tolerability in a BALB/c mouse model and 87 oligonucleotides were selected for *in vivo* efficacy in a PNPLA3 transgenic mouse model.

Of the 87 oligonucleotides tested in the transgenic mouse model, 23 oligonucleotides were selected to be further tested for tolerability in preclinical rodel models. In the *in vivo* rodent tolerability models, body weights and organ weights, liver function markers (such as alanine transaminase, aspartate transaminase and bilirubin), and kidney function markers (such as BUN and creatinine) were measured. In the CD1 mouse model and in the Sprague-Dawley rat model, ION 975591, 975605, 975612, 975613, 975616, 975617, 975735, 975736, 994282, and 994284 were found tolerable (Examples 5 and 6).

These compounds were further tested for efficacy in multi-dose assays in PNPLA3 transgenic mice (Example 7).

IONs 994284, 97605, 975616, 994282, 975613, 975617, 975735, 975736, and 975612 were tested for tolerability in cynomolgus monkeys (Example 8). Treatment with the compounds was well tolerated in the monkeys.

Accordingly, provided herein are compounds with any one or more of the improved properties. In certain embodiments, the compounds as described herein are potent and tolerable.

EXAMPLES

The Examples below describe the screening process to identify lead compounds targeted to PNPLA3. ION 994284, 97605, 975616, 994282, 975613, 975617, 975735, 975736, and 975612 resulted in high potency and tolerability.

Non-limiting disclosure and incorporation by reference

Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or

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

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

Certain compounds described herein (e.g. modified oligonucleotides) have one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), as α or β, such as for sugar anomers, or as (D) or (L), such as for amino acids, etc. Compounds provided herein that are drawn or described as having certain stereoisomeric configurations include only the indicated compounds. Compounds provided herein that are drawn or described with undefined stereochemistry include all such possible isomers, including their stereorandom and optically pure forms. Likewise, all tautomeric forms of the compounds provided herein are included unless otherwise indicated. Unless otherwise indicated, oligomeric compounds and modified oligonucleotides described herein are intended to include corresponding salt forms.

Compounds described herein include variations in which one or more atoms are replaced with a non-radioactive isotope or radioactive isotope of the indicated element. For example, compounds herein that comprise hydrogen atoms encompass all possible deuterium substitutions for each of the ^{1}H hydrogen atoms. Isotopic substitutions encompassed by the compounds herein include, but are not limited to: ^{2}H or ^{3}H in place of ^{1}H, ^{13}C or ^{14}C in place of ^{12}C, ^{15}N in place of ^{14}N, ^{17}O or ^{18}O in place of ^{16}O, and ^{33}S, ^{34}S, ^{35}S, or ^{36}S in place of ^{32}S.

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

Example 1: Antisense inhibition of human PNPLA3 in A431 cells

Antisense oligonucleotides were designed targeting a PNPLA3 nucleic acid and were tested for their effects on PNPLA3 mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human PNPLA3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_025225.2) or the human PNPLA3 genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NC_000022.11 truncated from nucleotides 43921001 to 43954500). 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Study 1

Cultured A431 cells at a density of 20,000 cells per well were transfected by free uptake with 4,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and PNPLA3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS36070 (forward sequence CCTTGGTATGTTCCTGCTTCA, designated herein as SEQ ID NO: 11; reverse sequence GTTGTCACTCACTCCTCCATC, designated herein as SEQ ID NO: 12; probe sequence TGGCCTTATCCCTCCTTCCTTCAGA, designated herein as SEQ ID NO: 13) was used to measure mRNA levels. PNPLA3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PNPLA3, relative to untreated control cells.

Table 1

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

912709	27	42	2765	2780	GGCATTCCCAGCGCGA	0	17
912710	95	110	2833	2848	TCCTGATCCGCAGCAG	15	18
912711	103	118	2841	2856	GGCTCGGGTCCTGATC	0	19
912712	131	146	2869	2884	GTTAGGATCTGGGTCG	91	20
912713	164	179	2902	2917	GTACATGGCGGCGGCG	0	21
912714	183	198	2921	2936	TCCAGCCGCGCTCTGC	23	22
912715	196	211	2934	2949	GCGAAGGACAAGCTCC	60	23
912716	197	212	2935	2950	CGCGAAGGACAAGCTC	0	24
912717	272	287	3010	3025	GCGGAGGAGGTGCGGG	0	25
912718	273	288	3011	3026	CGCGGAGGAGGTGCGG	0	26
912719	274	289	3012	3027	TCGCGGAGGAGGTGCG	19	27
912720	290	305	3028	3043	GAACAACATGCGCGCG	0	28
912721	291	306	3029	3044	CGAACAACATGCGCGC	7	29
912722	292	307	3030	3045	CCGAACAACATGCGCG	21	30
912723	293	308	3031	3046	GCCGAACAACATGCGC	0	31
912724	294	309	3032	3047	CGCCGAACAACATGCG	0	32
912725	323	338	3061	3076	GCCGACGCAGTGCAAC	0	33
912726	324	339	3062	3077	CGCCGACGCAGTGCAA	0	34
912727	340	355	3078	3093	GGGATACCGGAGAGGA	43	35
912728	370	385	5944	5959	TCTGAGAGGACCTGCA	53	36
912729	375	390	5949	5964	CAAGATCTGAGAGGAC	64	37
912730	404	419	5978	5993	GCCAATGTTCCGACTC	71	38
912731	410	425	5984	5999	GAAGATGCCAATGTTC	51	39
912732	429	444	6003	6018	TTAAGTTGAAGGATGG	96	40
912733	432	447	6006	6021	TGCTTAAGTTGAAGGA	90	41
912734	478	493	6052	6067	TGGACATTGGCCGGGA	85	42
912735	479	494	6053	6068	GTGGACATTGGCCGGG	50	43
912736	484	499	6058	6073	AGCTGGTGGACATTGG	64	44
912737	528	543	6102	6117	CATCAGACACTCTGGT	5	45
912738	531	546	6105	6120	CCCCATCAGACACTCT	73	46
912739	552	567	6126	6141	AGTCAGACACCAGAAC	54	47
912755	693	708	11911	11926	TGGCATCAATGAAGGG	74	48
912756	698	713	11916	11931	TGTTTTGGCATCAATG	91	49
912757	746	761	11964	11979	TTTAGGGCAGATGTCG	89	50

912758	747	762	11965	11980	CTTTAGGGCAGATGTC	90	51
912759	795	810	12013	12028	GTAGACTGAGCTTGGT	98	52
912760	820	835	12038	12053	AGGTAGAGGTTCCCTG	0	53
912761	841	856	12059	12074	GGGACAAAAGCTCTCG	20	54
912762	873	888	13609	13624	GGCATATCTCTCCCAG	0	55
912763	874	889	13610	13625	AGGCATATCTCTCCCA	0	56
912764	886	901	13622	13637	AAATATCCTCGAAGGC	57	57
912765	888	903	13624	13639	CCAAATATCCTCGAAG	30	58
912766	889	904	13625	13640	TCCAAATATCCTCGAA	38	59
912767	894	909	13630	13645	ATGCATCCAAATATCC	58	60
912768	925	940	N/A	N/A	TTGCAGATGCCCTTCT	15	61
912769	968	983	16088	16103	ATCCATCCCTTCTGAG	34	62
912770	986	1001	16106	16121	GGGCATGGCGACCTCA	0	63
912771	1004	1019	16124	16139	ACTCATGTTTGCCCAG	67	64
912772	1068	1083	16188	16203	GGTCTAGCAGCTCATC	89	65
912773	1075	1090	16195	16210	CGCAGGTGGTCTAGCA	0	66
912774	1076	1091	16196	16211	ACGCAGGTGGTCTAGC	25	67
912775	1080	1095	16200	16215	TGAGACGCAGGTGGTC	50	68
912776	1086	1101	16206	16221	GGATGCTGAGACGCAG	67	69
912777	1172	1187	19012	19027	GTATCCACCTTTGTCT	78	70
912778	1178	1193	19018	19033	GCTCATGTATCCACCT	79	71
912779	1187	1202	19027	19042	GCAAATCTTGCTCATG	3	72
912780	1188	1203	19028	19043	TGCAAATCTTGCTCAT	13	73
912781	1189	1204	19029	19044	TTGCAAATCTTGCTCA	0	74
912782	1195	1210	19035	19050	AGCAAGTTGCAAATCT	77	75
912783	1199	1214	19039	19054	GGGTAGCAAGTTGCAA	74	76
912784	1205	1220	19045	19060	CCTAATGGGTAGCAAG	62	77
912785	1206	1221	19046	19061	TCCTAATGGGTAGCAA	79	78

Table 2

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 2	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

	Start Site	Stop Site	Start Site	Stop Site			
912786	1207	1222	19047	19062	ATCCTAATGGGTAGCA	81	79
912787	1211	1226	19051	19066	CATTATCCTAATGGGT	46	80
912788	1212	1227	19052	19067	ACATTATCCTAATGGG	0	81
912789	1213	1228	19053	19068	GACATTATCCTAATGG	70	82
912790	1220	1235	19060	19075	TACATAAGACATTATC	34	83
912791	1224	1239	19064	19079	GCATTACATAAGACAT	86	84
912792	1245	1260	19085	19100	CCACAGGCAGGGTACA	76	85
912793	1246	1261	19086	19101	TCCACAGGCAGGGTAC	28	86
912794	1253	1268	19093	19108	GGCAGATTCCACAGGC	75	87
912795	1259	1274	19099	19114	CGCAATGGCAGATTCC	92	88
912796	1265	1280	19105	19120	GACAATCGCAATGGCA	64	89
912797	1266	1281	19106	19121	GGACAATCGCAATGGC	75	90
912798	1267	1282	19107	19122	TGGACAATCGCAATGG	73	91
912799	1285	1300	23690	23705	AGCCATGTCACCAGTC	67	92
912800	1289	1304	23694	23709	TGGAAGCCATGTCACC	24	93
912801	1290	1305	23695	23710	CTGGAAGCCATGTCAC	72	94
912802	1297	1312	23702	23717	GGCATATCTGGAAGCC	0	95
912803	1298	1313	23703	23718	GGGCATATCTGGAAGC	0	96
912804	1351	1366	23756	23771	AGCACTCGAGTGAACA	0	97
912805	1386	1401	N/A	N/A	GCATTTGGGACCTGGA	77	98
912806	1387	1402	N/A	N/A	GGCATTTGGGACCTGG	60	99
912807	1388	1403	25151	25166	TGGCATTTGGGACCTG	41	100
912808	1394	1409	25157	25172	GCTCACTGGCATTTGG	44	101
912809	1523	1538	25286	25301	GTTCAGGCTGGACCTG	49	102
912810	1547	1562	25310	25325	AGGTACTTTATTGCCC	11	103
912811	1550	1565	25313	25328	AGCAGGTACTTTATTG	64	104
912812	1653	1668	25416	25431	AACTTTAGCACCTCTG	91	105
912813	1655	1670	25418	25433	GAAACTTTAGCACCTC	88	106
912814	1656	1671	25419	25434	GGAAACTTTAGCACCT	53	107
912815	1669	1684	25432	25447	CTGCACAAAGATGGGA	80	108
912816	1671	1686	25434	25449	AGCTGCACAAAGATGG	45	109

912817	1685	1700	25448	25463	AGCAATGCGGAGGTAG	15	110
912818	1740	1755	25503	25518	ACCAACTCAGCTCAGA	85	111
912819	1741	1756	25504	25519	AACCAACTCAGCTCAG	79	112
912820	1757	1772	25520	25535	TCCTAGCTTTTCATAA	23	113
912821	1788	1803	25551	25566	TGCTGGACCGCTGCAC	0	114
912822	1796	1811	25559	25574	GAGTTAAGTGCTGGAC	93	115
912823	1802	1817	25565	25580	GTATTAGAGTTAAGTG	92	116
912824	1803	1818	25566	25581	TGTATTAGAGTTAAGT	79	117
912825	1806	1821	25569	25584	TGATGTATTAGAGTTA	92	118
912826	1808	1823	25571	25586	GCTGATGTATTAGAGT	80	119
912827	1821	1836	25584	25599	TGAATTAACGCATGCT	83	120
912828	1822	1837	25585	25600	CTGAATTAACGCATGC	78	121
912829	1870	1885	25633	25648	AGTAAGGGACCCTCTG	17	122
912830	1871	1886	25634	25649	CAGTAAGGGACCCTCT	28	123
912831	1872	1887	25635	25650	TCAGTAAGGGACCCTC	77	124
912832	1874	1889	25637	25652	AGTCAGTAAGGGACCC	51	125
912833	1893	1908	25656	25671	ATTAATAGGGCCACGA	80	126
912834	1895	1910	25658	25673	CCATTAATAGGGCCAC	90	127
912835	1896	1911	25659	25674	ACCATTAATAGGGCCA	81	128
912836	1906	1921	25669	25684	GAACAGTCTGACCATT	82	129
912837	1908	1923	25671	25686	TGGAACAGTCTGACCA	31	130
912838	1909	1924	25672	25687	CTGGAACAGTCTGACC	83	131
912839	1911	1926	25674	25689	TGCTGGAACAGTCTGA	72	132
912840	1916	1931	25679	25694	CCTCATGCTGGAACAG	83	133
912841	1928	1943	25691	25706	TCATTCTAAGAACCTC	96	134
912842	1945	1960	25708	25723	ACCCATCCAAACACCT	16	135
912843	1982	1997	25745	25760	ACACATGGGCCAGCCT	70	136
912844	1989	2004	25752	25767	CAAGATCACACATGGG	70	137
912845	2057	2072	25820	25835	GGGACGAACTGCACCC	0	138
912846	2098	2113	25861	25876	TATCATCTTTGCAGAC	81	139
912847	2116	2131	25879	25894	GTTTTTAGTAGTCAAG	91	140
912848	2117	2132	25880	25895	CGTTTTTAGTAGTCAA	91	141
912849	2145	2160	25908	25923	TATCATCTTGTTACCC	85	142
912850	2148	2163	25911	25926	GATTATCATCTTGTTA	70	143

912851	2150	2165	25913	25928	TAGATTATCATCTTGT	53	144
912852	2151	2166	25914	25929	GTAGATTATCATCTTG	80	145
912853	2152	2167	25915	25930	AGTAGATTATCATCTT	84	146
912854	2175	2190	25938	25953	GTGAAAAAGGTGTTCT	77	147
912855	2182	2197	25945	25960	TAGTTAGGTGAAAAAG	92	148
912856	2188	2203	25951	25966	TTATTTTAGTTAGGTG	88	149
912857	2190	2205	25953	25968	CATTATTTTAGTTAGG	86	150
912858	2273	2288	26036	26051	CTACTAACATCTCACT	55	151
912859	2274	2289	26037	26052	TCTACTAACATCTCAC	89	152
912860	2278	2293	26041	26056	TTATTCTACTAACATC	27	153
912861	2280	2295	26043	26058	GCTTATTCTACTAACA	79	154
912862	2281	2296	26044	26059	GGCTTATTCTACTAAC	81	155
912863	2632	2647	26395	26410	GGTGAATGCCCTGCAC	41	156

Table 3

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
912864	2703	2718	26466	26481	TTCAAGTTGTGTGCTC	90	157
912865	2755	2770	26518	26533	GGGAGAAACTCACTGA	37	158
912866	N/A	N/A	4416	4431	TGCTACTTGCCCCAGC	2	159
912867	N/A	N/A	4421	4436	CACAATGCTACTTGCC	87	160
912868	N/A	N/A	4584	4599	CCCAATGGCAGGGCTT	58	161
912869	N/A	N/A	4592	4607	TGCTCCTACCCAATGG	46	162
912870	N/A	N/A	4766	4781	GACTTTTATTGTTGCT	95	163
912871	N/A	N/A	4883	4898	TTCTATACCAGAGTGA	89	164
912872	N/A	N/A	4884	4899	TTTCTATACCAGAGTG	89	165
912873	N/A	N/A	5405	5420	GTAGATGGCCTTAATG	83	166
912876	N/A	N/A	6155	6170	TACATCCACGACTTCG	94	167
912877	N/A	N/A	6156	6171	TTACATCCACGACTTC	76	168
912880	N/A	N/A	6606	6621	GGAACATTCAGGGTTT	13	169

912881	N/A	N/A	6834	6849	ATTACTTGGGTGCAGG	55	170
912884	N/A	N/A	6838	6853	GCAGATTACTTGGGTG	45	171
912885	N/A	N/A	6931	6946	TGCAGGACAGGTTCCT	30	172
912888	N/A	N/A	7549	7564	CACACTGGGTCACCAC	55	173
912889	N/A	N/A	7552	7567	AGTCACACTGGGTCAC	61	174
912928	N/A	N/A	12273	12288	GGTATATGTTCCCAGG	87	175
912929	N/A	N/A	12314	12329	TATAACCACAGCCTGG	29	176
912932	N/A	N/A	12321	12336	CTGACTATATAACCAC	81	177
912933	N/A	N/A	12666	12681	ATCTTAGTGGCTGGGT	91	178
912936	N/A	N/A	12767	12782	CTTACTATGGTAGAGT	88	179
912937	N/A	N/A	12768	12783	TCTTACTATGGTAGAG	74	180
912940	N/A	N/A	12835	12850	TGCATTGCATAGCCTT	97	181
912941	N/A	N/A	12836	12851	TTGCATTGCATAGCCT	96	182
912944	N/A	N/A	12907	12922	TGCTTATAAAGCACAC	61	183
912945	N/A	N/A	12988	13003	GGAATAAGCCTCCACC	14	184
912948	N/A	N/A	14055	14070	GAAATCTGATTGCTTC	59	185
912949	N/A	N/A	14393	14408	TACTTATCTGCTCACT	66	186
912952	N/A	N/A	14673	14688	TCTCTTAGTGTCCCCA	90	187
			14707	14722			
912953	N/A	N/A	14674	14689	ATCTCTTAGTGTCCCC	92	188
			14708	14723			
912956	N/A	N/A	15284	15299	TCACATTCATGCTTGC	82	189
912957	N/A	N/A	15291	15306	GATAACCTCACATTCA	0	190
912960	N/A	N/A	15712	15727	GAGCTAGGTGCTTCAC	6	191
912961	N/A	N/A	15753	15768	ATAACAACTGAACCAC	85	192
912964	N/A	N/A	15937	15952	GTTATTAGCCAAATGC	92	193
912965	N/A	N/A	16468	16483	GGAGACTTGGCAAGGT	87	194
912968	N/A	N/A	16960	16975	ATTCATGACAGCCCTT	46	195
912969	N/A	N/A	17128	17143	ATCGATTTTTCAGAGT	9	196
912972	N/A	N/A	17134	17149	ACAAACATCGATTTTT	52	197
912973	N/A	N/A	17769	17784	CTCTTTAATGACCTCG	90	198
912976	N/A	N/A	18865	18880	GTCAGAGGCACTCACA	25	199
912977	N/A	N/A	18959	18974	AGCTATTATCTCCCAC	0	200
912980	N/A	N/A	19315	19330	AGTTTCTGGGCTTGCA	90	201

912981	N/A	N/A	19382	19397	GGCAATCACAAGAGAC	73	202
912984	N/A	N/A	20286	20301	AGAGGAAGCCCAATCA	79	203
			20316	20331			
912985	N/A	N/A	20287	20302	CAGAGGAAGCCCAATC	93	204
			20317	20332			
912988	N/A	N/A	20658	20673	TAGAAATTGCAGTGCC	92	205
912989	N/A	N/A	20731	20746	TCCTATCCATATATTG	55	206
912992	N/A	N/A	21408	21423	GCAATTCTAGACATGG	88	207
912993	N/A	N/A	21558	21573	AGGACTTACACCAAGA	86	208
912996	N/A	N/A	21936	21951	TTCCTAATAAGAGCCC	24	209
912997	N/A	N/A	21946	21961	GTCAAACATCTTCCTA	66	210
913000	N/A	N/A	22077	22092	AAAACTGTAGGATAGG	47	211
913001	N/A	N/A	22162	22177	GTTACATCCATAAAAC	0	212
913004	N/A	N/A	22169	22184	AGAGAATGTTACATCC	62	213
913008	N/A	N/A	23083	23098	AAAGATTAATCAGGGC	61	214
913012	N/A	N/A	23788	23803	GTATTTACCTGGAGGC	0	215
913016	N/A	N/A	24426	24441	GGCCTATGATTTTCAG	0	216

Table 4

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
912874	N/A	N/A	5869	5884	ATACTTTTGGCAAGGC	96	217
912875	N/A	N/A	5870	5885	AATACTTTTGGCAAGG	91	218
912878	N/A	N/A	6159	6174	TGCTTACATCCACGAC	12	219
912879	N/A	N/A	6296	6311	CATCATGTTGGTCTCG	54	220
912882	N/A	N/A	6835	6850	GATTACTTGGGTGCAG	39	221
912883	N/A	N/A	6837	6852	CAGATTACTTGGGTGC	69	222
912886	N/A	N/A	7083	7098	TTTAATGGTGTTTTGG	87	223
912887	N/A	N/A	7478	7493	TCAAATGCCGGTATTC	52	224
912890	N/A	N/A	7587	7602	GTGAACTTCAACTTCC	56	225

912930	N/A	N/A	12317	12332	CTATATAACCACAGCC	77	226
912931	N/A	N/A	12319	12334	GACTATATAACCACAG	92	227
912934	N/A	N/A	12670	12685	AATCATCTTAGTGGCT	91	228
912935	N/A	N/A	12765	12780	TACTATGGTAGAGTGG	80	229
912938	N/A	N/A	12786	12801	GTACATGGTCTGCAAA	84	230
912939	N/A	N/A	12787	12802	TGTACATGGTCTGCAA	57	231
912942	N/A	N/A	12843	12858	GCATGCATTGCATTGC	16	232
912943	N/A	N/A	12885	12900	ACCAATCCTGTTAGAC	93	233
912946	N/A	N/A	13557	13572	GGAGACACCAAGCACC	42	234
912947	N/A	N/A	13751	13766	GCACTAAGTGTTAGAA	79	235
912950	N/A	N/A	14396	14411	GCTTACTTATCTGCTC	0	236
912951	N/A	N/A	14501	14516	GGAGATCCATCCTGCA	0	237
912954	N/A	N/A	14675	14690	CATCTCTTAGTGTCCC	92	238
			14709	14724			
912955	N/A	N/A	15122	15137	TCCTAATGTCCTCAAC	9	239
912958	N/A	N/A	15293	15308	AAGATAACCTCACATT	33	240
912959	N/A	N/A	15294	15309	CAAGATAACCTCACAT	22	241
912962	N/A	N/A	15754	15769	TATAACAACTGAACCA	82	242
912963	N/A	N/A	15856	15871	GCTTTAAAGCAGGACA	8	243
912966	N/A	N/A	16774	16789	AAAATTGTGGGTTTAG	68	244
912967	N/A	N/A	16850	16865	ATCATTTGGACCATAG	81	245
912970	N/A	N/A	17130	17145	ACATCGATTTTTCAGA	83	246
912971	N/A	N/A	17133	17148	CAAACATCGATTTTTC	62	247
912974	N/A	N/A	17843	17858	GCTTTACAAGCTGGTC	0	248
912975	N/A	N/A	17879	17894	ATCTATGTTCTCCTAG	0	249
912978	N/A	N/A	19125	19140	ACCTAAAATGCTCACC	0	250
912979	N/A	N/A	19198	19213	CCAGACTACATGCCAC	79	251
912982	N/A	N/A	19446	19461	TCTACTAGGCATCTCT	63	252
912983	N/A	N/A	19447	19462	TTCTACTAGGCATCTC	42	253
912986	N/A	N/A	20288	20303	TCAGAGGAAGCCCAAT	92	254
			20318	20333			
912987	N/A	N/A	20656	20671	GAAATTGCAGTGCCCT	92	255
912990	N/A	N/A	21393	21408	GCCAACCTATCACTGA	60	256
912991	N/A	N/A	21400	21415	AGACATGGCCAACCTA	32	257

912994	N/A	N/A	21565	21580	TGAAATAAGGACTTAC	67	258
912995	N/A	N/A	21934	21949	CCTAATAAGAGCCCCA	31	259
912998	N/A	N/A	22041	22056	GAAATCTGTCAGAGCA	33	260
912999	N/A	N/A	22072	22087	TGTAGGATAGGACTAG	0	261
913002	N/A	N/A	22166	22181	GAATGTTACATCCATA	53	262
913003	N/A	N/A	22168	22183	GAGAATGTTACATCCA	80	263
913005	N/A	N/A	22605	22620	GTGATAAATCTGCAAG	70	264
913006	N/A	N/A	23081	23096	AGATTAATCAGGGCCA	8	265
913007	N/A	N/A	23082	23097	AAGATTAATCAGGGCC	30	266
913009	N/A	N/A	23325	23340	GGTCACATGTGAGCCC	0	267
913010	N/A	N/A	23496	23511	CACTTCTGGTTCAAGA	13	268
913011	N/A	N/A	23580	23595	CCAATCTGATGACTTC	80	269
913013	N/A	N/A	23790	23805	AAGTATTTACCTGGAG	0	270
913014	N/A	N/A	24028	24043	CACTCAAAGAGACTCA	65	271
913015	N/A	N/A	24425	24440	GCCTATGATTTTCAGG	0	272
913017	N/A	N/A	24633	24648	CACTACTGCCCTCTTC	50	273
913018	N/A	N/A	24983	24998	TGCTGGGCTGATGTCA	0	274
913019	N/A	N/A	25150	25165	GGCATTTGGGACCTGA	67	275

Table 5

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
915343	1	16	2739	2754	GCCCCCCTCGGACCAT	0	276
915363	45	60	2783	2798	CCTCAGTGTCTCGGCC	0	277
915383	107	122	2845	2860	AATCGGCTCGGGTCCT	29	278
915403	190	205	2928	2943	GACAAGCTCCAGCCGC	64	279
915423	249	264	2987	3002	CGCTCAGGCAGCGGGT	0	280
915443	347	362	N/A	N/A	CTCCAGCGGGATACCG	6	281
915463	386	401	5960	5975	GGCCTTCCGCACAAGA	0	282
915483	416	431	5990	6005	TGGATGGAAGATGCCA	28	283

915503	452	467	6026	6041	GAGACCCTGTCGGAGG	45	284
915523	488	503	6062	6077	GATGAGCTGGTGGACA	70	285
915543	510	525	6084	6099	GAGAGATGCCTATTTT	92	286
915563	559	574	6133	6148	GACCGAAAGTCAGACA	7	287
915603	697	712	11915	11930	GTTTTGGCATCAATGA	94	288
915623	754	769	11972	11987	GACTTGACTTTAGGGC	98	289
915643	827	842	12045	12060	CGAGAGAAGGTAGAGG	97	290
915663	879	894	13615	13630	CTCGAAGGCATATCTC	66	291
915683	932	947	16052	16067	GGGCCTGTTGCAGATG	0	292
915703	985	1000	16105	16120	GGCATGGCGACCTCAG	6	293
915723	1037	1052	16157	16172	AGCCAAGGCAGCCGAC	0	294
915743	1132	1147	16252	16267	GCGAGCCTGGGCGAGA	0	295
915763	1177	1192	19017	19032	CTCATGTATCCACCTT	88	296
915783	1229	1244	19069	19084	GGGCAGCATTACATAA	73	297
915803	1286	1301	23691	23706	AAGCCATGTCACCAGT	34	298
915823	1348	1363	23753	23768	ACTCGAGTGAACACCT	12	299
915843	1405	1420	25168	25183	GCCTGTTGGCTGCTCA	1	300
915863	1473	1488	25236	25251	CTGCTGGACAGCCCTT	0	301
915883	1542	1557	25305	25320	CTTTATTGCCCAAGAA	72	302
915903	1601	1616	25364	25379	CAGACTCTTCTCTAGT	49	303
915923	1633	1648	25396	25411	AATCTGCTAGACTCGC	88	304
915943	1686	1701	25449	25464	CAGCAATGCGGAGGTA	80	305
915963	1768	1783	25531	25546	GAAAGGTTGCTTCCTA	84	306
915983	1789	1804	25552	25567	GTGCTGGACCGCTGCA	11	307
916003	1815	1830	25578	25593	AACGCATGCTGATGTA	69	308
916023	1848	1863	25611	25626	GCTTCCTGGTGTCATT	81	309
916043	1884	1899	25647	25662	GCCACGAAACAGTCAG	67	310
916063	1913	1928	25676	25691	CATGCTGGAACAGTCT	20	311
916083	1954	1969	25717	25732	AAGGCCCCCACCCATC	0	312
916103	1977	1992	25740	25755	TGGGCCAGCCTACCCC	0	313
916123	2026	2041	25789	25804	GGAAGTGGGATCATGC	55	314
916142	2100	2115	25863	25878	GTTATCATCTTTGCAG	57	315
916162	2139	2154	25902	25917	CTTGTTACCCCCGCCA	84	316
916182	2264	2279	26027	26042	TCTCACTGATTCACAT	83	317

916202	2624	2639	26387	26402	CCCTGCACACTAGATT	55	318
916222	2677	2692	26440	26455	GAGGCGGAAGCTCCTG	0	319
916242	2707	2722	26470	26485	CAGGTTCAAGTTGTGT	83	320
916282	N/A	N/A	4225	4240	AAATGTACGGAATCTC	79	321
916302	N/A	N/A	4822	4837	GTGTAAACATTTGTCC	74	322
916322	N/A	N/A	5414	5429	AGCTTTGGTGTAGATG	49	323
916342	N/A	N/A	5801	5816	TACTATGGGAGCCACA	42	324
916362	N/A	N/A	6866	6881	TGAAATTGTAACTGCC	70	325
916382	N/A	N/A	7492	7507	TAGATCGGTGCTGTTC	27	326
916402	N/A	N/A	7785	7800	GTTATAGGCGAGAGCA	0	327
916562	N/A	N/A	12316	12331	TATATAACCACAGCCT	58	328
916582	N/A	N/A	12932	12947	ATAAGAGCTGTCTCCT	94	329
916602	N/A	N/A	13703	13718	CTAGTAAATGCTTGTC	96	330
916622	N/A	N/A	14177	14192	CTAATATTTCTACAGC	0	331
916642	N/A	N/A	14672	14687	CTCTTAGTGTCCCCAT	95	332
916662	N/A	N/A	15542	15557	TTCCATCACAAGGCCT	50	333
916682	N/A	N/A	16317	16332	TCCATAATGCACAAGA	71	334
916702	N/A	N/A	17223	17238	TGTAGCTGGTTTGTGG	88	335
916722	N/A	N/A	18223	18238	AACAGCTACATCAGGC	44	336
916742	N/A	N/A	19249	19264	GGCATTGCACATAGAC	74	337
916761	N/A	N/A	20410	20425	GTAAGCAATGCAGCCA	88	338
916781	N/A	N/A	20659	20674	TTAGAAATTGCAGTGC	91	339
916801	N/A	N/A	20989	21004	AGGTATTAAACTGCCA	25	340
916821	N/A	N/A	21506	21521	GTCCTAAGAGCACTCA	57	341
916841	N/A	N/A	22603	22618	GATAAATCTGCAAGAG	49	342
916861	N/A	N/A	23472	23487	GGGACTTACACTGAAA	66	343
916881	N/A	N/A	24314	24329	GTCAACGCAGACTGCT	33	344

Table 6

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 2	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

	Start Site	Stop Site	Start Site	Stop Site			
915344	2	17	2740	2755	CGCCCCCCTCGGACCA	0	345
915364	46	61	2784	2799	GCCTCAGTGTCTCGGC	0	346
915384	108	123	2846	2861	GAATCGGCTCGGGTCC	49	347
915404	191	206	2929	2944	GGACAAGCTCCAGCCG	9	348
915424	250	265	2988	3003	TCGCTCAGGCAGCGGG	0	349
915444	348	363	N/A	N/A	GCTCCAGCGGGATACC	0	350
915464	387	402	5961	5976	TGGCCTTCCGCACAAG	0	351
915484	428	443	6002	6017	TAAGTTGAAGGATGGA	96	352
915504	453	468	6027	6042	AGAGACCCTGTCGGAG	80	353
915524	489	504	6063	6078	AGATGAGCTGGTGGAC	81	354
915544	512	527	6086	6101	AAGAGAGATGCCTATT	77	355
915564	560	575	6134	6149	GGACCGAAAGTCAGAC	0	356
915604	700	715	11918	11933	GTTGTTTTGGCATCAA	91	357
915624	755	770	11973	11988	GGACTTGACTTTAGGG	81	358
915644	828	843	12046	12061	TCGAGAGAAGGTAGAG	24	359
915664	880	895	13616	13631	CCTCGAAGGCATATCT	41	360
915684	952	967	16072	16087	GATGACTTCAGGCCTG	0	361
915704	987	1002	16107	16122	TGGGCATGGCGACCTC	0	362
915724	1038	1053	16158	16173	CAGCCAAGGCAGCCGA	0	363
915744	1133	1148	16253	16268	AGCGAGCCTGGGCGAG	0	364
915764	1179	1194	19019	19034	TGCTCATGTATCCACC	56	365
915784	1230	1245	19070	19085	AGGGCAGCATTACATA	69	366
915804	1293	1308	23698	23713	TATCTGGAAGCCATGT	6	367
915824	1349	1364	23754	23769	CACTCGAGTGAACACC	0	368
915844	1406	1421	25169	25184	GGCCTGTTGGCTGCTC	0	369
915864	1477	1492	25240	25255	GTCTCTGCTGGACAGC	0	370
915884	1545	1560	25308	25323	GTACTTTATTGCCCAA	73	371
915904	1607	1622	25370	25385	GACTCACAGACTCTTC	92	372
915924	1634	1649	25397	25412	GAATCTGCTAGACTCG	65	373
915944	1687	1702	25450	25465	ACAGCAATGCGGAGGT	83	374
915964	1769	1784	25532	25547	CGAAAGGTTGCTTCCT	79	375

915984	1790	1805	25553	25568	AGTGCTGGACCGCTGC	38	376
916004	1816	1831	25579	25594	TAACGCATGCTGATGT	79	377
916024	1849	1864	25612	25627	GGCTTCCTGGTGTCAT	73	378
916044	1885	1900	25648	25663	GGCCACGAAACAGTCA	40	379
916064	1914	1929	25677	25692	TCATGCTGGAACAGTC	80	380
916084	1958	1973	25721	25736	TCACAAGGCCCCCACC	35	381
916104	1978	1993	25741	25756	ATGGGCCAGCCTACCC	0	382
916124	2053	2068	25816	25831	CGAACTGCACCCCTTC	38	383
916143	2101	2116	25864	25879	GGTTATCATCTTTGCA	81	384
916163	2140	2155	25903	25918	TCTTGTTACCCCCGCC	84	385
916183	2265	2280	26028	26043	ATCTCACTGATTCACA	86	386
916203	2625	2640	26388	26403	GCCCTGCACACTAGAT	65	387
916223	2678	2693	26441	26456	GGAGGCGGAAGCTCCT	0	388
916243	2709	2724	26472	26487	GCCAGGTTCAAGTTGT	62	389
916283	N/A	N/A	4226	4241	CAAATGTACGGAATCT	52	390
916303	N/A	N/A	4864	4879	TACTTTAGGCTCCTGG	90	391
916323	N/A	N/A	5422	5437	AGCATTAGAGCTTTGG	75	392
916343	N/A	N/A	5803	5818	TCTACTATGGGAGCCA	89	393
916363	N/A	N/A	6927	6942	GGACAGGTTCCTTGGA	0	394
916383	N/A	N/A	7493	7508	CTAGATCGGTGCTGTT	14	395
916403	N/A	N/A	7786	7801	AGTTATAGGCGAGAGC	0	396
916563	N/A	N/A	12318	12333	ACTATATAACCACAGC	90	397
916583	N/A	N/A	12936	12951	GACAATAAGAGCTGTC	0	398
916603	N/A	N/A	13704	13719	GCTAGTAAATGCTTGT	73	399
916623	N/A	N/A	14231	14246	CCAACTTTTAGTATTA	92	400
916643	N/A	N/A	14678	14693	AGCCATCTCTTAGTGT	50	401
916663	N/A	N/A	15566	15581	TCTGATGTCGAAGAGG	68	402
916683	N/A	N/A	16341	16356	TCCCATGTGGCAGTAC	0	403
916703	N/A	N/A	17239	17254	TCCAAATGCCCAACTC	37	404
916723	N/A	N/A	18241	18256	GCAAATAATGTGCACA	22	405
916743	N/A	N/A	19250	19265	GGGCATTGCACATAGA	59	406
916762	N/A	N/A	20413	20428	GTAGTAAGCAATGCAG	69	407
916782	N/A	N/A	20660	20675	CTTAGAAATTGCAGTG	91	408
916802	N/A	N/A	21002	21017	ATTTTAACAGCTCAGG	95	409

916822	N/A	N/A	21540	21555	TATGACATTTCAGAGT	88	410
916842	N/A	N/A	22629	22644	AGTACAAGCGCAGCCT	14	411
916862	N/A	N/A	23538	23553	ACAAGGACAAGCCCAC	37	412
916882	N/A	N/A	24339	24354	GAAGTAGCGGCATCCC	68	413

Table 7

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915345	3	18	2741	2756	CCGCCCCCCTCGGACC	0	414
915365	47	62	2785	2800	TGCCTCAGTGTCTCGG	0	415
915385	109	124	2847	2862	GGAATCGGCTCGGGTC	72	416
915405	193	208	2931	2946	AAGGACAAGCTCCAGC	41	417
915425	251	266	2989	3004	CTCGCTCAGGCAGCGG	0	418
915445	349	364	N/A	N/A	TGCTCCAGCGGGATAC	0	419
915465	388	403	5962	5977	CTGGCCTTCCGCACAA	16	420
915485	430	445	6004	6019	CTTAAGTTGAAGGATG	27	421
915505	454	469	6028	6043	CAGAGACCCTGTCGGA	72	422
915525	492	507	6066	6081	CGGAGATGAGCTGGTG	92	423
915545	513	528	6087	6102	TAAGAGAGATGCCTAT	57	424
915565	561	576	6135	6150	TGGACCGAAAGTCAGA	0	425
915605	701	716	11919	11934	GGTTGTTTTGGCATCA	97	426
915625	756	771	11974	11989	TGGACTTGACTTTAGG	93	427
915645	829	844	12047	12062	CTCGAGAGAAGGTAGA	0	428
915665	881	896	13617	13632	TCCTCGAAGGCATATC	0	429
915685	953	968	16073	16088	GGATGACTTCAGGCCT	0	430
915705	988	1003	16108	16123	CTGGGCATGGCGACCT	0	431
915725	1039	1054	16159	16174	ACAGCCAAGGCAGCCG	0	432
915745	1134	1149	16254	16269	TAGCGAGCCTGGGCGA	0	433
915765	1193	1208	19033	19048	CAAGTTGCAAATCTTG	0	434
915785	1231	1246	19071	19086	CAGGGCAGCATTACAT	74	435

915805	1300	1315	23705	23720	TCGGGCATATCTGGAA	21	436
915825	1350	1365	23755	23770	GCACTCGAGTGAACAC	0	437
915845	1407	1422	25170	25185	AGGCCTGTTGGCTGCT	0	438
915865	1480	1495	25243	25258	TTGGTCTCTGCTGGAC	21	439
915885	1546	1561	25309	25324	GGTACTTTATTGCCCA	62	440
915905	1609	1624	25372	25387	GTGACTCACAGACTCT	81	441
915925	1635	1650	25398	25413	AGAATCTGCTAGACTC	74	442
915945	1688	1703	25451	25466	CACAGCAATGCGGAGG	56	443
915965	1770	1785	25533	25548	GCGAAAGGTTGCTTCC	66	444
915985	1791	1806	25554	25569	AAGTGCTGGACCGCTG	71	445
916005	1817	1832	25580	25595	TTAACGCATGCTGATG	69	446
916025	1850	1865	25613	25628	GGGCTTCCTGGTGTCA	58	447
916045	1886	1901	25649	25664	GGGCCACGAAACAGTC	9	448
916065	1915	1930	25678	25693	CTCATGCTGGAACAGT	86	449
916085	1959	1974	25722	25737	ATCACAAGGCCCCCAC	82	450
916105	1979	1994	25742	25757	CATGGGCCAGCCTACC	0	451
916125	2054	2069	25817	25832	ACGAACTGCACCCCTT	84	452
916144	2102	2117	25865	25880	AGGTTATCATCTTTGC	90	453
916164	2141	2156	25904	25919	ATCTTGTTACCCCCGC	88	454
916184	2266	2281	26029	26044	CATCTCACTGATTCAC	91	455
916204	2626	2641	26389	26404	TGCCCTGCACACTAGA	47	456
916224	2680	2695	26443	26458	GAGGAGGCGGAAGCTC	0	457
916244	2710	2725	26473	26488	AGCCAGGTTCAAGTTG	71	458
916284	N/A	N/A	4227	4242	TCAAATGTACGGAATC	40	459
916304	N/A	N/A	4865	4880	GTACTTTAGGCTCCTG	89	460
916324	N/A	N/A	5429	5444	ACATATCAGCATTAGA	87	461
916344	N/A	N/A	5804	5819	GTCTACTATGGGAGCC	90	462
916364	N/A	N/A	6966	6981	GAAGATGCATAGAGGA	0	463
916384	N/A	N/A	7550	7565	TCACACTGGGTCACCA	43	464
916544	N/A	N/A	12135	12150	GGCAATCAGGGAGGCA	32	465
916564	N/A	N/A	12320	12335	TGACTATATAACCACA	92	466
916584	N/A	N/A	12951	12966	CCCAATTGCCACTAGG	83	467
916604	N/A	N/A	13718	13733	TCTTTACCAAGACCGC	92	468
916624	N/A	N/A	14245	14260	GACAAATTCATCAACC	87	469

916644	N/A	N/A	14778	14793	CTGTATCCAAAAGGCC	0	470
916664	N/A	N/A	15597	15612	ATACATAGCAGAGCCA	44	471
916684	N/A	N/A	16352	16367	CACCCTATCGCTCCCA	43	472
916704	N/A	N/A	17267	17282	AGTTATGTCTGACTCA	72	473
916724	N/A	N/A	18254	18269	AATATACCCCACAGCA	40	474
916744	N/A	N/A	19288	19303	GTGCATGTGTGGCTTG	82	475
916763	N/A	N/A	20414	20429	TGTAGTAAGCAATGCA	85	476
916783	N/A	N/A	20724	20739	CATATATTGCGGATGA	24	477
916803	N/A	N/A	21005	21020	GTTATTTTAACAGCTC	95	478
916823	N/A	N/A	21561	21576	ATAAGGACTTACACCA	83	479
916843	N/A	N/A	22679	22694	CAGCATGCAACCACCC	8	480
916863	N/A	N/A	23550	23565	TGGGATGCTAGGACAA	72	481
916883	N/A	N/A	24340	24355	GGAAGTAGCGGCATCC	0	482

Table 8

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915346	25	40	2763	2778	CATTCCCAGCGCGACG	0	483
915366	52	67	2790	2805	TACCCTGCCTCAGTGT	0	484
915386	112	127	2850	2865	TCGGGAATCGGCTCGG	26	485
915406	195	210	2933	2948	CGAAGGACAAGCTCCA	69	486
915426	252	267	2990	3005	GCTCGCTCAGGCAGCG	0	487
915446	350	365	N/A	N/A	CTGCTCCAGCGGGATA	0	488
915466	389	404	5963	5978	CCTGGCCTTCCGCACA	40	489
915486	431	446	6005	6020	GCTTAAGTTGAAGGAT	88	490
915506	455	470	6029	6044	GCAGAGACCCTGTCGG	32	491
915526	493	508	6067	6082	CCGGAGATGAGCTGGT	4	492
915546	514	529	6088	6103	GTAAGAGAGATGCCTA	94	493
915566	562	577	6136	6151	TTGGACCGAAAGTCAG	56	494
915606	702	717	11920	11935	TGGTTGTTTTGGCATC	99	495

915626	757	772	11975	11990	GTGGACTTGACTTTAG	89	496
915646	830	845	12048	12063	TCTCGAGAGAAGGTAG	0	497
915666	882	897	13618	13633	ATCCTCGAAGGCATAT	0	498
915686	956	971	16076	16091	TGAGGATGACTTCAGG	10	499
915706	989	1004	16109	16124	GCTGGGCATGGCGACC	10	500
915726	1064	1079	16184	16199	TAGCAGCTCATCTCCC	67	501
915746	1135	1150	16255	16270	GTAGCGAGCCTGGGCG	0	502
915766	1196	1211	19036	19051	TAGCAAGTTGCAAATC	78	503
915786	1232	1247	19072	19087	ACAGGGCAGCATTACA	87	504
915806	1302	1317	23707	23722	CGTCGGGCATATCTGG	53	505
915826	1352	1367	23757	23772	CAGCACTCGAGTGAAC	24	506
915846	1408	1423	25171	25186	GAGGCCTGTTGGCTGC	0	507
915866	1508	1523	25271	25286	GAGGATGGACCGCGGG	0	508
915886	1549	1564	25312	25327	GCAGGTACTTTATTGC	0	509
915906	1610	1625	25373	25388	AGTGACTCACAGACTC	35	510
915926	1636	1651	25399	25414	AAGAATCTGCTAGACT	69	511
915946	1689	1704	25452	25467	ACACAGCAATGCGGAG	69	512
915966	1771	1786	25534	25549	GGCGAAAGGTTGCTTC	58	513
915986	1792	1807	25555	25570	TAAGTGCTGGACCGCT	70	514
916006	1818	1833	25581	25596	ATTAACGCATGCTGAT	73	515
916026	1851	1866	25614	25629	TGGGCTTCCTGGTGTC	71	516
916046	1887	1902	25650	25665	AGGGCCACGAAACAGT	61	517
916066	1917	1932	25680	25695	ACCTCATGCTGGAACA	81	518
916086	1960	1975	25723	25738	CATCACAAGGCCCCCA	48	519
916106	1980	1995	25743	25758	ACATGGGCCAGCCTAC	54	520
916126	2055	2070	25818	25833	GACGAACTGCACCCCT	77	521
916145	2105	2120	25868	25883	TCAAGGTTATCATCTT	89	522
916165	2142	2157	25905	25920	CATCTTGTTACCCCCG	89	523
916185	2270	2285	26033	26048	CTAACATCTCACTGAT	66	524
916205	2627	2642	26390	26405	ATGCCCTGCACACTAG	62	525
916225	2681	2696	26444	26459	AGAGGAGGCGGAAGCT	25	526
916245	2711	2726	26474	26489	AAGCCAGGTTCAAGTT	83	527
916285	N/A	N/A	4240	4255	ATTAGGACAAGATTCA	75	528
916305	N/A	N/A	4866	4881	TGTACTTTAGGCTCCT	93	529

916325	N/A	N/A	5430	5445	AACATATCAGCATTAG	85	530
916345	N/A	N/A	5839	5854	CAAGGATGCCACCAAC	84	531
916365	N/A	N/A	6974	6989	TCATTATGGAAGATGC	0	532
916385	N/A	N/A	7602	7617	TTAACAACCCTGTCAG	1	533
916545	N/A	N/A	12151	12166	GTAACTGGTAGCTCCT	93	534
916565	N/A	N/A	12338	12353	ACCCATACTGCACCCC	79	535
916585	N/A	N/A	12957	12972	GCCTATCCCAATTGCC	70	536
916605	N/A	N/A	13719	13734	GTCTTTACCAAGACCG	23	537
916625	N/A	N/A	14248	14263	AACGACAAATTCATCA	84	538
916645	N/A	N/A	14788	14803	TGCAATCCCCCTGTAT	17	539
916665	N/A	N/A	15598	15613	AATACATAGCAGAGCC	68	540
916685	N/A	N/A	16366	16381	TGTCATGGTTGCCTCA	70	541
916705	N/A	N/A	17273	17288	ATAAGGAGTTATGTCT	80	542
916725	N/A	N/A	18255	18270	GAATATACCCCACAGC	58	543
916745	N/A	N/A	19295	19310	GTTACAGGTGCATGTG	75	544
916764	N/A	N/A	20435	20450	AGTCATCTGGAGTCAC	69	545
916784	N/A	N/A	20756	20771	TCAGACAACCCACTGA	24	546
916804	N/A	N/A	21046	21061	AGGAATCTGAATCCTA	0	547
916824	N/A	N/A	21640	21655	GATAATTTCCTAGAGC	29	548
916844	N/A	N/A	22699	22714	GAAATAAGTGCTCAGG	73	549
916864	N/A	N/A	23582	23597	CTCCAATCTGATGACT	53	550
916884	N/A	N/A	24347	24362	GAATTCAGGAAGTAGC	50	551

Table 9

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915347	26	41	2764	2779	GCATTCCCAGCGCGAC	0	552
915367	58	73	2796	2811	GCTCTCTACCCTGCCT	9	553
915387	113	128	2851	2866	ATCGGGAATCGGCTCG	28	554
915407	198	213	2936	2951	CCGCGAAGGACAAGCT	0	555

915427	253	268	2991	3006	TGCTCGCTCAGGCAGC	0	556
915447	351	366	N/A	N/A	TCTGCTCCAGCGGGAT	1	557
915467	391	406	5965	5980	CTCCTGGCCTTCCGCA	29	558
915487	433	448	6007	6022	TTGCTTAAGTTGAAGG	94	559
915507	456	471	6030	6045	TGCAGAGACCCTGTCG	31	560
915527	494	509	6068	6083	GCCGGAGATGAGCTGG	0	561
915547	515	530	6089	6104	GGTAAGAGAGATGCCT	0	562
915567	563	578	6137	6152	TTTGGACCGAAAGTCA	0	563
915607	703	718	11921	11936	ATGGTTGTTTTGGCAT	35	564
915627	758	773	11976	11991	CGTGGACTTGACTTTA	85	565
915647	831	846	12049	12064	CTCTCGAGAGAAGGTA	7	566
915667	883	898	13619	13634	TATCCTCGAAGGCATA	0	567
915687	959	974	16079	16094	TTCTGAGGATGACTTC	39	568
915707	996	1011	16116	16131	TTGCCCAGCTGGGCAT	0	569
915727	1065	1080	16185	16200	CTAGCAGCTCATCTCC	58	570
915747	1136	1151	16256	16271	TGTAGCGAGCCTGGGC	16	571
915767	1197	1212	19037	19052	GTAGCAAGTTGCAAAT	80	572
915787	1233	1248	19073	19088	TACAGGGCAGCATTAC	71	573
915807	1316	1331	23721	23736	CAACCACAGGACATCG	0	574
915827	1353	1368	23758	23773	TCAGCACTCGAGTGAA	0	575
915847	1409	1424	25172	25187	GGAGGCCTGTTGGCTG	0	576
915867	1509	1524	25272	25287	TGAGGATGGACCGCGG	14	577
915887	1553	1568	25316	25331	ACCAGCAGGTACTTTA	29	578
915907	1611	1626	25374	25389	AAGTGACTCACAGACT	29	579
915927	1637	1652	25400	25415	AAAGAATCTGCTAGAC	60	580
915947	1690	1705	25453	25468	TACACAGCAATGCGGA	69	581
915967	1772	1787	25535	25550	AGGCGAAAGGTTGCTT	0	582
915987	1793	1808	25556	25571	TTAAGTGCTGGACCGC	82	583
916007	1819	1834	25582	25597	AATTAACGCATGCTGA	61	584
916027	1864	1879	25627	25642	GGACCCTCTGCACTGG	43	585
916047	1888	1903	25651	25666	TAGGGCCACGAAACAG	80	586
916067	1918	1933	25681	25696	AACCTCATGCTGGAAC	72	587
916087	1961	1976	25724	25739	CCATCACAAGGCCCCC	63	588
916107	1981	1996	25744	25759	CACATGGGCCAGCCTA	74	589

916127	2056	2071	25819	25834	GGACGAACTGCACCCC	5	590
916146	2106	2121	25869	25884	GTCAAGGTTATCATCT	88	591
916166	2143	2158	25906	25921	TCATCTTGTTACCCCC	90	592
916186	2272	2287	26035	26050	TACTAACATCTCACTG	1	593
916206	2628	2643	26391	26406	AATGCCCTGCACACTA	56	594
916226	2682	2697	26445	26460	GAGAGGAGGCGGAAGC	10	595
916246	2712	2727	26475	26490	TAAGCCAGGTTCAAGT	81	596
916286	N/A	N/A	4244	4259	TTTCATTAGGACAAGA	61	597
916306	N/A	N/A	4867	4882	GTGTACTTTAGGCTCC	97	598
916326	N/A	N/A	5431	5446	GAACATATCAGCATTA	52	599
916346	N/A	N/A	5872	5887	GTAATACTTTTGGCAA	75	600
916366	N/A	N/A	7069	7084	GGTATTACAAATTATC	10	601
916386	N/A	N/A	7603	7618	CTTAACAACCCTGTCA	0	602
916546	N/A	N/A	12152	12167	AGTAACTGGTAGCTCC	88	603
916566	N/A	N/A	12343	12358	CTAATACCCATACTGC	84	604
916586	N/A	N/A	12966	12981	AACTTTGCAGCCTATC	85	605
916606	N/A	N/A	13739	13754	AGAACTAAGGCAAATC	85	606
916626	N/A	N/A	14257	14272	GTCTTGGCCAACGACA	0	607
916646	N/A	N/A	14793	14808	CAGGATGCAATCCCCC	45	608
916666	N/A	N/A	15601	15616	GCCAATACATAGCAGA	75	609
916686	N/A	N/A	16630	16645	GTCCATGAAATCCAGG	0	610
916706	N/A	N/A	17293	17308	TCTCTTAGGGCACCTC	87	611
916726	N/A	N/A	18256	18271	TGAATATACCCCACAG	24	612
916746	N/A	N/A	19337	19352	AGCTCTAGGAGTCCCC	63	613
916765	N/A	N/A	20513	20528	CCAGATTGAGTCTCCT	91	614
916785	N/A	N/A	20775	20790	AATCAAGTGCCCTCCA	73	615
916805	N/A	N/A	21211	21226	TGTAGCTGTGTGGTGG	85	616
916825	N/A	N/A	21760	21775	TACCATGATCAGGTCA	0	617
916845	N/A	N/A	22713	22728	GTAAAGATGTGAGTGA	85	618
916865	N/A	N/A	23606	23621	GTTTACAAAAGCTGCC	17	619
916885	N/A	N/A	24375	24390	TGAACTCCGGCTCAGT	0	620

Table 10

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915348	28	43	2766	2781	GGGCATTCCCAGCGCG	0	621
915368	59	74	2797	2812	CGCTCTCTACCCTGCC	0	622
915388	114	129	2852	2867	GATCGGGAATCGGCTC	32	623
915408	199	214	2937	2952	CCCGCGAAGGACAAGC	6	624
915428	275	290	3013	3028	GTCGCGGAGGAGGTGC	0	625
915448	352	367	N/A	N/A	GTCTGCTCCAGCGGGA	4	626
915468	392	407	5966	5981	ACTCCTGGCCTTCCGC	87	627
915488	434	449	6008	6023	CTTGCTTAAGTTGAAG	0	628
915508	457	472	6031	6046	TTGCAGAGACCCTGTC	63	629
915528	495	510	6069	6084	TGCCGGAGATGAGCTG	0	630
915548	516	531	6090	6105	TGGTAAGAGAGATGCC	18	631
915568	564	579	6138	6153	CTTTGGACCGAAAGTC	10	632
915608	704	719	11922	11937	GATGGTTGTTTTGGCA	98	633
915628	772	787	11990	12005	ACATGAAGAAAGTTCG	41	634
915648	832	847	12050	12065	GCTCTCGAGAGAAGGT	55	635
915668	884	899	13620	13635	ATATCCTCGAAGGCAT	33	636
915688	962	977	16082	16097	CCCTTCTGAGGATGAC	11	637
915708	998	1013	16118	16133	GTTTGCCCAGCTGGGC	0	638
915728	1067	1082	16187	16202	GTCTAGCAGCTCATCT	68	639
915748	1137	1152	16257	16272	CTGTAGCGAGCCTGGG	0	640
915768	1198	1213	19038	19053	GGTAGCAAGTTGCAAA	90	641
915788	1234	1249	19074	19089	GTACAGGGCAGCATTA	69	642
915808	1317	1332	23722	23737	GCAACCACAGGACATC	51	643
915828	1354	1369	23759	23774	ATCAGCACTCGAGTGA	0	644
915848	1410	1425	25173	25188	GGGAGGCCTGTTGGCT	17	645
915868	1510	1525	25273	25288	CTGAGGATGGACCGCG	53	646
915888	1554	1569	25317	25332	CACCAGCAGGTACTTT	0	647
915908	1612	1627	25375	25390	CAAGTGACTCACAGAC	91	648
915928	1639	1654	25402	25417	TGAAAGAATCTGCTAG	59	649

915948	1691	1706	25454	25469	CTACACAGCAATGCGG	20	650
915968	1773	1788	25536	25551	CAGGCGAAAGGTTGCT	60	651
915988	1794	1809	25557	25572	GTTAAGTGCTGGACCG	86	652
916008	1820	1835	25583	25598	GAATTAACGCATGCTG	88	653
916028	1865	1880	25628	25643	GGGACCCTCTGCACTG	0	654
916048	1889	1904	25652	25667	ATAGGGCCACGAAACA	75	655
916068	1919	1934	25682	25697	GAACCTCATGCTGGAA	72	656
916088	1962	1977	25725	25740	CCCATCACAAGGCCCC	37	657
916108	1984	1999	25747	25762	TCACACATGGGCCAGC	84	658
916128	2079	2094	25842	25857	CTGACAGGCAGTGTCG	10	659
916147	2107	2122	25870	25885	AGTCAAGGTTATCATC	81	660
916167	2144	2159	25907	25922	ATCATCTTGTTACCCC	88	661
916187	2276	2291	26039	26054	ATTCTACTAACATCTC	90	662
916207	2629	2644	26392	26407	GAATGCCCTGCACACT	72	663
916227	2691	2706	26454	26469	GCTCCAGTGGAGAGGA	14	664
916247	2713	2728	26476	26491	ATAAGCCAGGTTCAAG	88	665
916287	N/A	N/A	4308	4323	GTGAGAAACAAACCCT	92	666
916307	N/A	N/A	4882	4897	TCTATACCAGAGTGAG	84	667
916327	N/A	N/A	5514	5529	AGGAATGAGTCTCCCA	17	668
916347	N/A	N/A	5873	5888	GGTAATACTTTTGGCA	70	669
916367	N/A	N/A	7106	7121	CGCTTATGAAAGCATC	0	670
916387	N/A	N/A	7605	7620	CCCTTAACAACCCTGT	28	671
916547	N/A	N/A	12167	12182	TTTGATTGTGCAGACA	98	672
916567	N/A	N/A	12345	12360	TCCTAATACCCATACT	74	673
916587	N/A	N/A	12969	12984	ACAAACTTTGCAGCCT	95	674
916607	N/A	N/A	13742	13757	GTTAGAACTAAGGCAA	94	675
916627	N/A	N/A	14301	14316	GAGCAGATAAATACAC	91	676
916647	N/A	N/A	14892	14907	TGGTATCTCGCTTCCT	0	677
916667	N/A	N/A	15613	15628	TAAAGCCACGCAGCCA	46	678
916687	N/A	N/A	16656	16671	CCAGATGCAGGACCCC	0	679
916707	N/A	N/A	17326	17341	AAACTAATGCACCTGG	43	680
916727	N/A	N/A	18257	18272	CTGAATATACCCCACA	75	681
916747	N/A	N/A	19360	19375	AGCTGCTATGTGAGGC	12	682
916766	N/A	N/A	20520	20535	TCAGTAACCAGATTGA	25	683

916786	N/A	N/A	20778	20793	TTTAATCAAGTGCCCT	81	684
916806	N/A	N/A	21216	21231	CAGGATGTAGCTGTGT	84	685
916826	N/A	N/A	21887	21902	TAAGATCCCATCTTAC	13	686
916846	N/A	N/A	22739	22754	AAAGTAAACACCCACC	42	687
916866	N/A	N/A	23625	23640	GCTTACAACACTACCC	57	688
916886	N/A	N/A	24393	24408	GTAATGGGAGCCAGGC	38	689

Table 11

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915349	29	44	2767	2782	AGGGCATTCCCAGCGC	0	690
915369	60	75	2798	2813	GCGCTCTCTACCCTGC	23	691
915389	115	130	2853	2868	GGATCGGGAATCGGCT	54	692
915409	200	215	2938	2953	GCCCGCGAAGGACAAG	32	693
915429	276	291	3014	3029	CGTCGCGGAGGAGGTG	0	694
915449	364	379	5938	5953	AGGACCTGCAGAGTCT	21	695
915469	394	409	5968	5983	CGACTCCTGGCCTTCC	59	696
915489	435	450	6009	6024	ACTTGCTTAAGTTGAA	86	697
915509	466	481	6040	6055	GGGAGGCATTTGCAGA	57	698
915529	496	511	6070	6085	TTGCCGGAGATGAGCT	40	699
915549	518	533	6092	6107	TCTGGTAAGAGAGATG	61	700
915569	565	580	6139	6154	TCTTTGGACCGAAAGT	9	701
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	99	702
915629	776	791	11994	12009	GTCCACATGAAGAAAG	32	703
915649	833	848	12051	12066	AGCTCTCGAGAGAAGG	36	704
915669	885	900	13621	13636	AATATCCTCGAAGGCA	55	705
915689	969	984	16089	16104	GATCCATCCCTTCTGA	20	706
915709	999	1014	16119	16134	TGTTTGCCCAGCTGGG	5	707
915729	1077	1092	16197	16212	GACGCAGGTGGTCTAG	0	708
915749	1138	1153	N/A	N/A	GCTGTAGCGAGCCTGG	71	709

915769	1200	1215	19040	19055	TGGGTAGCAAGTTGCA	81	710
915789	1235	1250	19075	19090	GGTACAGGGCAGCATT	88	711
915809	1318	1333	23723	23738	TGCAACCACAGGACAT	40	712
915829	1355	1370	23760	23775	CATCAGCACTCGAGTG	0	713
915849	1424	1439	25187	25202	CTCAGGTGTGCATGGG	61	714
915869	1511	1526	25274	25289	CCTGAGGATGGACCGC	70	715
915889	1556	1571	25319	25334	AGCACCAGCAGGTACT	35	716
915909	1613	1628	25376	25391	TCAAGTGACTCACAGA	84	717
915929	1645	1660	25408	25423	CACCTCTGAAAGAATC	89	718
915949	1692	1707	25455	25470	ACTACACAGCAATGCG	33	719
915969	1774	1789	25537	25552	ACAGGCGAAAGGTTGC	88	720
915989	1795	1810	25558	25573	AGTTAAGTGCTGGACC	84	721
916009	1823	1838	25586	25601	GCTGAATTAACGCATG	67	722
916029	1866	1881	25629	25644	AGGGACCCTCTGCACT	15	723
916049	1890	1905	25653	25668	AATAGGGCCACGAAAC	52	724
916069	1920	1935	25683	25698	AGAACCTCATGCTGGA	85	725
916089	1963	1978	25726	25741	CCCCATCACAAGGCCC	20	726
916109	1985	2000	25748	25763	ATCACACATGGGCCAG	72	727
916129	2080	2095	25843	25858	CCTGACAGGCAGTGTC	15	728
916148	2108	2123	25871	25886	TAGTCAAGGTTATCAT	87	729
916168	2146	2161	25909	25924	TTATCATCTTGTTACC	82	730
916188	2279	2294	26042	26057	CTTATTCTACTAACAT	87	731
916208	2630	2645	26393	26408	TGAATGCCCTGCACAC	68	732
916228	2692	2707	26455	26470	TGCTCCAGTGGAGAGG	80	733
916248	2726	2741	26489	26504	GTCCCTGCAGAAAATA	0	734
916288	N/A	N/A	4337	4352	AGCATACCACACCCCA	75	735
916308	N/A	N/A	5086	5101	GGACATGCTCAGCAGC	68	736
916328	N/A	N/A	5533	5548	TGCTGTAGGCCTCAGC	0	737
916348	N/A	N/A	5874	5889	TGGTAATACTTTTGGC	86	738
916368	N/A	N/A	7132	7147	GTAAATGGAGTCCTTC	80	739
916388	N/A	N/A	7612	7627	CATAATCCCCTTAACA	32	740
916548	N/A	N/A	12195	12210	TTAACCATCAAGGACA	77	741
916568	N/A	N/A	12665	12680	TCTTAGTGGCTGGGTA	85	742
916588	N/A	N/A	12973	12988	CCTAACAAACTTTGCA	32	743

916608	N/A	N/A	13749	13764	ACTAAGTGTTAGAACT	76	744
916628	N/A	N/A	14338	14353	CTGCAGTATCCCTAGC	0	745
916648	N/A	N/A	15012	15027	TCCCATCGGTCATTTC	45	746
916668	N/A	N/A	15682	15697	GAAACCACTATCATCA	62	747
916688	N/A	N/A	16671	16686	GTAATAGGCCAAGTCC	0	748
916708	N/A	N/A	17327	17342	CAAACTAATGCACCTG	66	749
916728	N/A	N/A	18332	18347	CCAATATCATAGCTGA	85	750
916748	N/A	N/A	19376	19391	CACAAGAGACTGGACC	64	751
916767	N/A	N/A	20551	20566	TACTATGGGATGAGTA	0	752
916787	N/A	N/A	20779	20794	TTTTAATCAAGTGCCC	38	753
916807	N/A	N/A	21218	21233	GGCAGGATGTAGCTGT	63	754
916827	N/A	N/A	21947	21962	AGTCAAACATCTTCCT	50	755
916847	N/A	N/A	22759	22774	CAGACTAACTTACTAA	77	756
916867	N/A	N/A	23626	23641	AGCTTACAACACTACC	13	757
916887	N/A	N/A	24505	24520	ATGCTACGGGCTCTCA	0	758

Table 12

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915350	30	45	2768	2783	CAGGGCATTCCCAGCG	0	759
915370	82	97	2820	2835	CAGCTCCGCCCGGCGC	14	760
915390	130	145	2868	2883	TTAGGATCTGGGTCGG	88	761
915410	201	216	2939	2954	AGCCCGCGAAGGACAA	0	762
915430	295	310	3033	3048	GCGCCGAACAACATGC	0	763
915450	366	381	5940	5955	AGAGGACCTGCAGAGT	83	764
915470	395	410	5969	5984	CCGACTCCTGGCCTTC	68	765
915490	436	451	6010	6025	AACTTGCTTAAGTTGA	41	766
915510	467	482	6041	6056	CGGGAGGCATTTGCAG	44	767
915530	497	512	6071	6086	TTTGCCGGAGATGAGC	92	768
915550	519	534	6093	6108	CTCTGGTAAGAGAGAT	20	769

915570	566	581	6140	6155	GTCTTTGGACCGAAAG	19	770
915590	627	642	7857	7872	GAGGGATAAGGCCACT	83	771
915610	706	721	11924	11939	GTGATGGTTGTTTTGG	97	772
915630	782	797	12000	12015	GGTGATGTCCACATGA	87	773
915650	834	849	12052	12067	AAGCTCTCGAGAGAAG	44	774
915670	887	902	13623	13638	CAAATATCCTCGAAGG	0	775
915690	970	985	16090	16105	GGATCCATCCCTTCTG	0	776
915710	1003	1018	16123	16138	CTCATGTTTGCCCAGC	68	777
915730	1078	1093	16198	16213	AGACGCAGGTGGTCTA	0	778
915750	1139	1154	N/A	N/A	TGCTGTAGCGAGCCTG	56	779
915770	1201	1216	19041	19056	ATGGGTAGCAAGTTGC	79	780
915790	1247	1262	19087	19102	TTCCACAGGCAGGGTA	48	781
915810	1320	1335	23725	23740	ACTGCAACCACAGGAC	22	782
915830	1356	1371	23761	23776	ACATCAGCACTCGAGT	0	783
915850	1427	1442	25190	25205	CTGCTCAGGTGTGCAT	10	784
915870	1512	1527	25275	25290	ACCTGAGGATGGACCG	69	785
915890	1557	1572	25320	25335	CAGCACCAGCAGGTAC	62	786
915910	1617	1632	25380	25395	CTCCTCAAGTGACTCA	83	787
915930	1648	1663	25411	25426	TAGCACCTCTGAAAGA	55	788
915950	1693	1708	25456	25471	CACTACACAGCAATGC	74	789
915970	1775	1790	25538	25553	CACAGGCGAAAGGTTG	72	790
915990	1797	1812	25560	25575	AGAGTTAAGTGCTGGA	92	791
916010	1824	1839	25587	25602	AGCTGAATTAACGCAT	0	792
916030	1867	1882	25630	25645	AAGGGACCCTCTGCAC	38	793
916050	1891	1906	25654	25669	TAATAGGGCCACGAAA	53	794
916070	1921	1936	25684	25699	AAGAACCTCATGCTGG	24	795
916090	1964	1979	25727	25742	CCCCCATCACAAGGCC	24	796
916110	1986	2001	25749	25764	GATCACACATGGGCCA	0	797
916130	2081	2096	25844	25859	ACCTGACAGGCAGTGT	54	798
916149	2109	2124	25872	25887	GTAGTCAAGGTTATCA	87	799
916169	2154	2169	25917	25932	TAAGTAGATTATCATC	79	800
916189	2282	2297	26045	26060	AGGCTTATTCTACTAA	85	801
916209	2631	2646	26394	26409	GTGAATGCCCTGCACA	59	802
916229	2693	2708	26456	26471	GTGCTCCAGTGGAGAG	54	803

916249	2727	2742	26490	26505	GGTCCCTGCAGAAAAT	38	804
916289	N/A	N/A	4338	4353	AAGCATACCACACCCC	79	805
916309	N/A	N/A	5278	5293	AATCTTGGGATGCACA	95	806
916329	N/A	N/A	5569	5584	CATCATGGCTTCCAGT	79	807
916349	N/A	N/A	5879	5894	TGGGATGGTAATACTT	0	808
916369	N/A	N/A	7134	7149	AAGTAAATGGAGTCCT	5	809
916389	N/A	N/A	7615	7630	TTGCATAATCCCCTTA	33	810
916409	N/A	N/A	8165	8180	TTAACTAGATCACTGA	58	811
916429	N/A	N/A	9109	9124	TCCTAATGCGAGTCCC	86	812
916449	N/A	N/A	9522	9537	TGCTGCTGGGTGCACT	45	813
916469	N/A	N/A	10199	10214	GGTGATGACACAGCAT	94	814
916489	N/A	N/A	10382	10397	GCCATGTACAACTTTT	52	815
916509	N/A	N/A	11152	11167	TACAATTTGGACAGAG	71	816
916529	N/A	N/A	11546	11561	ACCTATAGGAGTGCCC	35	817
916549	N/A	N/A	12204	12219	TTATTTCCGTTAACCA	97	818
916569	N/A	N/A	12672	12687	AGAATCATCTTAGTGG	94	819
916589	N/A	N/A	12989	13004	CGGAATAAGCCTCCAC	0	820
916609	N/A	N/A	13752	13767	GGCACTAAGTGTTAGA	57	821
916629	N/A	N/A	14375	14390	TCTCACAAGGCTGGCA	84	822
916649	N/A	N/A	15137	15152	GCCATACCGGCTCCCT	30	823
916669	N/A	N/A	15691	15706	GGCCTTACAGAAACCA	15	824
916689	N/A	N/A	16672	16687	AGTAATAGGCCAAGTC	16	825
916709	N/A	N/A	17328	17343	ACAAACTAATGCACCT	42	826
916729	N/A	N/A	18333	18348	TCCAATATCATAGCTG	32	827
916749	N/A	N/A	19445	19460	CTACTAGGCATCTCTA	32	828
916768	N/A	N/A	20553	20568	CTTACTATGGGATGAG	83	829
916788	N/A	N/A	20808	20823	TAATATTCAGACCAGG	94	830
916808	N/A	N/A	21252	21267	CCATGCATGGCACAGT	4	831
916828	N/A	N/A	21968	21983	AGACAGGAATCCAACC	0	832
916848	N/A	N/A	22767	22782	GGACATGACAGACTAA	96	833
916868	N/A	N/A	23637	23652	GCAGACACAACAGCTT	40	834
916888	N/A	N/A	24507	24522	CCATGCTACGGGCTCT	0	835

Table 13

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915351	33	48	2771	2786	GGCCAGGGCATTCCCA	0	836
915371	83	98	2821	2836	GCAGCTCCGCCCGGCG	2	837
915391	132	147	2870	2885	GGTTAGGATCTGGGTC	54	838
915411	222	237	2960	2975	GGTAGAAGCCCAGGAA	57	839
915431	321	336	3059	3074	CGACGCAGTGCAACGC	49	840
915451	368	383	5942	5957	TGAGAGGACCTGCAGA	0	841
915471	400	415	5974	5989	ATGTTCCGACTCCTGG	82	842
915491	437	452	6011	6026	GAACTTGCTTAAGTTG	23	843
915511	468	483	6042	6057	CCGGGAGGCATTTGCA	0	844
915531	498	513	6072	6087	TTTTGCCGGAGATGAG	84	845
915551	520	535	6094	6109	ACTCTGGTAAGAGAGA	5	846
915571	567	582	6141	6156	CGTCTTTGGACCGAAA	64	847
915611	708	723	11926	11941	CGGTGATGGTTGTTTT	98	848
915631	783	798	12001	12016	TGGTGATGTCCACATG	0	849
915651	835	850	12053	12068	AAAGCTCTCGAGAGAA	35	850
915671	890	905	13626	13641	ATCCAAATATCCTCGA	42	851
915691	971	986	16091	16106	AGGATCCATCCCTTCT	0	852
915711	1005	1020	16125	16140	GACTCATGTTTGCCCA	73	853
915731	1079	1094	16199	16214	GAGACGCAGGTGGTCT	0	854
915751	1140	1155	N/A	N/A	GTGCTGTAGCGAGCCT	0	855
915771	1202	1217	19042	19057	AATGGGTAGCAAGTTG	80	856
915791	1248	1263	19088	19103	ATTCCACAGGCAGGGT	37	857
915811	1327	1342	23732	23747	GTCACCCACTGCAACC	52	858
915831	1357	1372	23762	23777	CACATCAGCACTCGAG	29	859
915851	1429	1444	25192	25207	TCCTGCTCAGGTGTGC	2	860
915871	1513	1528	25276	25291	GACCTGAGGATGGACC	20	861
915891	1558	1573	25321	25336	TCAGCACCAGCAGGTA	68	862
915911	1620	1635	25383	25398	CGCCTCCTCAAGTGAC	48	863

915931	1652	1667	25415	25430	ACTTTAGCACCTCTGA	93	864
915951	1695	1710	25458	25473	GTCACTACACAGCAAT	84	865
915971	1776	1791	25539	25554	GCACAGGCGAAAGGTT	74	866
915991	1798	1813	25561	25576	TAGAGTTAAGTGCTGG	84	867
916011	1825	1840	25588	25603	CAGCTGAATTAACGCA	0	868
916031	1868	1883	25631	25646	TAAGGGACCCTCTGCA	54	869
916051	1892	1907	25655	25670	TTAATAGGGCCACGAA	75	870
916071	1922	1937	25685	25700	TAAGAACCTCATGCTG	56	871
916091	1965	1980	25728	25743	CCCCCCATCACAAGGC	9	872
916111	1987	2002	25750	25765	AGATCACACATGGGCC	26	873
916131	2084	2099	25847	25862	ACCACCTGACAGGCAG	80	874
916150	2110	2125	25873	25888	AGTAGTCAAGGTTATC	92	875
916170	2174	2189	25937	25952	TGAAAAAGGTGTTCTA	49	876
916190	2283	2298	26046	26061	AAGGCTTATTCTACTA	79	877
916210	2633	2648	26396	26411	AGGTGAATGCCCTGCA	71	878
916230	2694	2709	26457	26472	TGTGCTCCAGTGGAGA	75	879
916250	2728	2743	26491	26506	TGGTCCCTGCAGAAAA	79	880
916290	N/A	N/A	4397	4412	TGCCTACTGGCTCACA	14	881
916310	N/A	N/A	5279	5294	AAATCTTGGGATGCAC	94	882
916330	N/A	N/A	5572	5587	TGACATCATGGCTTCC	93	883
916350	N/A	N/A	6158	6173	GCTTACATCCACGACT	0	884
916370	N/A	N/A	7135	7150	CAAGTAAATGGAGTCC	77	885
916390	N/A	N/A	7620	7635	ATCTATTGCATAATCC	86	886
916550	N/A	N/A	12205	12220	TTTATTTCCGTTAACC	96	887
916570	N/A	N/A	12694	12709	TTCTTGACCGTGTTTC	98	888
916590	N/A	N/A	12990	13005	CCGGAATAAGCCTCCA	47	889
916610	N/A	N/A	13822	13837	TGTACAATGGGACGGA	69	890
916630	N/A	N/A	14418	14433	ATCGACACAGCATCAC	92	891
916650	N/A	N/A	15138	15153	TGCCATACCGGCTCCC	0	892
916670	N/A	N/A	15758	15773	GGTTTATAACAACTGA	89	893
916690	N/A	N/A	16722	16737	GCCTTGAGGTGGGTGG	0	894
916710	N/A	N/A	17512	17527	AGTCATGGGATGTGCA	58	895
916730	N/A	N/A	18395	18410	ATGTTTGGAAGTCGCC	92	896
916750	N/A	N/A	19473	19488	AAGGATCCTGCTTCTA	9	897

916769	N/A	N/A	20554	20569	GCTTACTATGGGATGA	62	898
916789	N/A	N/A	20809	20824	GTAATATTCAGACCAG	96	899
916809	N/A	N/A	21254	21269	ATCCATGCATGGCACA	72	900
916829	N/A	N/A	21979	21994	GTCAGACACGGAGACA	0	901
916849	N/A	N/A	23110	23125	GGCTTTTGAAGGAGAG	84	902
916869	N/A	N/A	23787	23802	TATTTACCTGGAGGCG	0	903
916889	N/A	N/A	24612	24627	CAAATCGGATCTTTGC	44	904

Table 14

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915352	34	49	2772	2787	CGGCCAGGGCATTCCC	0	905
915372	86	101	2824	2839	GCAGCAGCTCCGCCCG	48	906
915392	135	150	2873	2888	GCGGGTTAGGATCTGG	25	907
915412	225	240	2963	2978	CGTGGTAGAAGCCCAG	2	908
915432	322	337	3060	3075	CCGACGCAGTGCAACG	31	909
915452	371	386	5945	5960	ATCTGAGAGGACCTGC	72	910
915472	401	416	5975	5990	AATGTTCCGACTCCTG	77	911
915492	438	453	6012	6027	GGAACTTGCTTAAGTT	55	912
915512	469	484	6043	6058	GCCGGGAGGCATTTGC	3	913
915532	499	514	6073	6088	ATTTTGCCGGAGATGA	86	914
915552	521	536	6095	6110	CACTCTGGTAAGAGAG	8	915
915612	709	724	11927	11942	ACGGTGATGGTTGTTT	87	916
915632	787	802	12005	12020	AGCTTGGTGATGTCCA	38	917
915652	836	851	12054	12069	AAAAGCTCTCGAGAGA	0	918
915672	891	906	13627	13642	CATCCAAATATCCTCG	82	919
915692	972	987	16092	16107	CAGGATCCATCCCTTC	0	920
915712	1006	1021	16126	16141	AGACTCATGTTTGCCC	77	921
915732	1081	1096	16201	16216	CTGAGACGCAGGTGGT	87	922
915752	1141	1156	N/A	N/A	AGTGCTGTAGCGAGCC	1	923

915772	1203	1218	19043	19058	TAATGGGTAGCAAGTT	77	924
915792	1257	1272	19097	19112	CAATGGCAGATTCCAC	56	925
915812	1328	1343	23733	23748	GGTCACCCACTGCAAC	58	926
915832	1358	1373	23763	23778	ACACATCAGCACTCGA	66	927
915852	1430	1445	25193	25208	GTCCTGCTCAGGTGTG	52	928
915872	1518	1533	25281	25296	GGCTGGACCTGAGGAT	0	929
915892	1570	1585	25333	25348	GTGGAGAGCCCCTCAG	47	930
915912	1621	1636	25384	25399	TCGCCTCCTCAAGTGA	8	931
915932	1654	1669	25417	25432	AAACTTTAGCACCTCT	90	932
915952	1696	1711	25459	25474	GGTCACTACACAGCAA	82	933
915972	1777	1792	25540	25555	TGCACAGGCGAAAGGT	64	934
915992	1799	1814	25562	25577	TTAGAGTTAAGTGCTG	91	935
916012	1826	1841	25589	25604	CCAGCTGAATTAACGC	32	936
916032	1869	1884	25632	25647	GTAAGGGACCCTCTGC	73	937
916052	1894	1909	25657	25672	CATTAATAGGGCCACG	81	938
916072	1923	1938	25686	25701	CTAAGAACCTCATGCT	70	939
916092	1966	1981	25729	25744	ACCCCCCATCACAAGG	30	940
916112	1988	2003	25751	25766	AAGATCACACATGGGC	86	941
916132	2085	2100	25848	25863	GACCACCTGACAGGCA	61	942
916151	2111	2126	25874	25889	TAGTAGTCAAGGTTAT	88	943
916171	2176	2191	25939	25954	GGTGAAAAAGGTGTTC	84	944
916191	2284	2299	26047	26062	TAAGGCTTATTCTACT	76	945
916211	2634	2649	26397	26412	GAGGTGAATGCCCTGC	0	946
916231	2695	2710	26458	26473	GTGTGCTCCAGTGGAG	87	947
916251	2729	2744	26492	26507	CTGGTCCCTGCAGAAA	67	948
916291	N/A	N/A	4419	4434	CAATGCTACTTGCCCC	68	949
916311	N/A	N/A	5280	5295	TAAATCTTGGGATGCA	94	950
916331	N/A	N/A	5576	5591	ACAATGACATCATGGC	97	951
916351	N/A	N/A	6165	6180	GCAAACTGCTTACATC	0	952
916371	N/A	N/A	7172	7187	GTTAGACGCGCCAGGC	7	953
916391	N/A	N/A	7624	7639	TCTCATCTATTGCATA	0	954
916551	N/A	N/A	12206	12221	TTTTATTTCCGTTAAC	73	955
916571	N/A	N/A	12714	12729	TAAACTACCGAACGCA	96	956
916591	N/A	N/A	12991	13006	CCCGGAATAAGCCTCC	47	957

916611	N/A	N/A	13823	13838	CTGTACAATGGGACGG	23	958
916631	N/A	N/A	14422	14437	TCCCATCGACACAGCA	95	959
916651	N/A	N/A	15206	15221	GGAATATTGCCAGGTA	95	960
916671	N/A	N/A	15759	15774	TGGTTTATAACAACTG	29	961
916691	N/A	N/A	16746	16761	ATTAGGAGAGGTCTCA	55	962
916711	N/A	N/A	17602	17617	CTTGATAGTGAATGTG	90	963
916731	N/A	N/A	18859	18874	GGCACTCACAAAAGCG	10	964
916751	N/A	N/A	20182	20197	CCCTATGTTCTACTTT	54	965
916770	N/A	N/A	20572	20587	CAACATCTCTAGCTGG	82	966
916790	N/A	N/A	20810	20825	GGTAATATTCAGACCA	0	967
916810	N/A	N/A	21265	21280	TGAAGCTACAGATCCA	74	968
916830	N/A	N/A	22042	22057	GGAAATCTGTCAGAGC	18	969
916850	N/A	N/A	23142	23157	GAATCTAGGAAGGCGA	77	970
916870	N/A	N/A	23789	23804	AGTATTTACCTGGAGG	0	971
916890	N/A	N/A	24738	24753	AGCCTTAGGAAGCCTC	16	972

Table 15

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915353	35	50	2773	2788	TCGGCCAGGGCATTCC	0	973
915373	87	102	2825	2840	CGCAGCAGCTCCGCCC	0	974
915393	136	151	2874	2889	CGCGGGTTAGGATCTG	0	975
915413	239	254	2977	2992	GCGGGTCGCCCCGACG	0	976
915433	325	340	3063	3078	ACGCCGACGCAGTGCA	0	977
915453	372	387	5946	5961	GATCTGAGAGGACCTG	24	978
915473	402	417	5976	5991	CAATGTTCCGACTCCT	73	979
915493	441	456	6015	6030	GGAGGAACTTGCTTAA	87	980
915513	470	485	6044	6059	GGCCGGGAGGCATTTG	0	981
915533	500	515	6074	6089	TATTTTGCCGGAGATG	75	982
915553	522	537	6096	6111	ACACTCTGGTAAGAGA	0	983

915613	710	725	11928	11943	CACGGTGATGGTTGTT	64	984
915633	788	803	12006	12021	GAGCTTGGTGATGTCC	74	985
915653	837	852	12055	12070	CAAAAGCTCTCGAGAG	0	986
915673	892	907	13628	13643	GCATCCAAATATCCTC	81	987
915693	973	988	16093	16108	TCAGGATCCATCCCTT	10	988
915713	1007	1022	16127	16142	CAGACTCATGTTTGCC	0	989
915733	1082	1097	16202	16217	GCTGAGACGCAGGTGG	64	990
915753	1142	1157	N/A	N/A	CAGTGCTGTAGCGAGC	0	991
915773	1204	1219	19044	19059	CTAATGGGTAGCAAGT	72	992
915793	1258	1273	19098	19113	GCAATGGCAGATTCCA	57	993
915813	1329	1344	23734	23749	AGGTCACCCACTGCAA	56	994
915833	1359	1374	23764	23779	GACACATCAGCACTCG	43	995
915853	1431	1446	25194	25209	AGTCCTGCTCAGGTGT	66	996
915873	1525	1540	25288	25303	AAGTTCAGGCTGGACC	54	997
915893	1571	1586	25334	25349	GGTGGAGAGCCCCTCA	0	998
915913	1622	1637	25385	25400	CTCGCCTCCTCAAGTG	52	999
915933	1660	1675	25423	25438	GATGGGAAACTTTAGC	85	1000
915953	1697	1712	25460	25475	GGGTCACTACACAGCA	78	1001
915973	1778	1793	25541	25556	CTGCACAGGCGAAAGG	35	1002
915993	1800	1815	25563	25578	ATTAGAGTTAAGTGCT	63	1003
916013	1827	1842	25590	25605	ACCAGCTGAATTAACG	66	1004
916033	1873	1888	25636	25651	GTCAGTAAGGGACCCT	52	1005
916053	1897	1912	25660	25675	GACCATTAATAGGGCC	51	1006
916073	1924	1939	25687	25702	TCTAAGAACCTCATGC	55	1007
916093	1967	1982	25730	25745	TACCCCCCATCACAAG	15	1008
916113	1990	2005	25753	25768	ACAAGATCACACATGG	72	1009
916133	2086	2101	25849	25864	AGACCACCTGACAGGC	79	1010
916152	2112	2127	25875	25890	TTAGTAGTCAAGGTTA	84	1011
916172	2177	2192	25940	25955	AGGTGAAAAAGGTGTT	88	1012
916192	2285	2300	26048	26063	TTAAGGCTTATTCTAC	82	1013
916212	2635	2650	26398	26413	TGAGGTGAATGCCCTG	58	1014
916232	2696	2711	26459	26474	TGTGTGCTCCAGTGGA	89	1015
916252	2730	2745	26493	26508	GCTGGTCCCTGCAGAA	44	1016
916272	N/A	N/A	3328	3343	GGGACGCACGAGAGTC	0	1017

916292	N/A	N/A	4432	4447	GTCAATAGCTTCACAA	86	1018
916312	N/A	N/A	5281	5296	ATAAATCTTGGGATGC	92	1019
916332	N/A	N/A	5577	5592	CACAATGACATCATGG	95	1020
916352	N/A	N/A	6170	6185	GATAAGCAAACTGCTT	19	1021
916372	N/A	N/A	7192	7207	GAGGATGCAACTGGCT	84	1022
916392	N/A	N/A	7644	7659	TCGGACTTCAGGCCCA	0	1023
916552	N/A	N/A	12208	12223	CCTTTTATTTCCGTTA	97	1024
916572	N/A	N/A	12745	12760	GCATACTAAAACCACC	85	1025
916592	N/A	N/A	13375	13390	GACTTTGCAGGCACCC	92	1026
916612	N/A	N/A	13909	13924	TGACATCCCAGTTCAA	30	1027
916632	N/A	N/A	14427	14442	TACTTTCCCATCGACA	81	1028
916652	N/A	N/A	15207	15222	AGGAATATTGCCAGGT	88	1029
916672	N/A	N/A	15768	15783	GGTTAGTGTTGGTTTA	92	1030
916692	N/A	N/A	16790	16805	CATTCGATGGAGGTTC	58	1031
916712	N/A	N/A	17629	17644	GGCGGATTTCCCCACT	11	1032
916732	N/A	N/A	18894	18909	TAAAATACGCCCGTCC	7	1033
916752	N/A	N/A	20183	20198	TCCCTATGTTCTACTT	32	1034
916771	N/A	N/A	20574	20589	ATCAACATCTCTAGCT	46	1035
916791	N/A	N/A	20811	20826	GGGTAATATTCAGACC	43	1036
916811	N/A	N/A	21313	21328	TTTACTAGAGACTCTG	69	1037
916831	N/A	N/A	22071	22086	GTAGGATAGGACTAGA	45	1038
916851	N/A	N/A	23219	23234	ATAAATGCCTGACCAC	64	1039
916871	N/A	N/A	23861	23876	TGTTTCTAGAATGTCG	68	1040
916891	N/A	N/A	24873	24888	GCCTATCAGTTTCCCC	0	1041

Table 16

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915354	36	51	2774	2789	CTCGGCCAGGGCATTC	0	1042
915374	89	104	2827	2842	TCCGCAGCAGCTCCGC	60	1043

915394	137	152	2875	2890	GCGCGGGTTAGGATCT	0	1044
915414	240	255	2978	2993	AGCGGGTCGCCCCGAC	21	1045
915434	337	352	3075	3090	ATACCGGAGAGGACGC	85	1046
915454	374	389	5948	5963	AAGATCTGAGAGGACC	24	1047
915474	403	418	5977	5992	CCAATGTTCCGACTCC	95	1048
915494	442	457	6016	6031	CGGAGGAACTTGCTTA	93	1049
915514	471	486	6045	6060	TGGCCGGGAGGCATTT	0	1050
915534	501	516	6075	6090	CTATTTTGCCGGAGAT	87	1051
915554	523	538	6097	6112	GACACTCTGGTAAGAG	26	1052
915614	711	726	11929	11944	ACACGGTGATGGTTGT	46	1053
915634	791	806	12009	12024	ACTGAGCTTGGTGATG	87	1054
915654	838	853	12056	12071	ACAAAAGCTCTCGAGA	0	1055
915674	900	915	13636	13651	ACCTGAATGCATCCAA	93	1056
915694	974	989	16094	16109	CTCAGGATCCATCCCT	43	1057
915714	1008	1023	16128	16143	CCAGACTCATGTTTGC	0	1058
915734	1083	1098	16203	16218	TGCTGAGACGCAGGTG	50	1059
915754	1143	1158	N/A	N/A	TCAGTGCTGTAGCGAG	42	1060
915774	1208	1223	19048	19063	TATCCTAATGGGTAGC	53	1061
915794	1260	1275	19100	19115	TCGCAATGGCAGATTC	67	1062
915814	1333	1348	23738	23753	TGTGAGGTCACCCACT	0	1063
915834	1360	1375	23765	23780	AGACACATCAGCACTC	24	1064
915854	1432	1447	25195	25210	CAGTCCTGCTCAGGTG	54	1065
915874	1526	1541	25289	25304	GAAGTTCAGGCTGGAC	75	1066
915894	1572	1587	25335	25350	AGGTGGAGAGCCCCTC	0	1067
915914	1623	1638	25386	25401	ACTCGCCTCCTCAAGT	0	1068
915934	1661	1676	25424	25439	AGATGGGAAACTTTAG	84	1069
915954	1724	1739	25487	25502	GGCTGGGATCCTCCAC	24	1070
915974	1779	1794	25542	25557	GCTGCACAGGCGAAAG	56	1071
915994	1801	1816	25564	25579	TATTAGAGTTAAGTGC	75	1072
916014	1828	1843	25591	25606	AACCAGCTGAATTAAC	55	1073
916034	1875	1890	25638	25653	CAGTCAGTAAGGGACC	70	1074
916054	1898	1913	25661	25676	TGACCATTAATAGGGC	74	1075
916074	1925	1940	25688	25703	TTCTAAGAACCTCATG	22	1076
916094	1968	1983	25731	25746	CTACCCCCCATCACAA	0	1077

916114	1992	2007	25755	25770	CCACAAGATCACACAT	0	1078
916134	2087	2102	25850	25865	CAGACCACCTGACAGG	78	1079
916153	2113	2128	25876	25891	TTTAGTAGTCAAGGTT	93	1080
916173	2178	2193	25941	25956	TAGGTGAAAAAGGTGT	89	1081
916193	2306	2321	26069	26084	ACCCAACCGATTTTTT	61	1082
916213	2636	2651	26399	26414	CTGAGGTGAATGCCCT	73	1083
916233	2697	2712	26460	26475	TTGTGTGCTCCAGTGG	92	1084
916253	2746	2761	26509	26524	TCACTGACCATGTGGG	16	1085
916273	N/A	N/A	3362	3377	CTTCATGCACGGGCGC	37	1086
916293	N/A	N/A	4462	4477	GCATAATCTCCTGCCT	0	1087
916313	N/A	N/A	5284	5299	GCCATAAATCTTGGGA	37	1088
916333	N/A	N/A	5605	5620	CTTTATTCAATGTGGC	97	1089
916353	N/A	N/A	6529	6544	TACAACTGCCTGTGTT	0	1090
916373	N/A	N/A	7218	7233	AAAGCTTCCGCAAACA	51	1091
916393	N/A	N/A	7657	7672	CTAACATACACCCTCG	0	1092
916553	N/A	N/A	12225	12240	AGCTTCTGGGACAAGC	10	1093
916573	N/A	N/A	12746	12761	GGCATACTAAAACCAC	55	1094
916593	N/A	N/A	13397	13412	TTGAATGTCACCCTTC	91	1095
916613	N/A	N/A	13914	13929	AGTCATGACATCCCAG	93	1096
916633	N/A	N/A	14442	14457	TCTCATTGGCACCTGT	86	1097
916653	N/A	N/A	15252	15267	CCCTATCAGATGCCCT	81	1098
916673	N/A	N/A	15799	15814	CATATCTGGTTTCATG	0	1099
916693	N/A	N/A	16842	16857	GACCATAGCACTGTCT	0	1100
916713	N/A	N/A	17737	17752	ATTAATCTGGTCATAT	0	1101
916733	N/A	N/A	18898	18913	TCCATAAAATACGCCC	69	1102
916753	N/A	N/A	20195	20210	GAAAGATGGAATTCCC	86	1103
916772	N/A	N/A	20604	20619	TACGATCATCATTATT	91	1104
916792	N/A	N/A	20841	20856	GTATTAGCTCAATATT	0	1105
916812	N/A	N/A	21314	21329	GTTTACTAGAGACTCT	64	1106
916832	N/A	N/A	22080	22095	GTAAAAACTGTAGGAT	0	1107
916852	N/A	N/A	23220	23235	GATAAATGCCTGACCA	29	1108
916872	N/A	N/A	24011	24026	CCGACGGGAAGTCTTC	0	1109
916892	N/A	N/A	24874	24889	GGCCTATCAGTTTCCC	0	1110

Table17

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915355	37	52	2775	2790	TCTCGGCCAGGGCATT	0	1111
915375	90	105	2828	2843	ATCCGCAGCAGCTCCG	52	1112
915395	138	153	2876	2891	GGCGCGGGTTAGGATC	0	1113
915415	241	256	2979	2994	CAGCGGGTCGCCCCGA	8	1114
915435	338	353	3076	3091	GATACCGGAGAGGACG	30	1115
915455	378	393	5952	5967	GCACAAGATCTGAGAG	72	1116
915475	405	420	5979	5994	TGCCAATGTTCCGACT	0	1117
915495	443	458	6017	6032	TCGGAGGAACTTGCTT	69	1118
915515	472	487	6046	6061	TTGGCCGGGAGGCATT	9	1119
915535	502	517	6076	6091	CCTATTTTGCCGGAGA	96	1120
915555	524	539	6098	6113	AGACACTCTGGTAAGA	2	1121
915615	712	727	11930	11945	GACACGGTGATGGTTG	32	1122
915635	792	807	12010	12025	GACTGAGCTTGGTGAT	93	1123
915655	839	854	12057	12072	GACAAAAGCTCTCGAG	40	1124
915675	901	916	13637	13652	AACCTGAATGCATCCA	92	1125
915695	975	990	16095	16110	CCTCAGGATCCATCCC	0	1126
915715	1011	1026	16131	16146	AATCCAGACTCATGTT	67	1127
915735	1088	1103	16208	16223	CAGGATGCTGAGACGC	86	1128
915755	1144	1159	N/A	N/A	CTCAGTGCTGTAGCGA	25	1129
915775	1209	1224	19049	19064	TTATCCTAATGGGTAG	23	1130
915795	1261	1276	19101	19116	ATCGCAATGGCAGATT	0	1131
915815	1337	1352	23742	23757	CACCTGTGAGGTCACC	35	1132
915835	1361	1376	23766	23781	CAGACACATCAGCACT	54	1133
915855	1433	1448	25196	25211	CCAGTCCTGCTCAGGT	23	1134
915875	1530	1545	25293	25308	AGAAGAAGTTCAGGCT	81	1135
915895	1574	1589	25337	25352	AAAGGTGGAGAGCCCC	76	1136
915915	1624	1639	25387	25402	GACTCGCCTCCTCAAG	75	1137

915935	1674	1689	25437	25452	GGTAGCTGCACAAAGA	76	1138
915955	1726	1741	25489	25504	GAGGCTGGGATCCTCC	0	1139
915975	1780	1795	25543	25558	CGCTGCACAGGCGAAA	0	1140
915995	1805	1820	25568	25583	GATGTATTAGAGTTAA	82	1141
916015	1829	1844	25592	25607	CAACCAGCTGAATTAA	59	1142
916035	1876	1891	25639	25654	ACAGTCAGTAAGGGAC	81	1143
916055	1899	1914	25662	25677	CTGACCATTAATAGGG	49	1144
916075	1929	1944	25692	25707	GTCATTCTAAGAACCT	81	1145
916095	1969	1984	25732	25747	CCTACCCCCCATCACA	21	1146
916115	1995	2010	25758	25773	ACCCCACAAGATCACA	0	1147
916135	2088	2103	25851	25866	GCAGACCACCTGACAG	44	1148
916154	2131	2146	25894	25909	CCCCGCCATGGAGACG	68	1149
916174	2180	2195	25943	25958	GTTAGGTGAAAAAGGT	90	1150
916194	2308	2323	26071	26086	GCACCCAACCGATTTT	83	1151
916214	2637	2652	26400	26415	GCTGAGGTGAATGCCC	52	1152
916234	2698	2713	26461	26476	GTTGTGTGCTCCAGTG	88	1153
916254	2747	2762	26510	26525	CTCACTGACCATGTGG	13	1154
916274	N/A	N/A	3524	3539	GCAAATCGGCCCCTCG	3	1155
916294	N/A	N/A	4463	4478	GGCATAATCTCCTGCC	0	1156
916314	N/A	N/A	5324	5339	TGGCATGCAAGACCAC	0	1157
916334	N/A	N/A	5606	5621	ACTTTATTCAATGTGG	95	1158
916354	N/A	N/A	6556	6571	GTTTATGTCACTCTGG	68	1159
916374	N/A	N/A	7245	7260	GAACAGACAAGTGCTG	38	1160
916394	N/A	N/A	7658	7673	ACTAACATACACCCTC	31	1161
916554	N/A	N/A	12249	12264	ATAATCAGGGTGGTGC	0	1162
916574	N/A	N/A	12747	12762	AGGCATACTAAAACCA	47	1163
916594	N/A	N/A	13500	13515	GAATCATGCAAGCTCT	50	1164
916614	N/A	N/A	13996	14011	TAAACTAAGGGTCACA	37	1165
916634	N/A	N/A	14497	14512	ATCCATCCTGCATGAG	76	1166
916654	N/A	N/A	15254	15269	GGCCCTATCAGATGCC	0	1167
916674	N/A	N/A	15802	15817	CTACATATCTGGTTTC	0	1168
916694	N/A	N/A	16844	16859	TGGACCATAGCACTGT	60	1169
916714	N/A	N/A	17738	17753	TATTAATCTGGTCATA	18	1170
916734	N/A	N/A	18926	18941	CCACTTTACTCTGTTG	64	1171

916754	N/A	N/A	20210	20225	AACTATGCCTAGAACG	43	1172
916773	N/A	N/A	20606	20621	TTTACGATCATCATTA	77	1173
916793	N/A	N/A	20842	20857	TGTATTAGCTCAATAT	0	1174
916813	N/A	N/A	21319	21334	TGGGAGTTTACTAGAG	66	1175
916833	N/A	N/A	22118	22133	AGAGAGTACTCTTGGA	11	1176
916853	N/A	N/A	23222	23237	CTGATAAATGCCTGAC	78	1177
916873	N/A	N/A	24038	24053	ATCAATGCTGCACTCA	88	1178
916893	N/A	N/A	24889	24904	ACGAATCCCTGGAGGG	0	1179

Table 18

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
915356	38	53	2776	2791	GTCTCGGCCAGGGCAT	0	1180
915376	93	108	2831	2846	CTGATCCGCAGCAGCT	28	1181
915396	165	180	2903	2918	CGTACATGGCGGCGGC	0	1182
915416	242	257	2980	2995	GCAGCGGGTCGCCCCG	0	1183
915436	339	354	3077	3092	GGATACCGGAGAGGAC	64	1184
915456	379	394	5953	5968	CGCACAAGATCTGAGA	79	1185
915476	406	421	5980	5995	ATGCCAATGTTCCGAC	83	1186
915496	444	459	6018	6033	GTCGGAGGAACTTGCT	35	1187
915516	473	488	6047	6062	ATTGGCCGGGAGGCAT	0	1188
915536	503	518	6077	6092	GCCTATTTTGCCGGAG	77	1189
915556	525	540	6099	6114	CAGACACTCTGGTAAG	62	1190
915616	730	745	11948	11963	TACTCCCCATAGAAGG	8	1191
915636	794	809	12012	12027	TAGACTGAGCTTGGTG	90	1192
915656	840	855	12058	12073	GGACAAAAGCTCTCGA	60	1193
915676	902	917	13638	13653	GAACCTGAATGCATCC	72	1194
915696	978	993	16098	16113	CGACCTCAGGATCCAT	0	1195
915716	1012	1027	16132	16147	GAATCCAGACTCATGT	0	1196
915736	1089	1104	16209	16224	GCAGGATGCTGAGACG	55	1197

915756	1145	1160	N/A	N/A	ACTCAGTGCTGTAGCG	12	1198
915776	1210	1225	19050	19065	ATTATCCTAATGGGTA	28	1199
915796	1262	1277	19102	19117	AATCGCAATGGCAGAT	0	1200
915816	1339	1354	23744	23759	AACACCTGTGAGGTCA	56	1201
915836	1365	1380	23770	23785	GGAGCAGACACATCAG	53	1202
915856	1434	1449	25197	25212	GCCAGTCCTGCTCAGG	21	1203
915876	1531	1546	25294	25309	AAGAAGAAGTTCAGGC	85	1204
915896	1575	1590	25338	25353	GAAAGGTGGAGAGCCC	78	1205
915916	1626	1641	25389	25404	TAGACTCGCCTCCTCA	32	1206
915936	1676	1691	25439	25454	GAGGTAGCTGCACAAA	91	1207
915956	1737	1752	25500	25515	AACTCAGCTCAGAGGC	46	1208
915976	1781	1796	25544	25559	CCGCTGCACAGGCGAA	0	1209
915996	1807	1822	25570	25585	CTGATGTATTAGAGTT	93	1210
916016	1830	1845	25593	25608	CCAACCAGCTGAATTA	21	1211
916036	1877	1892	25640	25655	AACAGTCAGTAAGGGA	82	1212
916056	1900	1915	25663	25678	TCTGACCATTAATAGG	13	1213
916076	1930	1945	25693	25708	TGTCATTCTAAGAACC	40	1214
916096	1970	1985	25733	25748	GCCTACCCCCCATCAC	18	1215
916116	1996	2011	25759	25774	CACCCCACAAGATCAC	50	1216
916136	2089	2104	25852	25867	TGCAGACCACCTGACA	58	1217
916155	2132	2147	25895	25910	CCCCCGCCATGGAGAC	33	1218
916175	2224	2239	25987	26002	CGCTTCCTTACATTTT	89	1219
916195	2309	2324	26072	26087	TGCACCCAACCGATTT	64	1220
916215	2638	2653	26401	26416	GGCTGAGGTGAATGCC	0	1221
916235	2699	2714	26462	26477	AGTTGTGTGCTCCAGT	85	1222
916255	2748	2763	26511	26526	ACTCACTGACCATGTG	0	1223
916275	N/A	N/A	3555	3570	GGCCAAAGCCCCACTC	0	1224
916295	N/A	N/A	4464	4479	GGGCATAATCTCCTGC	0	1225
916315	N/A	N/A	5342	5357	GGCTGATCTGCACTCT	84	1226
916335	N/A	N/A	5626	5641	TAATTCTACCTGTGTC	92	1227
916355	N/A	N/A	6557	6572	AGTTTATGTCACTCTG	27	1228
916375	N/A	N/A	7321	7336	ACACTTTGCGAAGCAC	27	1229
916395	N/A	N/A	7660	7675	GAACTAACATACACCC	1	1230
916555	N/A	N/A	12252	12267	CCCATAATCAGGGTGG	0	1231

916575	N/A	N/A	12758	12773	GTAGAGTGGTAAGGCA	95	1232
916595	N/A	N/A	13502	13517	AAGAATCATGCAAGCT	34	1233
916615	N/A	N/A	13997	14012	TTAAACTAAGGGTCAC	65	1234
916635	N/A	N/A	14549	14564	TTAATGTGGATTCACG	76	1235
916655	N/A	N/A	15295	15310	CCAAGATAACCTCACA	64	1236
916675	N/A	N/A	15806	15821	CCATCTACATATCTGG	26	1237
916695	N/A	N/A	16854	16869	CACAATCATTTGGACC	72	1238
916715	N/A	N/A	17739	17754	GTATTAATCTGGTCAT	87	1239
916735	N/A	N/A	19113	19128	CACCTCTGGACAATCG	29	1240
916755	N/A	N/A	20212	20227	CAAACTATGCCTAGAA	70	1241
916774	N/A	N/A	20608	20623	ATTTTACGATCATCAT	91	1242
916794	N/A	N/A	20846	20861	TGCCTGTATTAGCTCA	90	1243
916814	N/A	N/A	21345	21360	CACATAAAGTCAAACG	87	1244
916834	N/A	N/A	22124	22139	AGAACAAGAGAGTACT	3	1245
916854	N/A	N/A	23250	23265	CACATAAAGGACCCCC	54	1246
916874	N/A	N/A	24126	24141	CGCTATCTGACACTCC	87	1247
916894	N/A	N/A	24896	24911	TCCACCAACGAATCCC	50	1248

Table 19

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
915357	39	54	2777	2792	TGTCTCGGCCAGGGCA	0	1249
915377	94	109	2832	2847	CCTGATCCGCAGCAGC	0	1250
915397	182	197	2920	2935	CCAGCCGCGCTCTGCG	0	1251
915417	243	258	2981	2996	GGCAGCGGGTCGCCCC	0	1252
915437	341	356	3079	3094	CGGGATACCGGAGAGG	57	1253
915457	380	395	5954	5969	CCGCACAAGATCTGAG	71	1254
915477	407	422	5981	5996	GATGCCAATGTTCCGA	93	1255
915497	445	460	6019	6034	TGTCGGAGGAACTTGC	85	1256
915517	474	489	6048	6063	CATTGGCCGGGAGGCA	0	1257

915537	504	519	6078	6093	TGCCTATTTTGCCGGA	44	1258
915557	526	541	6100	6115	TCAGACACTCTGGTAA	26	1259
915617	732	747	11950	11965	CGTACTCCCCATAGAA	66	1260
915637	796	811	12014	12029	CGTAGACTGAGCTTGG	98	1261
915657	857	872	12075	12090	CACCTTGAGATCCGGG	0	1262
915677	903	918	13639	13654	AGAACCTGAATGCATC	78	1263
915697	979	994	16099	16114	GCGACCTCAGGATCCA	1	1264
915717	1031	1046	16151	16166	GGCAGCCGACTCCGGG	19	1265
915737	1109	1124	16229	16244	CAGGATGCTCTCATCC	0	1266
915757	1146	1161	N/A	N/A	CACTCAGTGCTGTAGC	33	1267
915777	1214	1229	19054	19069	AGACATTATCCTAATG	42	1268
915797	1263	1278	19103	19118	CAATCGCAATGGCAGA	49	1269
915817	1340	1355	23745	23760	GAACACCTGTGAGGTC	44	1270
915837	1398	1413	25161	25176	GGCTGCTCACTGGCAT	18	1271
915857	1435	1450	25198	25213	GGCCAGTCCTGCTCAG	0	1272
915877	1534	1549	25297	25312	CCCAAGAAGAAGTTCA	76	1273
915897	1576	1591	25339	25354	GGAAAGGTGGAGAGCC	24	1274
915917	1627	1642	25390	25405	CTAGACTCGCCTCCTC	77	1275
915937	1679	1694	25442	25457	GCGGAGGTAGCTGCAC	16	1276
915957	1738	1753	25501	25516	CAACTCAGCTCAGAGG	61	1277
915977	1782	1797	25545	25560	ACCGCTGCACAGGCGA	34	1278
915997	1809	1824	25572	25587	TGCTGATGTATTAGAG	83	1279
916017	1831	1846	25594	25609	CCCAACCAGCTGAATT	42	1280
916037	1878	1893	25641	25656	AAACAGTCAGTAAGGG	92	1281
916057	1901	1916	25664	25679	GTCTGACCATTAATAG	64	1282
916077	1931	1946	25694	25709	CTGTCATTCTAAGAAC	41	1283
916097	1971	1986	25734	25749	AGCCTACCCCCCATCA	0	1284
916117	1997	2012	25760	25775	CCACCCCACAAGATCA	0	1285
916137	2090	2105	25853	25868	TTGCAGACCACCTGAC	65	1286
916156	2133	2148	25896	25911	ACCCCCGCCATGGAGA	54	1287
916176	2225	2240	25988	26003	ACGCTTCCTTACATTT	84	1288
916196	2310	2325	26073	26088	CTGCACCCAACCGATT	58	1289
916216	2639	2654	26402	26417	GGGCTGAGGTGAATGC	46	1290
916236	2700	2715	26463	26478	AAGTTGTGTGCTCCAG	86	1291

916256	2751	2766	26514	26529	GAAACTCACTGACCAT	41	1292
916276	N/A	N/A	4068	4083	GGAAACAACTTTCCTC	0	1293
916296	N/A	N/A	4730	4745	GATCATGTGGCGGTCT	68	1294
916316	N/A	N/A	5364	5379	CACTTACTGGCCTGGC	30	1295
916336	N/A	N/A	5645	5660	ATATTGGGCTCAATGA	89	1296
916356	N/A	N/A	6575	6590	ATCACTGGAGGTGTAC	0	1297
916376	N/A	N/A	7328	7343	CAGGATCACACTTTGC	17	1298
916396	N/A	N/A	7661	7676	GGAACTAACATACACC	0	1299
916556	N/A	N/A	12272	12287	GTATATGTTCCCAGGT	81	1300
916576	N/A	N/A	12788	12803	GTGTACATGGTCTGCA	94	1301
916596	N/A	N/A	13529	13544	ATCATTGGAAGACCGC	89	1302
916616	N/A	N/A	13998	14013	GTTAAACTAAGGGTCA	85	1303
916636	N/A	N/A	14550	14565	CTTAATGTGGATTCAC	91	1304
916656	N/A	N/A	15351	15366	TCCAACTTCAGGCTGA	74	1305
916676	N/A	N/A	15819	15834	AGCTTTGTGGGCTCCA	69	1306
916696	N/A	N/A	16982	16997	GTTTAATAAGGGCACC	63	1307
916716	N/A	N/A	17740	17755	CGTATTAATCTGGTCA	93	1308
916736	N/A	N/A	19126	19141	CACCTAAAATGCTCAC	17	1309
916756	N/A	N/A	20213	20228	ACAAACTATGCCTAGA	58	1310
916775	N/A	N/A	20609	20624	AATTTTACGATCATCA	78	1311
916795	N/A	N/A	20927	20942	GACAGATCAGCACTCG	80	1312
916815	N/A	N/A	21407	21422	CAATTCTAGACATGGC	88	1313
916835	N/A	N/A	22338	22353	TGCACCTACCCTTTTC	39	1314
916855	N/A	N/A	23251	23266	ACACATAAAGGACCCC	48	1315
916875	N/A	N/A	24241	24256	GCATTACCAGGCACCT	61	1316
916895	N/A	N/A	24912	24927	GACATCACAGGTGTTG	5	1317

Table 20

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

915358	40	55	2778	2793	GTGTCTCGGCCAGGGC	0	1318
915378	96	111	2834	2849	GTCCTGATCCGCAGCA	0	1319
915398	184	199	2922	2937	CTCCAGCCGCGCTCTG	14	1320
915418	244	259	2982	2997	AGGCAGCGGGTCGCCC	0	1321
915438	342	357	3080	3095	GCGGGATACCGGAGAG	44	1322
915458	381	396	5955	5970	TCCGCACAAGATCTGA	41	1323
915478	408	423	5982	5997	AGATGCCAATGTTCCG	95	1324
915498	446	461	6020	6035	CTGTCGGAGGAACTTG	40	1325
915518	475	490	6049	6064	ACATTGGCCGGGAGGC	5	1326
915538	505	520	6079	6094	ATGCCTATTTTGCCGG	61	1327
915558	527	542	6101	6116	ATCAGACACTCTGGTA	0	1328
915618	748	763	11966	11981	ACTTTAGGGCAGATGT	87	1329
915638	818	833	12036	12051	GTAGAGGTTCCCTGTG	87	1330
915658	859	874	N/A	N/A	AGCACCTTGAGATCCG	0	1331
915678	926	941	N/A	N/A	GTTGCAGATGCCCTTC	24	1332
915698	980	995	16100	16115	GGCGACCTCAGGATCC	0	1333
915718	1032	1047	16152	16167	AGGCAGCCGACTCCGG	8	1334
915738	1114	1129	16234	16249	GTGTCCAGGATGCTCT	41	1335
915758	1147	1162	N/A	N/A	TCACTCAGTGCTGTAG	59	1336
915778	1217	1232	19057	19072	ATAAGACATTATCCTA	85	1337
915798	1264	1279	19104	19119	ACAATCGCAATGGCAG	66	1338
915818	1342	1357	23747	23762	GTGAACACCTGTGAGG	58	1339
915838	1400	1415	25163	25178	TTGGCTGCTCACTGGC	79	1340
915858	1436	1451	25199	25214	GGGCCAGTCCTGCTCA	0	1341
915878	1535	1550	25298	25313	GCCCAAGAAGAAGTTC	54	1342
915898	1590	1605	25353	25368	CTAGTGAAAAACTGGG	34	1343
915918	1628	1643	25391	25406	GCTAGACTCGCCTCCT	33	1344
915938	1680	1695	25443	25458	TGCGGAGGTAGCTGCA	0	1345
915958	1756	1771	25519	25534	CCTAGCTTTTCATAAA	0	1346
915978	1783	1798	25546	25561	GACCGCTGCACAGGCG	24	1347
915998	1810	1825	25573	25588	ATGCTGATGTATTAGA	86	1348
916018	1832	1847	25595	25610	TCCCAACCAGCTGAAT	3	1349
916038	1879	1894	25642	25657	GAAACAGTCAGTAAGG	64	1350
916058	1902	1917	25665	25680	AGTCTGACCATTAATA	86	1351

916078	1933	1948	25696	25711	ACCTGTCATTCTAAGA	18	1352
916098	1972	1987	25735	25750	CAGCCTACCCCCCATC	41	1353
916118	1999	2014	25762	25777	CTCCACCCCACAAGAT	0	1354
916138	2092	2107	25855	25870	CTTTGCAGACCACCTG	65	1355
916157	2134	2149	25897	25912	TACCCCCGCCATGGAG	57	1356
916177	2237	2252	26000	26015	CAACAGGTAACAACGC	88	1357
916197	2579	2594	26342	26357	GTCAGACTTTCACTCA	81	1358
916217	2659	2674	26422	26437	GTGCTTGGCTCCTGCC	43	1359
916237	2701	2716	26464	26479	CAAGTTGTGTGCTCCA	73	1360
916257	2769	2784	26532	26547	CATCGCCACACATGGG	61	1361
916277	N/A	N/A	4105	4120	AGGAAGGGTCCCAAAC	0	1362
916297	N/A	N/A	4731	4746	TGATCATGTGGCGGTC	80	1363
916317	N/A	N/A	5391	5406	TGCTATCAGGTGCAGG	60	1364
916337	N/A	N/A	5646	5661	TATATTGGGCTCAATG	71	1365
916357	N/A	N/A	6594	6609	GTTTACAAACATGGAC	26	1366
916377	N/A	N/A	7464	7479	TCATTAGCATCACCGG	33	1367
916397	N/A	N/A	7662	7677	GGGAACTAACATACAC	0	1368
916557	N/A	N/A	12274	12289	GGGTATATGTTCCCAG	0	1369
916577	N/A	N/A	12830	12845	TGCATAGCCTTCTTTC	84	1370
916597	N/A	N/A	13530	13545	CATCATTGGAAGACCG	62	1371
916617	N/A	N/A	14016	14031	TCTTTAACTTCGGCCC	70	1372
916637	N/A	N/A	14551	14566	TCTTAATGTGGATTCA	88	1373
916657	N/A	N/A	15388	15403	TCAGACAACCACAGCT	66	1374
916677	N/A	N/A	15852	15867	TAAAGCAGGACACACG	74	1375
916697	N/A	N/A	17077	17092	AGACATGTTGGTGTCT	0	1376
916717	N/A	N/A	17788	17803	CCCCAGTCTTTTATTC	0	1377
916737	N/A	N/A	19140	19155	GGAAGACACGGAGCCA	20	1378
916757	N/A	N/A	20240	20255	CCTAACTGCTGGCTCT	85	1379
916776	N/A	N/A	20610	20625	TAATTTTACGATCATC	76	1380
916796	N/A	N/A	20939	20954	CTCTTTGTAGCAGACA	90	1381
916816	N/A	N/A	21439	21454	CAATATACTGAGAGGA	92	1382
916836	N/A	N/A	22392	22407	GTAGACATCCTTCCCG	65	1383
916856	N/A	N/A	23252	23267	GACACATAAAGGACCC	59	1384
916876	N/A	N/A	24242	24257	TGCATTACCAGGCACC	37	1385

916896	N/A	N/A	24913	24928	GGACATCACAGGTGTT	19	1386

Table 21

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915359	41	56	2779	2794	AGTGTCTCGGCCAGGG	0	1387
915379	97	112	2835	2850	GGTCCTGATCCGCAGC	0	1388
915399	185	200	2923	2938	GCTCCAGCCGCGCTCT	26	1389
915419	245	260	2983	2998	CAGGCAGCGGGTCGCC	0	1390
915439	343	358	3081	3096	AGCGGGATACCGGAGA	69	1391
915459	382	397	5956	5971	TTCCGCACAAGATCTG	71	1392
915479	409	424	5983	5998	AAGATGCCAATGTTCC	93	1393
915499	448	463	6022	6037	CCCTGTCGGAGGAACT	30	1394
915519	477	492	6051	6066	GGACATTGGCCGGGAG	88	1395
915539	506	521	6080	6095	GATGCCTATTTTGCCG	60	1396
915559	529	544	6103	6118	CCATCAGACACTCTGG	30	1397
915579	595	610	7825	7840	CAGGAACATACCAAGG	98	1398
915599	674	689	11892	11907	GTTGTCACTCACTCCT	98	1399
915619	749	764	11967	11982	GACTTTAGGGCAGATG	96	1400
915639	819	834	12037	12052	GGTAGAGGTTCCCTGT	92	1401
915659	860	875	N/A	N/A	CAGCACCTTGAGATCC	0	1402
915679	928	943	N/A	N/A	CTGTTGCAGATGCCCT	58	1403
915699	981	996	16101	16116	TGGCGACCTCAGGATC	40	1404
915719	1033	1048	16153	16168	AAGGCAGCCGACTCCG	0	1405
915739	1115	1130	16235	16250	GGTGTCCAGGATGCTC	40	1406
915759	1148	1163	N/A	N/A	TTCACTCAGTGCTGTA	29	1407
915779	1219	1234	19059	19074	ACATAAGACATTATCC	86	1408
915799	1268	1283	19108	19123	CTGGACAATCGCAATG	42	1409
915819	1344	1359	23749	23764	GAGTGAACACCTGTGA	81	1410
915839	1401	1416	25164	25179	GTTGGCTGCTCACTGG	85	1411

915859	1437	1452	25200	25215	AGGGCCAGTCCTGCTC	0	1412
915879	1538	1553	25301	25316	ATTGCCCAAGAAGAAG	54	1413
915899	1591	1606	25354	25369	TCTAGTGAAAAACTGG	0	1414
915919	1629	1644	25392	25407	TGCTAGACTCGCCTCC	72	1415
915939	1681	1696	25444	25459	ATGCGGAGGTAGCTGC	39	1416
915959	1764	1779	25527	25542	GGTTGCTTCCTAGCTT	87	1417
915979	1784	1799	25547	25562	GGACCGCTGCACAGGC	0	1418
915999	1811	1826	25574	25589	CATGCTGATGTATTAG	35	1419
916019	1833	1848	25596	25611	TTCCCAACCAGCTGAA	0	1420
916039	1880	1895	25643	25658	CGAAACAGTCAGTAAG	80	1421
916059	1905	1920	25668	25683	AACAGTCTGACCATTA	85	1422
916079	1934	1949	25697	25712	CACCTGTCATTCTAAG	45	1423
916099	1973	1988	25736	25751	CCAGCCTACCCCCCAT	53	1424
916119	2022	2037	25785	25800	GTGGGATCATGCTATT	76	1425
916139	2093	2108	25856	25871	TCTTTGCAGACCACCT	85	1426
916158	2135	2150	25898	25913	TTACCCCCGCCATGGA	0	1427
916178	2238	2253	26001	26016	TCAACAGGTAACAACG	87	1428
916198	2620	2635	26383	26398	GCACACTAGATTATTT	66	1429
916218	2673	2688	26436	26451	CGGAAGCTCCTGCTGT	27	1430
916238	2702	2717	26465	26480	TCAAGTTGTGTGCTCC	91	1431
916258	2770	2785	26533	26548	TCATCGCCACACATGG	49	1432
916278	N/A	N/A	4211	4226	TCATTTCCAGGAGTAC	75	1433
916298	N/A	N/A	4735	4750	CAAATGATCATGTGGC	93	1434
916318	N/A	N/A	5394	5409	TAATGCTATCAGGTGC	95	1435
916338	N/A	N/A	5648	5663	GATATATTGGGCTCAA	97	1436
916358	N/A	N/A	6596	6611	GGGTTTACAAACATGG	75	1437
916378	N/A	N/A	7465	7480	TTCATTAGCATCACCG	78	1438
916398	N/A	N/A	7686	7701	GTTAATCCATGGGTCA	49	1439
916418	N/A	N/A	8992	9007	AGCCTAAACTTCCTCC	63	1440
916438	N/A	N/A	9318	9333	AGAAGAGCCGCCCTGC	77	1441
916458	N/A	N/A	9795	9810	GCAAGACTAGCAAGTG	85	1442
916478	N/A	N/A	10301	10316	AGCATGCGGTATGTAC	67	1443
916498	N/A	N/A	10849	10864	CACACAATTTCTAGGG	82	1444
916518	N/A	N/A	11346	11361	TTGACAATTAGAACCA	96	1445

916538	N/A	N/A	11711	11726	ACAAATCCTTACCGAG	54	1446
916558	N/A	N/A	12285	12300	GTTTTAGGTCTGGGTA	94	1447
916578	N/A	N/A	12831	12846	TTGCATAGCCTTCTTT	93	1448
916598	N/A	N/A	13660	13675	CATACATACCCTTCTC	9	1449
916618	N/A	N/A	14025	14040	CGCAGAAACTCTTTAA	89	1450
916638	N/A	N/A	14552	14567	GTCTTAATGTGGATTC	93	1451
916658	N/A	N/A	15421	15436	AGCATTGGCACACTGG	70	1452
916678	N/A	N/A	15857	15872	GGCTTTAAAGCAGGAC	62	1453
916698	N/A	N/A	17079	17094	GCAGACATGTTGGTGT	2	1454
916718	N/A	N/A	17839	17854	TACAAGCTGGTCCTTG	0	1455
916738	N/A	N/A	19211	19226	GACAATCCAGGTCCCA	70	1456
916758	N/A	N/A	20285	20300	GAGGAAGCCCAATCAA	81	1457
916777	N/A	N/A	20611	20626	CTAATTTTACGATCAT	81	1458
916797	N/A	N/A	20984	20999	TTAAACTGCCAAGTCC	83	1459
916817	N/A	N/A	21440	21455	CCAATATACTGAGAGG	96	1460
916837	N/A	N/A	22406	22421	GGTAGCACCGCCAAGT	0	1461
916857	N/A	N/A	23301	23316	CACCATGGAGAGGTCT	0	1462
916877	N/A	N/A	24243	24258	TTGCATTACCAGGCAC	17	1463
916897	N/A	N/A	24934	24949	GCTACCTGGACACCTC	47	1464

Table 22

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
841947	2094	2109	25857	25872	ATCTTTGCAGACCACC	89	1464
912986	N/A	N/A	20288	20303	TCAGAGGAAGCCCAAT	92	254
			20318	20333			
915360	42	57	2780	2795	CAGTGTCTCGGCCAGG	0	1466
915380	98	113	2836	2851	GGGTCCTGATCCGCAG	0	1467
915400	186	201	2924	2939	AGCTCCAGCCGCGCTC	0	1468
915420	246	261	2984	2999	TCAGGCAGCGGGTCGC	78	1469

915440	344	359	3082	3097	CAGCGGGATACCGGAG	72	1470
915460	383	398	5957	5972	CTTCCGCACAAGATCT	0	1471
915480	411	426	5985	6000	GGAAGATGCCAATGTT	94	1472
915500	449	464	6023	6038	ACCCTGTCGGAGGAAC	40	1473
915520	480	495	6054	6069	GGTGGACATTGGCCGG	38	1474
915540	507	522	6081	6096	AGATGCCTATTTTGCC	76	1475
915560	556	571	6130	6145	CGAAAGTCAGACACCA	69	1476
915620	750	765	11968	11983	TGACTTTAGGGCAGAT	89	1477
915640	821	836	12039	12054	AAGGTAGAGGTTCCCT	10	1478
915660	875	890	13611	13626	AAGGCATATCTCTCCC	47	1479
915680	929	944	N/A	N/A	CCTGTTGCAGATGCCC	22	1480
915700	982	997	16102	16117	ATGGCGACCTCAGGAT	58	1481
915720	1034	1049	16154	16169	CAAGGCAGCCGACTCC	63	1482
915740	1121	1136	16241	16256	CGAGAGGGTGTCCAGG	0	1483
915760	1149	1164	N/A	N/A	CTTCACTCAGTGCTGT	13	1484
915780	1226	1241	19066	19081	CAGCATTACATAAGAC	94	1485
915800	1270	1285	19110	19125	CTCTGGACAATCGCAA	55	1486
915820	1345	1360	23750	23765	CGAGTGAACACCTGTG	83	1487
915840	1402	1417	25165	25180	TGTTGGCTGCTCACTG	77	1488
915860	1470	1485	25233	25248	CTGGACAGCCCTTGGG	29	1489
915880	1539	1554	25302	25317	TATTGCCCAAGAAGAA	18	1490
915900	1598	1613	25361	25376	ACTCTTCTCTAGTGAA	67	1491
915920	1630	1645	25393	25408	CTGCTAGACTCGCCTC	88	1492
915940	1682	1697	25445	25460	AATGCGGAGGTAGCTG	0	1493
915960	1765	1780	25528	25543	AGGTTGCTTCCTAGCT	55	1494
915980	1785	1800	25548	25563	TGGACCGCTGCACAGG	82	1495
916000	1812	1827	25575	25590	GCATGCTGATGTATTA	52	1496
916020	1837	1852	25600	25615	TCATTTCCCAACCAGC	94	1497
916040	1881	1896	25644	25659	ACGAAACAGTCAGTAA	79	1498
916060	1907	1922	25670	25685	GGAACAGTCTGACCAT	22	1499
916080	1936	1951	25699	25714	AACACCTGTCATTCTA	71	1500
916100	1974	1989	25737	25752	GCCAGCCTACCCCCCA	23	1501
916120	2023	2038	25786	25801	AGTGGGATCATGCTAT	0	1502
916159	2136	2151	25899	25914	GTTACCCCCGCCATGG	47	1503

916179	2239	2254	26002	26017	TTCAACAGGTAACAAC	84	1504
916199	2621	2636	26384	26399	TGCACACTAGATTATT	0	1505
916219	2674	2689	26437	26452	GCGGAAGCTCCTGCTG	6	1506
916239	2704	2719	26467	26482	GTTCAAGTTGTGTGCT	85	1507
916259	2771	2786	26534	26549	CTCATCGCCACACATG	85	1508
916279	N/A	N/A	4218	4233	CGGAATCTCATTTCCA	0	1509
916299	N/A	N/A	4736	4751	GCAAATGATCATGTGG	93	1510
916319	N/A	N/A	5396	5411	CTTAATGCTATCAGGT	83	1511
916339	N/A	N/A	5649	5664	GGATATATTGGGCTCA	96	1512
916359	N/A	N/A	6597	6612	AGGGTTTACAAACATG	32	1513
916379	N/A	N/A	7466	7481	ATTCATTAGCATCACC	52	1514
916399	N/A	N/A	7687	7702	GGTTAATCCATGGGTC	0	1515
916559	N/A	N/A	12286	12301	AGTTTTAGGTCTGGGT	89	1516
916579	N/A	N/A	12833	12848	CATTGCATAGCCTTCT	96	1517
916599	N/A	N/A	13661	13676	CCATACATACCCTTCT	21	1518
916619	N/A	N/A	14077	14092	ACCCACACCTGACTGG	16	1519
916639	N/A	N/A	14572	14587	CGCTCCTACTTATCCC	96	1520
916659	N/A	N/A	15427	15442	TCTTACAGCATTGGCA	38	1521
916679	N/A	N/A	15973	15988	CATCTACCAAACTGCA	73	1522
916699	N/A	N/A	17135	17150	AACAAACATCGATTTT	47	1523
916719	N/A	N/A	17844	17859	AGCTTTACAAGCTGGT	0	1524
916739	N/A	N/A	19213	19228	ACGACAATCCAGGTCC	14	1525
916778	N/A	N/A	20612	20627	TCTAATTTTACGATCA	92	1526
916798	N/A	N/A	20985	21000	ATTAAACTGCCAAGTC	72	1527
916818	N/A	N/A	21441	21456	ACCAATATACTGAGAG	92	1528
916838	N/A	N/A	22409	22424	AGCGGTAGCACCGCCA	0	1529
916858	N/A	N/A	23323	23338	TCACATGTGAGCCCAG	46	1530
916878	N/A	N/A	24271	24286	GTACAACAGAGGGTGG	19	1531
916898	N/A	N/A	24978	24993	GGCTGATGTCACCACC	32	1532

Table 23

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915361	43	58	2781	2796	TCAGTGTCTCGGCCAG	0	1533
915381	105	120	2843	2858	TCGGCTCGGGTCCTGA	0	1534
915401	188	203	2926	2941	CAAGCTCCAGCCGCGC	22	1535
915421	247	262	2985	3000	CTCAGGCAGCGGGTCG	48	1536
915441	345	360	3083	3098	CCAGCGGGATACCGGA	0	1537
915461	384	399	5958	5973	CCTTCCGCACAAGATC	59	1538
915481	414	429	5988	6003	GATGGAAGATGCCAAT	83	1539
915501	450	465	6024	6039	GACCCTGTCGGAGGAA	26	1540
915521	482	497	6056	6071	CTGGTGGACATTGGCC	0	1541
915541	508	523	6082	6097	GAGATGCCTATTTTGC	92	1542
915561	557	572	6131	6146	CCGAAAGTCAGACACC	0	1543
915601	691	706	11909	11924	GCATCAATGAAGGGTA	91	1544
915621	751	766	11969	11984	TTGACTTTAGGGCAGA	85	1545
915641	822	837	12040	12055	GAAGGTAGAGGTTCCC	74	1546
915661	876	891	13612	13627	GAAGGCATATCTCTCC	32	1547
915681	930	945	16050	16065	GCCTGTTGCAGATGCC	0	1548
915701	983	998	16103	16118	CATGGCGACCTCAGGA	0	1549
915721	1035	1050	16155	16170	CCAAGGCAGCCGACTC	79	1550
915741	1122	1137	16242	16257	GCGAGAGGGTGTCCAG	0	1551
915761	1150	1165	18990	19005	TCTTCACTCAGTGCTG	41	1552
915781	1227	1242	19067	19082	GCAGCATTACATAAGA	75	1553
915801	1273	1288	N/A	N/A	AGTCTCTGGACAATCG	0	1554
915821	1346	1361	23751	23766	TCGAGTGAACACCTGT	52	1555
915841	1403	1418	25166	25181	CTGTTGGCTGCTCACT	80	1556
915861	1471	1486	25234	25249	GCTGGACAGCCCTTGG	0	1557
915881	1540	1555	25303	25318	TTATTGCCCAAGAAGA	75	1558
915901	1599	1614	25362	25377	GACTCTTCTCTAGTGA	67	1559
915921	1631	1646	25394	25409	TCTGCTAGACTCGCCT	50	1560
915941	1683	1698	25446	25461	CAATGCGGAGGTAGCT	39	1561

915961	1766	1781	25529	25544	AAGGTTGCTTCCTAGC	71	1562
915981	1786	1801	25549	25564	CTGGACCGCTGCACAG	0	1563
916001	1813	1828	25576	25591	CGCATGCTGATGTATT	73	1564
916021	1840	1855	25603	25618	GTGTCATTTCCCAACC	61	1565
916041	1882	1897	25645	25660	CACGAAACAGTCAGTA	34	1566
916061	1910	1925	25673	25688	GCTGGAACAGTCTGAC	82	1567
916081	1942	1957	25705	25720	CATCCAAACACCTGTC	69	1568
916101	1975	1990	25738	25753	GGCCAGCCTACCCCCC	2	1569
916121	2024	2039	25787	25802	AAGTGGGATCATGCTA	26	1570
916140	2095	2110	25858	25873	CATCTTTGCAGACCAC	92	1571
916160	2137	2152	25900	25915	TGTTACCCCCGCCATG	51	1572
916180	2257	2272	26020	26035	GATTCACATAATACAA	87	1573
916200	2622	2637	26385	26400	CTGCACACTAGATTAT	0	1574
916220	2675	2690	26438	26453	GGCGGAAGCTCCTGCT	0	1575
916240	2705	2720	26468	26483	GGTTCAAGTTGTGTGC	69	1576
916260	2772	2787	26535	26550	TCTCATCGCCACACAT	72	1577
916280	N/A	N/A	4220	4235	TACGGAATCTCATTTC	80	1578
916300	N/A	N/A	4791	4806	GGCCACCTTGGGATAC	17	1579
916320	N/A	N/A	5398	5413	GCCTTAATGCTATCAG	67	1580
916340	N/A	N/A	5650	5665	TGGATATATTGGGCTC	97	1581
916360	N/A	N/A	6603	6618	ACATTCAGGGTTTACA	18	1582
916380	N/A	N/A	7468	7483	GTATTCATTAGCATCA	51	1583
916400	N/A	N/A	7688	7703	AGGTTAATCCATGGGT	29	1584
916560	N/A	N/A	12287	12302	GAGTTTTAGGTCTGGG	95	1585
916580	N/A	N/A	12905	12920	CTTATAAAGCACACGG	95	1586
916600	N/A	N/A	13683	13698	GGGCATGGCTGATCCT	8	1587
916620	N/A	N/A	14099	14114	CAAACTTGTCTAGTGG	67	1588
916640	N/A	N/A	14600	14615	TCGCATCCATGGGTCC	83	1589
916660	N/A	N/A	15429	15444	GCTCTTACAGCATTGG	51	1590
916680	N/A	N/A	15974	15989	CCATCTACCAAACTGC	61	1591
916700	N/A	N/A	17195	17210	GACTTAGTCCGTGTTC	49	1592
916720	N/A	N/A	17883	17898	CAGCATCTATGTTCTC	67	1593
916740	N/A	N/A	19239	19254	ATAGACTGTGAGCTGT	82	1594
916759	N/A	N/A	20354	20369	GACCATTCTGCTCCCC	20	1595

916779	N/A	N/A	20632	20647	GCCCATACCTTTTATC	47	1596
916799	N/A	N/A	20987	21002	GTATTAAACTGCCAAG	81	1597
916819	N/A	N/A	21444	21459	CTAACCAATATACTGA	73	1598
916839	N/A	N/A	22506	22521	GGCTGGTGATGAAACA	0	1599
916859	N/A	N/A	23345	23360	CCTCATGGTTTGCTGT	31	1600
916879	N/A	N/A	24273	24288	CAGTACAACAGAGGGT	81	1601
916899	N/A	N/A	25064	25079	CACATTGCCGGCCAGT	58	1602

Table 24

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915362	44	59	2782	2797	CTCAGTGTCTCGGCCA	0	1603
915382	106	121	2844	2859	ATCGGCTCGGGTCCTG	30	1604
915402	189	204	2927	2942	ACAAGCTCCAGCCGCG	24	1605
915422	248	263	2986	3001	GCTCAGGCAGCGGGTC	33	1606
915442	346	361	N/A	N/A	TCCAGCGGGATACCGG	0	1607
915462	385	400	5959	5974	GCCTTCCGCACAAGAT	60	1608
915482	415	430	5989	6004	GGATGGAAGATGCCAA	60	1609
915502	451	466	6025	6040	AGACCCTGTCGGAGGA	75	1610
915522	487	502	6061	6076	ATGAGCTGGTGGACAT	74	1611
915542	509	524	6083	6098	AGAGATGCCTATTTTG	91	1612
915562	558	573	6132	6147	ACCGAAAGTCAGACAC	0	1613
915602	692	707	11910	11925	GGCATCAATGAAGGGT	88	1614
915622	752	767	11970	11985	CTTGACTTTAGGGCAG	86	1615
915642	824	839	12042	12057	GAGAAGGTAGAGGTTC	81	1616
915662	878	893	13614	13629	TCGAAGGCATATCTCT	16	1617
915682	931	946	16051	16066	GGCCTGTTGCAGATGC	0	1618
915702	984	999	16104	16119	GCATGGCGACCTCAGG	24	1619
915722	1036	1051	16156	16171	GCCAAGGCAGCCGACT	0	1620
915742	1123	1138	16243	16258	GGCGAGAGGGTGTCCA	0	1621

915762	1173	1188	19013	19028	TGTATCCACCTTTGTC	85	1622
915782	1228	1243	19068	19083	GGCAGCATTACATAAG	73	1623
915802	1283	1298	N/A	N/A	CCATGTCACCAGTCTC	59	1624
915822	1347	1362	23752	23767	CTCGAGTGAACACCTG	0	1625
915842	1404	1419	25167	25182	CCTGTTGGCTGCTCAC	88	1626
915862	1472	1487	25235	25250	TGCTGGACAGCCCTTG	0	1627
915882	1541	1556	25304	25319	TTTATTGCCCAAGAAG	38	1628
915902	1600	1615	25363	25378	AGACTCTTCTCTAGTG	60	1629
915922	1632	1647	25395	25410	ATCTGCTAGACTCGCC	86	1630
915942	1684	1699	25447	25462	GCAATGCGGAGGTAGC	38	1631
915962	1767	1782	25530	25545	AAAGGTTGCTTCCTAG	77	1632
915982	1787	1802	25550	25565	GCTGGACCGCTGCACA	79	1633
916002	1814	1829	25577	25592	ACGCATGCTGATGTAT	72	1634
916022	1841	1856	25604	25619	GGTGTCATTTCCCAAC	80	1635
916042	1883	1898	25646	25661	CCACGAAACAGTCAGT	87	1636
916062	1912	1927	25675	25690	ATGCTGGAACAGTCTG	78	1637
916082	1943	1958	25706	25721	CCATCCAAACACCTGT	64	1638
916102	1976	1991	25739	25754	GGGCCAGCCTACCCCC	0	1639
916122	2025	2040	25788	25803	GAAGTGGGATCATGCT	71	1640
916141	2097	2112	25860	25875	ATCATCTTTGCAGACC	91	1641
916161	2138	2153	25901	25916	TTGTTACCCCCGCCAT	89	1642
916181	2259	2274	26022	26037	CTGATTCACATAATAC	91	1643
916201	2623	2638	26386	26401	CCTGCACACTAGATTA	59	1644
916221	2676	2691	26439	26454	AGGCGGAAGCTCCTGC	0	1645
916241	2706	2721	26469	26484	AGGTTCAAGTTGTGTG	87	1646
916281	N/A	N/A	4224	4239	AATGTACGGAATCTCA	83	1647
916301	N/A	N/A	4810	4825	GTCCATGTGGGTGTCC	74	1648
916321	N/A	N/A	5399	5414	GGCCTTAATGCTATCA	13	1649
916341	N/A	N/A	5711	5726	TAGTATGAAATATCTC	96	1650
916361	N/A	N/A	6862	6877	ATTGTAACTGCCAGGC	0	1651
916381	N/A	N/A	7471	7486	CCGGTATTCATTAGCA	0	1652
916401	N/A	N/A	7728	7743	GAGCAGGGCAACAAAC	22	1653
916561	N/A	N/A	12315	12330	ATATAACCACAGCCTG	54	1654
916581	N/A	N/A	12906	12921	GCTTATAAAGCACACG	94	1655

916601	N/A	N/A	13702	13717	TAGTAAATGCTTGTCA	95	1656
916621	N/A	N/A	14123	14138	GGCAGAAATGTGCTCT	60	1657
916641	N/A	N/A	14632	14647	CTTCATGCCATCCTGT	83	1658
916661	N/A	N/A	15430	15445	TGCTCTTACAGCATTG	0	1659
916681	N/A	N/A	16262	16277	GGTACCTGTAGCGAGC	0	1660
916701	N/A	N/A	17197	17212	TTGACTTAGTCCGTGT	93	1661
916721	N/A	N/A	18220	18235	AGCTACATCAGGCTGG	0	1662
916741	N/A	N/A	19244	19259	TGCACATAGACTGTGA	0	1663
916760	N/A	N/A	20373	20388	GACTGCTGAGCCAAGC	61	1664
916780	N/A	N/A	20657	20672	AGAAATTGCAGTGCCC	92	1665
916800	N/A	N/A	20988	21003	GGTATTAAACTGCCAA	0	1666
916820	N/A	N/A	21447	21462	TCACTAACCAATATAC	47	1667
916840	N/A	N/A	22602	22617	ATAAATCTGCAAGAGC	62	1668
916860	N/A	N/A	23369	23384	TCTCATGGTCAAGACC	52	1669
916880	N/A	N/A	24305	24320	GACTGCTAGGCTTCAC	54	1670
916900	N/A	N/A	25100	25115	CGCTGCTGCAGTGTGC	34	1671

Human primer probe set RTS36075 (forward sequence TGAGGCTGGAGGGAGATG, designated herein as SEQ ID NO: 14; reverse sequence GCTCATGTATCCACCTTTGTCT, designated herein as SEQ ID NO: 15; probe sequence CTAGACCACCTGCGTCTCAGCATC, designated herein as SEQ ID NO: 16) was also used to measure mRNA levels. PNPLA3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PNPLA3, relative to untreated control cells.

Table 25

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
898558	581	596	N/A	N/A	GGCATCCACGACTTCG	87	1672
912709	27	42	2765	2780	GGCATTCCCAGCGCGA	0	17
912710	95	110	2833	2848	TCCTGATCCGCAGCAG	0	18
912711	103	118	2841	2856	GGCTCGGGTCCTGATC	0	19

912712	131	146	2869	2884	GTTAGGATCTGGGTCG	76	20
912713	164	179	2902	2917	GTACATGGCGGCGGCG	0	21
912714	183	198	2921	2936	TCCAGCCGCGCTCTGC	29	22
912715	196	211	2934	2949	GCGAAGGACAAGCTCC	31	23
912716	197	212	2935	2950	CGCGAAGGACAAGCTC	0	24
912717	272	287	3010	3025	GCGGAGGAGGTGCGGG	0	25
912718	273	288	3011	3026	CGCGGAGGAGGTGCGG	0	26
912719	274	289	3012	3027	TCGCGGAGGAGGTGCG	16	27
912720	290	305	3028	3043	GAACAACATGCGCGCG	0	28
912721	291	306	3029	3044	CGAACAACATGCGCGC	2	29
912722	292	307	3030	3045	CCGAACAACATGCGCG	0	30
912723	293	308	3031	3046	GCCGAACAACATGCGC	0	31
912724	294	309	3032	3047	CGCCGAACAACATGCG	0	32
912725	323	338	3061	3076	GCCGACGCAGTGCAAC	0	33
912726	324	339	3062	3077	CGCCGACGCAGTGCAA	0	34
912727	340	355	3078	3093	GGGATACCGGAGAGGA	32	35
912728	370	385	5944	5959	TCTGAGAGGACCTGCA	31	36
912729	375	390	5949	5964	CAAGATCTGAGAGGAC	60	37
912730	404	419	5978	5993	GCCAATGTTCCGACTC	52	38
912731	410	425	5984	5999	GAAGATGCCAATGTTC	31	39
912732	429	444	6003	6018	TTAAGTTGAAGGATGG	93	40
912733	432	447	6006	6021	TGCTTAAGTTGAAGGA	82	41
912734	478	493	6052	6067	TGGACATTGGCCGGGA	73	42
912735	479	494	6053	6068	GTGGACATTGGCCGGG	44	43
912736	484	499	6058	6073	AGCTGGTGGACATTGG	29	44
912737	528	543	6102	6117	CATCAGACACTCTGGT	0	45
912738	531	546	6105	6120	CCCCATCAGACACTCT	55	46
912739	552	567	6126	6141	AGTCAGACACCAGAAC	23	47
912740	582	597	N/A	N/A	AGGCATCCACGACTTC	40	1673
912741	584	599	N/A	N/A	CAAGGCATCCACGACT	55	1674
912742	591	606	N/A	N/A	AACATACCAAGGCATC	59	1675
912743	593	608	N/A	N/A	GGAACATACCAAGGCA	69	1676
912744	594	609	7824	7839	AGGAACATACCAAGGC	85	1677
912745	625	640	7855	7870	GGGATAAGGCCACTGT	71	1678

912746	626	641	7856	7871	AGGGATAAGGCCACTG	12	1679
912747	630	645	7860	7875	AAGGAGGGATAAGGCC	0	1680
912748	652	667	N/A	N/A	ACATATCGCACGCCTC	35	1681
912749	653	668	N/A	N/A	CACATATCGCACGCCT	3	1682
912750	654	669	N/A	N/A	CCACATATCGCACGCC	27	1683
912751	656	671	N/A	N/A	ATCCACATATCGCACG	24	1684
912752	660	675	11878	11893	CTCCATCCACATATCG	87	1685
912753	689	704	11907	11922	ATCAATGAAGGGTACG	79	1686
912754	690	705	11908	11923	CATCAATGAAGGGTAC	63	1687
912755	693	708	11911	11926	TGGCATCAATGAAGGG	68	48
912756	698	713	11916	11931	TGTTTTGGCATCAATG	88	49
912757	746	761	11964	11979	TTTAGGGCAGATGTCG	75	50
912758	747	762	11965	11980	CTTTAGGGCAGATGTC	82	51
912759	795	810	12013	12028	GTAGACTGAGCTTGGT	96	52
912760	820	835	12038	12053	AGGTAGAGGTTCCCTG	0	53
912761	841	856	12059	12074	GGGACAAAAGCTCTCG	0	54
912762	873	888	13609	13624	GGCATATCTCTCCCAG	0	55
912763	874	889	13610	13625	AGGCATATCTCTCCCA	0	56
912764	886	901	13622	13637	AAATATCCTCGAAGGC	71	57
912765	888	903	13624	13639	CCAAATATCCTCGAAG	37	58
912766	889	904	13625	13640	TCCAAATATCCTCGAA	0	59
912767	894	909	13630	13645	ATGCATCCAAATATCC	42	60
912768	925	940	N/A	N/A	TTGCAGATGCCCTTCT	5	61
912769	968	983	16088	16103	ATCCATCCCTTCTGAG	6	62
912770	986	1001	16106	16121	GGGCATGGCGACCTCA	0	63
912771	1004	1019	16124	16139	ACTCATGTTTGCCCAG	67	64
912782	1195	1210	19035	19050	AGCAAGTTGCAAATCT	71	75
912783	1199	1214	19039	19054	GGGTAGCAAGTTGCAA	37	76
912784	1205	1220	19045	19060	CCTAATGGGTAGCAAG	25	77
912785	1206	1221	19046	19061	TCCTAATGGGTAGCAA	64	78

Table 26

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
912786	1207	1222	19047	19062	ATCCTAATGGGTAGCA	65	79
912787	1211	1226	19051	19066	CATTATCCTAATGGGT	43	80
912788	1212	1227	19052	19067	ACATTATCCTAATGGG	0	81
912789	1213	1228	19053	19068	GACATTATCCTAATGG	59	82
912790	1220	1235	19060	19075	TACATAAGACATTATC	8	83
912791	1224	1239	19064	19079	GCATTACATAAGACAT	86	84
912792	1245	1260	19085	19100	CCACAGGCAGGGTACA	58	85
912793	1246	1261	19086	19101	TCCACAGGCAGGGTAC	5	86
912794	1253	1268	19093	19108	GGCAGATTCCACAGGC	68	87
912795	1259	1274	19099	19114	CGCAATGGCAGATTCC	84	88
912796	1265	1280	19105	19120	GACAATCGCAATGGCA	63	89
912797	1266	1281	19106	19121	GGACAATCGCAATGGC	54	90
912798	1267	1282	19107	19122	TGGACAATCGCAATGG	59	91
912799	1285	1300	23690	23705	AGCCATGTCACCAGTC	51	92
912800	1289	1304	23694	23709	TGGAAGCCATGTCACC	32	93
912801	1290	1305	23695	23710	CTGGAAGCCATGTCAC	44	94
912802	1297	1312	23702	23717	GGCATATCTGGAAGCC	0	95
912803	1298	1313	23703	23718	GGGCATATCTGGAAGC	0	96
912804	1351	1366	23756	23771	AGCACTCGAGTGAACA	6	97
912805	1386	1401	N/A	N/A	GCATTTGGGACCTGGA	54	98
912806	1387	1402	N/A	N/A	GGCATTTGGGACCTGG	33	99
912807	1388	1403	25151	25166	TGGCATTTGGGACCTG	0	100
912808	1394	1409	25157	25172	GCTCACTGGCATTTGG	7	101
912809	1523	1538	25286	25301	GTTCAGGCTGGACCTG	17	102
912810	1547	1562	25310	25325	AGGTACTTTATTGCCC	30	103
912811	1550	1565	25313	25328	AGCAGGTACTTTATTG	55	104
912812	1653	1668	25416	25431	AACTTTAGCACCTCTG	87	105
912813	1655	1670	25418	25433	GAAACTTTAGCACCTC	85	106
912814	1656	1671	25419	25434	GGAAACTTTAGCACCT	26	107

912815	1669	1684	25432	25447	CTGCACAAAGATGGGA	66	108
912816	1671	1686	25434	25449	AGCTGCACAAAGATGG	41	109
912817	1685	1700	25448	25463	AGCAATGCGGAGGTAG	35	110
912818	1740	1755	25503	25518	ACCAACTCAGCTCAGA	76	111
912819	1741	1756	25504	25519	AACCAACTCAGCTCAG	77	112
912820	1757	1772	25520	25535	TCCTAGCTTTTCATAA	18	113
912821	1788	1803	25551	25566	TGCTGGACCGCTGCAC	1	114
912822	1796	1811	25559	25574	GAGTTAAGTGCTGGAC	90	115
912823	1802	1817	25565	25580	GTATTAGAGTTAAGTG	86	116
912824	1803	1818	25566	25581	TGTATTAGAGTTAAGT	79	117
912825	1806	1821	25569	25584	TGATGTATTAGAGTTA	89	118
912826	1808	1823	25571	25586	GCTGATGTATTAGAGT	79	119
912827	1821	1836	25584	25599	TGAATTAACGCATGCT	73	120
912828	1822	1837	25585	25600	CTGAATTAACGCATGC	69	121
912829	1870	1885	25633	25648	AGTAAGGGACCCTCTG	0	122
912830	1871	1886	25634	25649	CAGTAAGGGACCCTCT	44	123
912831	1872	1887	25635	25650	TCAGTAAGGGACCCTC	67	124
912832	1874	1889	25637	25652	AGTCAGTAAGGGACCC	50	125
912833	1893	1908	25656	25671	ATTAATAGGGCCACGA	78	126
912834	1895	1910	25658	25673	CCATTAATAGGGCCAC	72	127
912835	1896	1911	25659	25674	ACCATTAATAGGGCCA	65	128
912836	1906	1921	25669	25684	GAACAGTCTGACCATT	82	129
912837	1908	1923	25671	25686	TGGAACAGTCTGACCA	39	130
912838	1909	1924	25672	25687	CTGGAACAGTCTGACC	84	131
912839	1911	1926	25674	25689	TGCTGGAACAGTCTGA	72	132
912840	1916	1931	25679	25694	CCTCATGCTGGAACAG	84	133
912841	1928	1943	25691	25706	TCATTCTAAGAACCTC	87	134
912842	1945	1960	25708	25723	ACCCATCCAAACACCT	18	135
912843	1982	1997	25745	25760	ACACATGGGCCAGCCT	46	136
912844	1989	2004	25752	25767	CAAGATCACACATGGG	71	137
912845	2057	2072	25820	25835	GGGACGAACTGCACCC	0	138
912846	2098	2113	25861	25876	TATCATCTTTGCAGAC	68	139
912847	2116	2131	25879	25894	GTTTTTAGTAGTCAAG	90	140
912848	2117	2132	25880	25895	CGTTTTTAGTAGTCAA	94	141

912849	2145	2160	25908	25923	TATCATCTTGTTACCC	87	142
912850	2148	2163	25911	25926	GATTATCATCTTGTTA	60	143
912851	2150	2165	25913	25928	TAGATTATCATCTTGT	50	144
912852	2151	2166	25914	25929	GTAGATTATCATCTTG	72	145
912853	2152	2167	25915	25930	AGTAGATTATCATCTT	79	146
912854	2175	2190	25938	25953	GTGAAAAAGGTGTTCT	64	147
912855	2182	2197	25945	25960	TAGTTAGGTGAAAAAG	77	148
912856	2188	2203	25951	25966	TTATTTTAGTTAGGTG	82	149
912857	2190	2205	25953	25968	CATTATTTTAGTTAGG	77	150
912858	2273	2288	26036	26051	CTACTAACATCTCACT	48	151
912859	2274	2289	26037	26052	TCTACTAACATCTCAC	91	152
912860	2278	2293	26041	26056	TTATTCTACTAACATC	37	153
912861	2280	2295	26043	26058	GCTTATTCTACTAACA	77	154
912862	2281	2296	26044	26059	GGCTTATTCTACTAAC	70	155
912863	2632	2647	26395	26410	GGTGAATGCCCTGCAC	42	156

Study 2

Cultured A431 cells at a density of 5,000 cells per well were transfected by free uptake with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and PNPLA3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS36070 was used to measure mRNA levels. PNPLA3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PNPLA3, relative to untreated control cells.

Table 27

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	97	702
959270	413	428	5987	6002	ATGGAAGATGCCAATG	32	1688

959280	491	506	6065	6080	GGAGATGAGCTGGTGG	66	1689
959290	793	808	12011	12026	AGACTGAGCTTGGTGA	78	1690
959300	899	914	13635	13650	CCTGAATGCATCCAAA	69	1691
959310	1084	1099	16204	16219	ATGCTGAGACGCAGGT	0	1692
959320	1256	1271	19096	19111	AATGGCAGATTCCACA	25	1693
959330	1642	1657	25405	25420	CTCTGAAAGAATCTGC	75	1694
959340	1659	1674	25422	25437	ATGGGAAACTTTAGCA	77	1695
959350	1839	1854	25602	25617	TGTCATTTCCCAACCA	79	1696
959360	2114	2129	25877	25892	TTTTAGTAGTCAAGGT	88	1697
959370	2223	2238	25986	26001	GCTTCCTTACATTTTT	85	1698
959380	2269	2284	26032	26047	TAACATCTCACTGATT	42	1699
959390	N/A	N/A	4311	4326	CTAGTGAGAAACAAAC	0	1700
959400	N/A	N/A	4761	4776	TTATTGTTGCTAAACC	32	1701
959410	N/A	N/A	4863	4878	ACTTTAGGCTCCTGGG	60	1702
959420	N/A	N/A	5285	5300	AGCCATAAATCTTGGG	24	1703
959430	N/A	N/A	5573	5588	ATGACATCATGGCTTC	93	1704
959440	N/A	N/A	5603	5618	TTATTCAATGTGGCTT	95	1705
959450	N/A	N/A	5640	5655	GGGCTCAATGAAATTA	12	1706
959460	N/A	N/A	5713	5728	CTTAGTATGAAATATC	86	1707
959470	N/A	N/A	5808	5823	TACTGTCTACTATGGG	91	1708
959480	N/A	N/A	6157	6172	CTTACATCCACGACTT	35	1709
959660	N/A	N/A	12153	12168	CAGTAACTGGTAGCTC	74	1710
959670	N/A	N/A	12169	12184	TGTTTGATTGTGCAGA	95	1711
959680	N/A	N/A	12210	12225	CGCCTTTTATTTCCGT	92	1712
959690	N/A	N/A	12313	12328	ATAACCACAGCCTGGG	66	1713
959700	N/A	N/A	12675	12690	ATAAGAATCATCTTAG	7	1714
959710	N/A	N/A	12711	12726	ACTACCGAACGCAGTT	41	1715
959720	N/A	N/A	12757	12772	TAGAGTGGTAAGGCAT	84	1716
959730	N/A	N/A	12793	12808	GGTTGGTGTACATGGT	96	1717
959740	N/A	N/A	12880	12895	TCCTGTTAGACAGCTT	93	1718
959750	N/A	N/A	12902	12917	ATAAAGCACACGGGAA	86	1719
959760	N/A	N/A	12931	12946	TAAGAGCTGTCTCCTC	85	1720
959770	N/A	N/A	12972	12987	CTAACAAACTTTGCAG	79	1721
959780	N/A	N/A	13392	13407	TGTCACCCTTCCACGG	15	1722

959790	N/A	N/A	13526	13541	ATTGGAAGACCGCAGA	43	1723
959800	N/A	N/A	13706	13721	CCGCTAGTAAATGCTT	44	1724
959810	N/A	N/A	13737	13752	AACTAAGGCAAATCTC	77	1725
959820	N/A	N/A	13915	13930	GAGTCATGACATCCCA	89	1726
959830	N/A	N/A	14299	14314	GCAGATAAATACACAT	93	1727
959840	N/A	N/A	14424	14439	TTTCCCATCGACACAG	78	1728
959850	N/A	N/A	14571	14586	GCTCCTACTTATCCCC	76	1729
959860	N/A	N/A	15202	15217	TATTGCCAGGTATCTG	64	1730
959870	N/A	N/A	15599	15614	CAATACATAGCAGAGC	23	1731
959880	N/A	N/A	17192	17207	TTAGTCCGTGTTCAGG	90	1732
959890	N/A	N/A	17222	17237	GTAGCTGGTTTGTGGG	20	1733
959900	N/A	N/A	17295	17310	CATCTCTTAGGGCACC	79	1734
959910	N/A	N/A	18393	18408	GTTTGGAAGTCGCCAT	77	1735
959920	N/A	N/A	20284	20299	AGGAAGCCCAATCAAG	85	1736
959930	N/A	N/A	20512	20527	CAGATTGAGTCTCCTG	10	1737
959940	N/A	N/A	20607	20622	TTTTACGATCATCATT	72	1738
959950	N/A	N/A	20661	20676	GCTTAGAAATTGCAGT	75	1739
959960	N/A	N/A	20812	20827	AGGGTAATATTCAGAC	86	1740
959970	N/A	N/A	20934	20949	TGTAGCAGACAGATCA	74	1741
959980	N/A	N/A	21000	21015	TTTAACAGCTCAGGTA	66	1742
959990	N/A	N/A	21405	21420	ATTCTAGACATGGCCA	51	1743
960000	N/A	N/A	21442	21457	AACCAATATACTGAGA	71	1744
960010	N/A	N/A	21545	21560	AGACATATGACATTTC	91	1745
960020	N/A	N/A	22765	22780	ACATGACAGACTAACT	55	1746
960030	N/A	N/A	24039	24054	CATCAATGCTGCACTC	13	1747

Table 28

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702

959271	425	440	5999	6014	GTTGAAGGATGGATGG	90	1748
959281	511	526	6085	6100	AGAGAGATGCCTATTT	73	1749
959291	813	828	12031	12046	GGTTCCCTGTGCAGAG	79	1750
959301	904	919	13640	13655	AAGAACCTGAATGCAT	48	1751
959311	1085	1100	16205	16220	GATGCTGAGACGCAGG	0	1752
959321	1602	1617	25365	25380	ACAGACTCTTCTCTAG	45	1753
959331	1643	1658	25406	25421	CCTCTGAAAGAATCTG	81	1754
959341	1673	1688	25436	25451	GTAGCTGCACAAAGAT	69	1755
959351	1842	1857	25605	25620	TGGTGTCATTTCCCAA	19	1756
959361	2115	2130	25878	25893	TTTTTAGTAGTCAAGG	91	1757
959371	2240	2255	26003	26018	ATTCAACAGGTAACAA	69	1758
959381	2271	2286	26034	26049	ACTAACATCTCACTGA	33	1759
959391	N/A	N/A	4313	4328	AGCTAGTGAGAAACAA	47	1760
959401	N/A	N/A	4764	4779	CTTTTATTGTTGCTAA	77	1761
959411	N/A	N/A	4868	4883	AGTGTACTTTAGGCTC	90	1762
959421	N/A	N/A	5286	5301	CAGCCATAAATCTTGG	0	1763
959431	N/A	N/A	5574	5589	AATGACATCATGGCTT	74	1764
959441	N/A	N/A	5604	5619	TTTATTCAATGTGGCT	96	1765
959451	N/A	N/A	5642	5657	TTGGGCTCAATGAAAT	0	1766
959461	N/A	N/A	5714	5729	GCTTAGTATGAAATAT	78	1767
959471	N/A	N/A	5809	5824	GTACTGTCTACTATGG	78	1768
959481	N/A	N/A	6160	6175	CTGCTTACATCCACGA	4	1769
959661	N/A	N/A	12154	12169	ACAGTAACTGGTAGCT	72	1770
959671	N/A	N/A	12170	12185	CTGTTTGATTGTGCAG	50	1771
959681	N/A	N/A	12211	12226	GCGCCTTTTATTTCCG	14	1772
959691	N/A	N/A	12322	12337	CCTGACTATATAACCA	56	1773
959701	N/A	N/A	12689	12704	GACCGTGTTTCCAAAT	97	1774
959711	N/A	N/A	12712	12727	AACTACCGAACGCAGT	48	1775
959721	N/A	N/A	12759	12774	GGTAGAGTGGTAAGGC	95	1776
959731	N/A	N/A	12828	12843	CATAGCCTTCTTTCTT	93	1777
959741	N/A	N/A	12882	12897	AATCCTGTTAGACAGC	90	1778
959751	N/A	N/A	12903	12918	TATAAAGCACACGGGA	82	1779
959761	N/A	N/A	12933	12948	AATAAGAGCTGTCTCC	87	1780
959771	N/A	N/A	12974	12989	CCCTAACAAACTTTGC	62	1781

959781	N/A	N/A	13394	13409	AATGTCACCCTTCCAC	90	1782
959791	N/A	N/A	13527	13542	CATTGGAAGACCGCAG	76	1783
959801	N/A	N/A	13707	13722	ACCGCTAGTAAATGCT	35	1784
959811	N/A	N/A	13740	13755	TAGAACTAAGGCAAAT	65	1785
959821	N/A	N/A	13916	13931	GGAGTCATGACATCCC	42	1786
959831	N/A	N/A	14302	14317	TGAGCAGATAAATACA	75	1787
959841	N/A	N/A	14425	14440	CTTTCCCATCGACACA	79	1788
959851	N/A	N/A	14573	14588	GCGCTCCTACTTATCC	0	1789
959861	N/A	N/A	15203	15218	ATATTGCCAGGTATCT	67	1790
959871	N/A	N/A	15763	15778	GTGTTGGTTTATAACA	13	1791
959881	N/A	N/A	17194	17209	ACTTAGTCCGTGTTCA	45	1792
959891	N/A	N/A	17224	17239	CTGTAGCTGGTTTGTG	42	1793
959901	N/A	N/A	17296	17311	CCATCTCTTAGGGCAC	53	1794
959911	N/A	N/A	18394	18409	TGTTTGGAAGTCGCCA	87	1795
959921	N/A	N/A	20289	20304	ATCAGAGGAAGCCCAA	84	1796
959931	N/A	N/A	20514	20529	ACCAGATTGAGTCTCC	91	1797
959941	N/A	N/A	20613	20628	CTCTAATTTTACGATC	70	1798
959951	N/A	N/A	20662	20677	AGCTTAGAAATTGCAG	0	1799
959961	N/A	N/A	20813	20828	CAGGGTAATATTCAGA	87	1800
959971	N/A	N/A	20936	20951	TTTGTAGCAGACAGAT	18	1801
959981	N/A	N/A	21001	21016	TTTTAACAGCTCAGGT	71	1802
959991	N/A	N/A	21406	21421	AATTCTAGACATGGCC	25	1803
960001	N/A	N/A	21443	21458	TAACCAATATACTGAG	72	1804
960011	N/A	N/A	22023	22038	CGCAAAAAGACAACGA	16	1805
960021	N/A	N/A	22766	22781	GACATGACAGACTAAC	76	1806
960031	N/A	N/A	24040	24055	CCATCAATGCTGCACT	61	1807

Table 29

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

915609	705	720	11923	11938	TGATGGTTGTTTTGGC	97	702
959272	426	441	6000	6015	AGTTGAAGGATGGATG	76	1808
959282	517	532	6091	6106	CTGGTAAGAGAGATGC	64	1809
959292	815	830	12033	12048	GAGGTTCCCTGTGCAG	81	1810
959302	905	920	13641	13656	CAAGAACCTGAATGCA	65	1811
959312	1087	1102	16207	16222	AGGATGCTGAGACGCA	66	1812
959322	1604	1619	25367	25382	TCACAGACTCTTCTCT	50	1813
959332	1644	1659	25407	25422	ACCTCTGAAAGAATCT	69	1814
959342	1675	1690	25438	25453	AGGTAGCTGCACAAAG	78	1815
959352	1903	1918	25666	25681	CAGTCTGACCATTAAT	77	1816
959362	2173	2188	25936	25951	GAAAAAGGTGTTCTAA	85	1817
959372	2242	2257	26005	26020	AAATTCAACAGGTAAC	62	1818
959382	2275	2290	26038	26053	TTCTACTAACATCTCA	80	1819
959392	N/A	N/A	4732	4747	ATGATCATGTGGCGGT	80	1820
959402	N/A	N/A	4765	4780	ACTTTTATTGTTGCTA	86	1821
959412	N/A	N/A	4869	4884	GAGTGTACTTTAGGCT	94	1822
959422	N/A	N/A	5389	5404	CTATCAGGTGCAGGAG	93	1823
959432	N/A	N/A	5575	5590	CAATGACATCATGGCT	90	1824
959442	N/A	N/A	5607	5622	TACTTTATTCAATGTG	0	1825
959452	N/A	N/A	5643	5658	ATTGGGCTCAATGAAA	35	1826
959462	N/A	N/A	5716	5731	TGGCTTAGTATGAAAT	80	1827
959472	N/A	N/A	5864	5879	TTTGGCAAGGCCAGAA	0	1828
959482	N/A	N/A	6960	6975	GCATAGAGGAAGCTCG	32	1829
959662	N/A	N/A	12155	12170	GACAGTAACTGGTAGC	92	1830
959672	N/A	N/A	12172	12187	TTCTGTTTGATTGTGC	97	1831
959682	N/A	N/A	12280	12295	AGGTCTGGGTATATGT	93	1832
959692	N/A	N/A	12323	12338	CCCTGACTATATAACC	32	1833
959702	N/A	N/A	12691	12706	TTGACCGTGTTTCCAA	94	1834
959712	N/A	N/A	12713	12728	AAACTACCGAACGCAG	92	1835
959722	N/A	N/A	12760	12775	TGGTAGAGTGGTAAGG	94	1836
959732	N/A	N/A	12829	12844	GCATAGCCTTCTTTCT	87	1837
959742	N/A	N/A	12883	12898	CAATCCTGTTAGACAG	13	1838
959752	N/A	N/A	12904	12919	TTATAAAGCACACGGG	87	1839
959762	N/A	N/A	12934	12949	CAATAAGAGCTGTCTC	81	1840

959772	N/A	N/A	13370	13385	TGCAGGCACCCCAGCA	0	1841
959782	N/A	N/A	13395	13410	GAATGTCACCCTTCCA	90	1842
959792	N/A	N/A	13528	13543	TCATTGGAAGACCGCA	84	1843
959802	N/A	N/A	13708	13723	GACCGCTAGTAAATGC	57	1844
959812	N/A	N/A	13741	13756	TTAGAACTAAGGCAAA	78	1845
959822	N/A	N/A	13917	13932	TGGAGTCATGACATCC	0	1846
959832	N/A	N/A	14303	14318	CTGAGCAGATAAATAC	74	1847
959842	N/A	N/A	14553	14568	AGTCTTAATGTGGATT	76	1848
959852	N/A	N/A	14667	14682	AGTGTCCCCATCCCCA	54	1849
959862	N/A	N/A	15204	15219	AATATTGCCAGGTATC	56	1850
959872	N/A	N/A	15765	15780	TAGTGTTGGTTTATAA	89	1851
959882	N/A	N/A	17196	17211	TGACTTAGTCCGTGTT	43	1852
959892	N/A	N/A	17225	17240	TCTGTAGCTGGTTTGT	61	1853
959902	N/A	N/A	17298	17313	TCCCATCTCTTAGGGC	24	1854
959912	N/A	N/A	18396	18411	TATGTTTGGAAGTCGC	91	1855
959922	N/A	N/A	20290	20305	AATCAGAGGAAGCCCA	40	1856
959932	N/A	N/A	20515	20530	AACCAGATTGAGTCTC	72	1857
959942	N/A	N/A	20614	20629	TCTCTAATTTTACGAT	23	1858
959952	N/A	N/A	20663	20678	CAGCTTAGAAATTGCA	24	1859
959962	N/A	N/A	20814	20829	CCAGGGTAATATTCAG	87	1860
959972	N/A	N/A	20937	20952	CTTTGTAGCAGACAGA	50	1861
959982	N/A	N/A	21003	21018	TATTTTAACAGCTCAG	94	1862
959992	N/A	N/A	21409	21424	TGCAATTCTAGACATG	12	1863
960002	N/A	N/A	21445	21460	ACTAACCAATATACTG	55	1864
960012	N/A	N/A	22541	22556	CAACAGATTACTGGAC	28	1865
960022	N/A	N/A	22768	22783	AGGACATGACAGACTA	66	1866
960032	N/A	N/A	24041	24056	ACCATCAATGCTGCAC	79	1867

Table 30

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 2	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO

	Start Site	Stop Site	Start Site	Stop Site			
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702
959273	427	442	6001	6016	AAGTTGAAGGATGGAT	86	1868
959283	694	709	11912	11927	TTGGCATCAATGAAGG	73	1869
959293	816	831	12034	12049	AGAGGTTCCCTGTGCA	80	1870
959303	906	921	13642	13657	CCAAGAACCTGAATGC	65	1871
959313	1090	1105	16210	16225	GGCAGGATGCTGAGAC	43	1872
959323	1605	1620	25368	25383	CTCACAGACTCTTCTC	81	1873
959333	1646	1661	25409	25424	GCACCTCTGAAAGAAT	51	1874
959343	1677	1692	25440	25455	GGAGGTAGCTGCACAA	73	1875
959353	1904	1919	25667	25682	ACAGTCTGACCATTAA	85	1876
959363	2179	2194	25942	25957	TTAGGTGAAAAAGGTG	91	1877
959373	2258	2273	26021	26036	TGATTCACATAATACA	71	1878
959383	2277	2292	26040	26055	TATTCTACTAACATCT	38	1879
959393	N/A	N/A	4733	4748	AATGATCATGTGGCGG	93	1880
959403	N/A	N/A	4767	4782	TGACTTTTATTGTTGC	87	1881
959413	N/A	N/A	4870	4885	TGAGTGTACTTTAGGC	94	1882
959423	N/A	N/A	5392	5407	ATGCTATCAGGTGCAG	0	1883
959433	N/A	N/A	5578	5593	GCACAATGACATCATG	86	1884
959443	N/A	N/A	5608	5623	TTACTTTATTCAATGT	9	1885
959453	N/A	N/A	5644	5659	TATTGGGCTCAATGAA	80	1886
959463	N/A	N/A	5798	5813	TATGGGAGCCACATGT	4	1887
959473	N/A	N/A	5866	5881	CTTTTGGCAAGGCCAG	0	1888
959483	N/A	N/A	7199	7214	TTAAACAGAGGATGCA	31	1889
959663	N/A	N/A	12156	12171	AGACAGTAACTGGTAG	75	1890
959673	N/A	N/A	12199	12214	TCCGTTAACCATCAAG	95	1891
959683	N/A	N/A	12282	12297	TTAGGTCTGGGTATAT	94	1892
959693	N/A	N/A	12324	12339	CCCCTGACTATATAAC	0	1893
959703	N/A	N/A	12692	12707	CTTGACCGTGTTTCCA	97	1894
959713	N/A	N/A	12715	12730	TTAAACTACCGAACGC	95	1895
959723	N/A	N/A	12761	12776	ATGGTAGAGTGGTAAG	77	1896
959733	N/A	N/A	12832	12847	ATTGCATAGCCTTCTT	95	1897

959743	N/A	N/A	12884	12899	CCAATCCTGTTAGACA	83	1898
959753	N/A	N/A	12908	12923	CTGCTTATAAAGCACA	2	1899
959763	N/A	N/A	12935	12950	ACAATAAGAGCTGTCT	85	1900
959773	N/A	N/A	13372	13387	TTTGCAGGCACCCCAG	63	1901
959783	N/A	N/A	13396	13411	TGAATGTCACCCTTCC	53	1902
959793	N/A	N/A	13531	13546	GCATCATTGGAAGACC	86	1903
959803	N/A	N/A	13713	13728	ACCAAGACCGCTAGTA	38	1904
959813	N/A	N/A	13743	13758	TGTTAGAACTAAGGCA	79	1905
959823	N/A	N/A	13919	13934	CCTGGAGTCATGACAT	7	1906
959833	N/A	N/A	14304	14319	TCTGAGCAGATAAATA	39	1907
959843	N/A	N/A	14554	14569	AAGTCTTAATGTGGAT	84	1908
959853	N/A	N/A	14669	14684	TTAGTGTCCCCATCCC	78	1909
959863	N/A	N/A	15205	15220	GAATATTGCCAGGTAT	86	1910
959873	N/A	N/A	15766	15781	TTAGTGTTGGTTTATA	92	1911
959883	N/A	N/A	17198	17213	TTTGACTTAGTCCGTG	86	1912
959893	N/A	N/A	17226	17241	CTCTGTAGCTGGTTTG	82	1913
959903	N/A	N/A	17601	17616	TTGATAGTGAATGTGT	83	1914
959913	N/A	N/A	18397	18412	ATATGTTTGGAAGTCG	91	1915
959923	N/A	N/A	20291	20306	CAATCAGAGGAAGCCC	34	1916
959933	N/A	N/A	20516	20531	TAACCAGATTGAGTCT	66	1917
959943	N/A	N/A	20615	20630	GTCTCTAATTTTACGA	53	1918
959953	N/A	N/A	20664	20679	ACAGCTTAGAAATTGC	89	1919
959963	N/A	N/A	20843	20858	CTGTATTAGCTCAATA	67	1920
959973	N/A	N/A	20938	20953	TCTTTGTAGCAGACAG	84	1921
959983	N/A	N/A	21004	21019	TTATTTTAACAGCTCA	92	1922
959993	N/A	N/A	21410	21425	CTGCAATTCTAGACAT	29	1923
960003	N/A	N/A	21535	21550	CATTTCAGAGTATAAG	46	1924
960013	N/A	N/A	22708	22723	GATGTGAGTGAAATAA	69	1925
960023	N/A	N/A	22769	22784	AAGGACATGACAGACT	68	1926
960033	N/A	N/A	24043	24058	CCACCATCAATGCTGC	58	1927

Table 31

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	99	702
959284	695	710	11913	11928	TTTGGCATCAATGAAG	69	1928
959294	817	832	12035	12050	TAGAGGTTCCCTGTGC	83	1929
959314	1091	1106	16211	16226	GGGCAGGATGCTGAGA	22	1930
959324	1606	1621	25369	25384	ACTCACAGACTCTTCT	68	1931
959334	1647	1662	25410	25425	AGCACCTCTGAAAGAA	67	1932
959344	1678	1693	25441	25456	CGGAGGTAGCTGCACA	85	1933
959354	1926	1941	25689	25704	ATTCTAAGAACCTCAT	51	1934
959384	N/A	N/A	4303	4318	AAACAAACCCTCCGTC	10	1935
959394	N/A	N/A	4734	4749	AAATGATCATGTGGCG	94	1936
959404	N/A	N/A	4768	4783	CTGACTTTTATTGTTG	74	1937
959414	N/A	N/A	4871	4886	GTGAGTGTACTTTAGG	98	1938
959424	N/A	N/A	5393	5408	AATGCTATCAGGTGCA	31	1939
959434	N/A	N/A	5579	5594	TGCACAATGACATCAT	88	1940
959444	N/A	N/A	5621	5636	CTACCTGTGTCTTTTA	90	1941
959454	N/A	N/A	5647	5662	ATATATTGGGCTCAAT	81	1942
959474	N/A	N/A	5867	5882	ACTTTTGGCAAGGCCA	17	1943
959484	N/A	N/A	7211	7226	CCGCAAACAAGGTTAA	22	1944
959684	N/A	N/A	12283	12298	TTTAGGTCTGGGTATA	93	1945
959694	N/A	N/A	12325	12340	CCCCCTGACTATATAA	18	1946
959704	N/A	N/A	12693	12708	TCTTGACCGTGTTTCC	97	1947
959734	N/A	N/A	12834	12849	GCATTGCATAGCCTTC	96	1948
959744	N/A	N/A	12886	12901	AACCAATCCTGTTAGA	59	1949
959754	N/A	N/A	12909	12924	TCTGCTTATAAAGCAC	100	1950
959774	N/A	N/A	13373	13388	CTTTGCAGGCACCCCA	72	1951
959784	N/A	N/A	13398	13413	CTTGAATGTCACCCTT	92	1952
959804	N/A	N/A	13715	13730	TTACCAAGACCGCTAG	47	1953
959824	N/A	N/A	14228	14243	ACTTTTAGTATTAAAG	0	1954
959844	N/A	N/A	14555	14570	AAAGTCTTAATGTGGA	88	1955

959854	N/A	N/A	14670	14685	CTTAGTGTCCCCATCC	76	1956
959874	N/A	N/A	15767	15782	GTTAGTGTTGGTTTAT	95	1957
959884	N/A	N/A	17199	17214	GTTTGACTTAGTCCGT	96	1958
959914	N/A	N/A	18398	18413	AATATGTTTGGAAGTC	87	1959
959934	N/A	N/A	20518	20533	AGTAACCAGATTGAGT	96	1960
959954	N/A	N/A	20665	20680	CACAGCTTAGAAATTG	88	1961
959964	N/A	N/A	20844	20859	CCTGTATTAGCTCAAT	92	1962
959974	N/A	N/A	20940	20955	CCTCTTTGTAGCAGAC	84	1963
959984	N/A	N/A	21006	21021	GGTTATTTTAACAGCT	85	1964
959994	N/A	N/A	21412	21427	ACCTGCAATTCTAGAC	61	1965
960014	N/A	N/A	22710	22725	AAGATGTGAGTGAAAT	70	1966
960024	N/A	N/A	22770	22785	AAAGGACATGACAGAC	87	1967

Table 32

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	**SEQ ID NO: 1 Start Site**	**SEQ ID NO: 1 Stop Site**	**SEQ ID NO: 2 Start Site**	**SEQ ID NO: 2 Stop Site**	**Sequence (5' to 3')**	**PNPLA3 % Inhibition**	**SEQ ID NO**
959275	440	455	6014	6029	GAGGAACTTGCTTAAG	81	1968
959285	696	711	11914	11929	TTTTGGCATCAATGAA	62	1969
959305	1066	1081	16186	16201	TCTAGCAGCTCATCTC	64	1970
959335	1649	1664	25412	25427	TTAGCACCTCTGAAAG	72	1971
959345	1804	1819	25567	25582	ATGTATTAGAGTTAAG	77	1972
959355	1927	1942	25690	25705	CATTCTAAGAACCTCA	71	1973
959365	2183	2198	25946	25961	TTAGTTAGGTGAAAAA	81	1974
959375	2261	2276	26024	26039	CACTGATTCACATAAT	70	1975
959395	N/A	N/A	4737	4752	TGCAAATGATCATGTG	66	1976
959405	N/A	N/A	4769	4784	GCTGACTTTTATTGTT	84	1977
959415	N/A	N/A	4872	4887	AGTGAGTGTACTTTAG	94	1978
959425	N/A	N/A	5395	5410	TTAATGCTATCAGGTG	81	1979
959435	N/A	N/A	5580	5595	ATGCACAATGACATCA	86	1980
959445	N/A	N/A	5624	5639	ATTCTACCTGTGTCTT	97	1981

959455	N/A	N/A	5651	5666	TTGGATATATTGGGCT	97	1982
959475	N/A	N/A	5868	5883	TACTTTTGGCAAGGCC	70	1983
959485	N/A	N/A	7697	7712	GCACAGAGTAGGTTAA	72	1984
959655	N/A	N/A	12146	12161	TGGTAGCTCCTGGCAA	55	1985
959675	N/A	N/A	12201	12216	TTTCCGTTAACCATCA	94	1986
959695	N/A	N/A	12667	12682	CATCTTAGTGGCTGGG	93	1987
959705	N/A	N/A	12695	12710	GTTCTTGACCGTGTTT	97	1988
959715	N/A	N/A	12717	12732	GGTTAAACTACCGAAC	11	1989
959725	N/A	N/A	12783	12798	CATGGTCTGCAAATTT	89	1990
959745	N/A	N/A	12887	12902	AAACCAATCCTGTTAG	46	1991
959755	N/A	N/A	12910	12925	ATCTGCTTATAAAGCA	42	1992
959775	N/A	N/A	13374	13389	ACTTTGCAGGCACCCC	87	1993
959785	N/A	N/A	13399	13414	GCTTGAATGTCACCCT	94	1994
959805	N/A	N/A	13716	13731	TTTACCAAGACCGCTA	86	1995
959825	N/A	N/A	14230	14245	CAACTTTTAGTATTAA	0	1996
959835	N/A	N/A	14417	14432	TCGACACAGCATCACC	62	1997
959855	N/A	N/A	14671	14686	TCTTAGTGTCCCCATC	78	1998
959865	N/A	N/A	15209	15224	TTAGGAATATTGCCAG	93	1999
959875	N/A	N/A	15769	15784	GGGTTAGTGTTGGTTT	94	2000
959895	N/A	N/A	17288	17303	TAGGGCACCTCAAGAA	0	2001
959915	N/A	N/A	18400	18415	CAAATATGTTTGGAAG	50	2002
959925	N/A	N/A	20293	20308	CCCAATCAGAGGAAGC	41	2003
959935	N/A	N/A	20599	20614	TCATCATTATTACCTG	91	2004
959955	N/A	N/A	20803	20818	TTCAGACCAGGGTAAT	91	2005
959965	N/A	N/A	20845	20860	GCCTGTATTAGCTCAA	90	2006
959975	N/A	N/A	20941	20956	GCCTCTTTGTAGCAGA	0	2007
959995	N/A	N/A	21434	21449	TACTGAGAGGAAATGA	64	2008
960015	N/A	N/A	22714	22729	TGTAAAGATGTGAGTG	78	2009
960025	N/A	N/A	22772	22787	TCAAAGGACATGACAG	80	2010
960037	N/A	N/A	22590	22605	GAGCAACGAGGAAGGA	68	2011

Table 33

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702
959276	447	462	6021	6036	CCTGTCGGAGGAACTT	37	2012
959286	699	714	11917	11932	TTGTTTTGGCATCAAT	73	2013
959296	895	910	13631	13646	AATGCATCCAAATATC	0	2014
959306	1069	1084	16189	16204	TGGTCTAGCAGCTCAT	25	2015
959316	1221	1236	19061	19076	TTACATAAGACATTAT	35	2016
959326	1614	1629	25377	25392	CTCAAGTGACTCACAG	85	2017
959336	1650	1665	25413	25428	TTTAGCACCTCTGAAA	24	2018
959346	1834	1849	25597	25612	TTTCCCAACCAGCTGA	60	2019
959356	2096	2111	25859	25874	TCATCTTTGCAGACCA	90	2020
959366	2184	2199	25947	25962	TTTAGTTAGGTGAAAA	57	2021
959376	2262	2277	26025	26040	TCACTGATTCACATAA	86	2022
959386	N/A	N/A	4306	4321	GAGAAACAAACCCTCC	0	2023
959396	N/A	N/A	4738	4753	GTGCAAATGATCATGT	69	2024
959406	N/A	N/A	4771	4786	AAGCTGACTTTTATTG	57	2025
959416	N/A	N/A	5276	5291	TCTTGGGATGCACAGG	49	2026
959426	N/A	N/A	5397	5412	CCTTAATGCTATCAGG	0	2027
959436	N/A	N/A	5581	5596	AATGCACAATGACATC	69	2028
959446	N/A	N/A	5625	5640	AATTCTACCTGTGTCT	82	2029
959456	N/A	N/A	5652	5667	TTTGGATATATTGGGC	95	2030
959466	N/A	N/A	5802	5817	CTACTATGGGAGCCAC	64	2031
959476	N/A	N/A	5871	5886	TAATACTTTTGGCAAG	29	2032
959486	N/A	N/A	7784	7799	TTATAGGCGAGAGCAC	0	2033
959656	N/A	N/A	12147	12162	CTGGTAGCTCCTGGCA	44	2034
959666	N/A	N/A	12164	12179	GATTGTGCAGACAGTA	95	2035
959676	N/A	N/A	12202	12217	ATTTCCGTTAACCATC	93	2036
959686	N/A	N/A	12288	12303	TGAGTTTTAGGTCTGG	96	2037
959696	N/A	N/A	12669	12684	ATCATCTTAGTGGCTG	91	2038
959706	N/A	N/A	12696	12711	TGTTCTTGACCGTGTT	98	2039

959716	N/A	N/A	12719	12734	AAGGTTAAACTACCGA	6	2040
959726	N/A	N/A	12785	12800	TACATGGTCTGCAAAT	90	2041
959736	N/A	N/A	12838	12853	CATTGCATTGCATAGC	96	2042
959746	N/A	N/A	12888	12903	AAAACCAATCCTGTTA	47	2043
959756	N/A	N/A	12911	12926	CATCTGCTTATAAAGC	81	2044
959766	N/A	N/A	12967	12982	AAACTTTGCAGCCTAT	93	2045
959776	N/A	N/A	13376	13391	AGACTTTGCAGGCACC	90	2046
959786	N/A	N/A	13400	13415	GGCTTGAATGTCACCC	69	2047
959796	N/A	N/A	13697	13712	AATGCTTGTCAAAAGG	70	2048
959806	N/A	N/A	13717	13732	CTTTACCAAGACCGCT	82	2049
959816	N/A	N/A	13747	13762	TAAGTGTTAGAACTAA	30	2050
959826	N/A	N/A	14232	14247	ACCAACTTTTAGTATT	79	2051
959836	N/A	N/A	14419	14434	CATCGACACAGCATCA	59	2052
959846	N/A	N/A	14557	14572	CCAAAGTCTTAATGTG	53	2053
959856	N/A	N/A	14676	14691	CCATCTCTTAGTGTCC	88	2054
959866	N/A	N/A	15210	15225	CTTAGGAATATTGCCA	87	2055
959876	N/A	N/A	15770	15785	AGGGTTAGTGTTGGTT	89	2056
959886	N/A	N/A	17202	17217	TCTGTTTGACTTAGTC	70	2057
959896	N/A	N/A	17290	17305	CTTAGGGCACCTCAAG	30	2058
959906	N/A	N/A	17735	17750	TAATCTGGTCATATGG	43	2059
959916	N/A	N/A	18445	18460	TGCTTACGGAGCATAG	0	2060
959926	N/A	N/A	20472	20487	CTCTAGACGGGAAGCT	31	2061
959936	N/A	N/A	20601	20616	GATCATCATTATTACC	71	2062
959946	N/A	N/A	20652	20667	TTGCAGTGCCCTGGCC	31	2063
959956	N/A	N/A	20804	20819	ATTCAGACCAGGGTAA	87	2064
959966	N/A	N/A	20847	20862	ATGCCTGTATTAGCTC	65	2065
959976	N/A	N/A	20942	20957	AGCCTCTTTGTAGCAG	5	2066
959986	N/A	N/A	21008	21023	GAGGTTATTTTAACAG	69	2067
959996	N/A	N/A	21435	21450	ATACTGAGAGGAAATG	68	2068
960006	N/A	N/A	21539	21554	ATGACATTTCAGAGTA	88	2069
960016	N/A	N/A	22715	22730	GTGTAAAGATGTGAGT	84	2070
960026	N/A	N/A	24033	24048	TGCTGCACTCAAAGAG	0	2071
960038	N/A	N/A	19377	19392	TCACAAGAGACTGGAC	31	2072

Table 34

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702
959277	481	496	6055	6070	TGGTGGACATTGGCCG	5	2073
959287	707	722	11925	11940	GGTGATGGTTGTTTTG	85	2074
959297	896	911	13632	13647	GAATGCATCCAAATAT	0	2075
959307	1070	1085	16190	16205	GTGGTCTAGCAGCTCA	66	2076
959317	1223	1238	19063	19078	CATTACATAAGACATT	46	2077
959327	1615	1630	25378	25393	CCTCAAGTGACTCACA	83	2078
959337	1651	1666	25414	25429	CTTTAGCACCTCTGAA	71	2079
959347	1835	1850	25598	25613	ATTTCCCAACCAGCTG	49	2080
959357	2099	2114	25862	25877	TTATCATCTTTGCAGA	48	2081
959367	2185	2200	25948	25963	TTTTAGTTAGGTGAAA	43	2082
959377	2263	2278	26026	26041	CTCACTGATTCACATA	79	2083
959387	N/A	N/A	4307	4322	TGAGAAACAAACCCTC	0	2084
959397	N/A	N/A	4739	4754	TGTGCAAATGATCATG	82	2085
959407	N/A	N/A	4859	4874	TAGGCTCCTGGGACCT	0	2086
959417	N/A	N/A	5277	5292	ATCTTGGGATGCACAG	89	2087
959427	N/A	N/A	5567	5582	TCATGGCTTCCAGTGT	78	2088
959437	N/A	N/A	5600	5615	TTCAATGTGGCTTCTA	96	2089
959447	N/A	N/A	5627	5642	TTAATTCTACCTGTGT	72	2090
959457	N/A	N/A	5706	5721	TGAAATATCTCATTAG	77	2091
959467	N/A	N/A	5805	5820	TGTCTACTATGGGAGC	83	2092
959477	N/A	N/A	5875	5890	ATGGTAATACTTTTGG	75	2093
959657	N/A	N/A	12148	12163	ACTGGTAGCTCCTGGC	78	2094
959667	N/A	N/A	12165	12180	TGATTGTGCAGACAGT	97	2095
959677	N/A	N/A	12203	12218	TATTTCCGTTAACCAT	91	2096
959687	N/A	N/A	12289	12304	CTGAGTTTTAGGTCTG	96	2097
959697	N/A	N/A	12671	12686	GAATCATCTTAGTGGC	94	2098

959707	N/A	N/A	12697	12712	TTGTTCTTGACCGTGT	98	2099
959717	N/A	N/A	12753	12768	GTGGTAAGGCATACTA	35	2100
959727	N/A	N/A	12789	12804	GGTGTACATGGTCTGC	97	2101
959737	N/A	N/A	12839	12854	GCATTGCATTGCATAG	92	2102
959747	N/A	N/A	12890	12905	GGAAAACCAATCCTGT	69	2103
959757	N/A	N/A	12927	12942	AGCTGTCTCCTCTACT	70	2104
959767	N/A	N/A	12968	12983	CAAACTTTGCAGCCTA	95	2105
959777	N/A	N/A	13377	13392	GAGACTTTGCAGGCAC	88	2106
959787	N/A	N/A	13402	13417	TCGGCTTGAATGTCAC	67	2107
959797	N/A	N/A	13700	13715	GTAAATGCTTGTCAAA	91	2108
959807	N/A	N/A	13720	13735	AGTCTTTACCAAGACC	0	2109
959817	N/A	N/A	13911	13926	CATGACATCCCAGTTC	29	2110
959827	N/A	N/A	14233	14248	AACCAACTTTTAGTAT	27	2111
959837	N/A	N/A	14420	14435	CCATCGACACAGCATC	89	2112
959847	N/A	N/A	14567	14582	CTACTTATCCCCAAAG	16	2113
959857	N/A	N/A	14677	14692	GCCATCTCTTAGTGTC	36	2114
959867	N/A	N/A	15211	15226	CCTTAGGAATATTGCC	75	2115
959877	N/A	N/A	15771	15786	GAGGGTTAGTGTTGGT	93	2116
959887	N/A	N/A	17218	17233	CTGGTTTGTGGGTTCT	75	2117
959897	N/A	N/A	17291	17306	TCTTAGGGCACCTCAA	53	2118
959907	N/A	N/A	17736	17751	TTAATCTGGTCATATG	0	2119
959917	N/A	N/A	18852	18867	ACAAAAGCGACAAGGT	33	2120
959927	N/A	N/A	20508	20523	TTGAGTCTCCTGACCA	65	2121
959937	N/A	N/A	20602	20617	CGATCATCATTATTAC	87	2122
959947	N/A	N/A	20653	20668	ATTGCAGTGCCCTGGC	69	2123
959957	N/A	N/A	20805	20820	TATTCAGACCAGGGTA	89	2124
959967	N/A	N/A	20848	20863	GATGCCTGTATTAGCT	72	2125
959977	N/A	N/A	20944	20959	GCAGCCTCTTTGTAGC	0	2126
959987	N/A	N/A	21010	21025	CTGAGGTTATTTTAAC	40	2127
959997	N/A	N/A	21436	21451	TATACTGAGAGGAAAT	47	2128
960007	N/A	N/A	21541	21556	ATATGACATTTCAGAG	91	2129
960017	N/A	N/A	22716	22731	CGTGTAAAGATGTGAG	87	2130
960027	N/A	N/A	24035	24050	AATGCTGCACTCAAAG	31	2131
960039	N/A	N/A	20215	20230	TAACAAACTATGCCTA	44	2132

Table 35

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702
959274	439	454	6013	6028	AGGAACTTGCTTAAGT	73	2133
959284	695	710	11913	11928	TTTGGCATCAATGAAG	60	1928
959294	817	832	12035	12050	TAGAGGTTCCCTGTGC	77	1929
959304	1063	1078	16183	16198	AGCAGCTCATCTCCCT	62	2134
959314	1091	1106	16211	16226	GGGCAGGATGCTGAGA	13	1930
959324	1606	1621	25369	25384	ACTCACAGACTCTTCT	72	1931
959334	1647	1662	25410	25425	AGCACCTCTGAAAGAA	65	1932
959344	1678	1693	25441	25456	CGGAGGTAGCTGCACA	86	1933
959354	1926	1941	25689	25704	ATTCTAAGAACCTCAT	54	1934
959364	2181	2196	25944	25959	AGTTAGGTGAAAAAGG	92	2135
959374	2260	2275	26023	26038	ACTGATTCACATAATA	78	2136
959384	N/A	N/A	4303	4318	AAACAAACCCTCCGTC	2	1935
959394	N/A	N/A	4734	4749	AAATGATCATGTGGCG	94	1936
959404	N/A	N/A	4768	4783	CTGACTTTTATTGTTG	72	1937
959414	N/A	N/A	4871	4886	GTGAGTGTACTTTAGG	97	1938
959424	N/A	N/A	5393	5408	AATGCTATCAGGTGCA	31	1939
959434	N/A	N/A	5579	5594	TGCACAATGACATCAT	87	1940
959444	N/A	N/A	5621	5636	CTACCTGTGTCTTTTA	90	1941
959454	N/A	N/A	5647	5662	ATATATTGGGCTCAAT	80	1942
959464	N/A	N/A	5799	5814	CTATGGGAGCCACATG	24	2137
959474	N/A	N/A	5867	5882	ACTTTTGGCAAGGCCA	23	1943
959484	N/A	N/A	7211	7226	CCGCAAACAAGGTTAA	0	1944
959664	N/A	N/A	12157	12172	CAGACAGTAACTGGTA	96	2138
959674	N/A	N/A	12200	12215	TTCCGTTAACCATCAA	97	2139
959684	N/A	N/A	12283	12298	TTTAGGTCTGGGTATA	93	1945

959694	N/A	N/A	12325	12340	CCCCCTGACTATATAA	26	1946
959704	N/A	N/A	12693	12708	TCTTGACCGTGTTTCC	98	1947
959714	N/A	N/A	12716	12731	GTTAAACTACCGAACG	35	2140
959724	N/A	N/A	12763	12778	CTATGGTAGAGTGGTA	93	2141
959734	N/A	N/A	12834	12849	GCATTGCATAGCCTTC	97	1948
959744	N/A	N/A	12886	12901	AACCAATCCTGTTAGA	55	1949
959754	N/A	N/A	12909	12924	TCTGCTTATAAAGCAC	0	1950
959764	N/A	N/A	12937	12952	GGACAATAAGAGCTGT	91	2142
959774	N/A	N/A	13373	13388	CTTTGCAGGCACCCCA	72	1951
959784	N/A	N/A	13398	13413	CTTGAATGTCACCCTT	92	1952
959794	N/A	N/A	13532	13547	AGCATCATTGGAAGAC	92	2143
959804	N/A	N/A	13715	13730	TTACCAAGACCGCTAG	57	1953
959814	N/A	N/A	13744	13759	GTGTTAGAACTAAGGC	94	2144
959824	N/A	N/A	14228	14243	ACTTTTAGTATTAAAG	0	1954
959834	N/A	N/A	14306	14321	TTTCTGAGCAGATAAA	66	2145
959844	N/A	N/A	14555	14570	AAAGTCTTAATGTGGA	87	1955
959854	N/A	N/A	14670	14685	CTTAGTGTCCCCATCC	77	1956
959864	N/A	N/A	15208	15223	TAGGAATATTGCCAGG	89	2146
959874	N/A	N/A	15767	15782	GTTAGTGTTGGTTTAT	94	1957
959884	N/A	N/A	17199	17214	GTTTGACTTAGTCCGT	95	1958
959894	N/A	N/A	17228	17243	AACTCTGTAGCTGGTT	41	2147
959904	N/A	N/A	17603	17618	TCTTGATAGTGAATGT	73	2148
959914	N/A	N/A	18398	18413	AATATGTTTGGAAGTC	89	1959
959924	N/A	N/A	20292	20307	CCAATCAGAGGAAGCC	58	2149
959934	N/A	N/A	20518	20533	AGTAACCAGATTGAGT	81	1960
959944	N/A	N/A	20617	20632	CTGTCTCTAATTTTAC	75	2150
959954	N/A	N/A	20665	20680	CACAGCTTAGAAATTG	87	1961
959964	N/A	N/A	20844	20859	CCTGTATTAGCTCAAT	92	1962
959974	N/A	N/A	20940	20955	CCTCTTTGTAGCAGAC	83	1963
959984	N/A	N/A	21006	21021	GGTTATTTTAACAGCT	85	1964
959994	N/A	N/A	21412	21427	ACCTGCAATTCTAGAC	54	1965
960004	N/A	N/A	21537	21552	GACATTTCAGAGTATA	93	2151
960014	N/A	N/A	22710	22725	AAGATGTGAGTGAAAT	69	1966
960024	N/A	N/A	22770	22785	AAAGGACATGACAGAC	75	1967

960034	N/A	N/A	24613	24628	GCAAATCGGATCTTTG	32	2152

Table 36

Inhibition of PNPLA3 mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

Compound Number	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	PNPLA3 % Inhibition	SEQ ID NO
915609	705	720	11923	11938	TGATGGTTGTTTTGGC	98	702
959275	440	455	6014	6029	GAGGAACTTGCTTAAG	80	1968
959285	696	711	11914	11929	TTTTGGCATCAATGAA	63	1969
959295	823	838	12041	12056	AGAAGGTAGAGGTTCC	48	2153
959305	1066	1081	16186	16201	TCTAGCAGCTCATCTC	66	1970
959315	1093	1108	16213	16228	CAGGGCAGGATGCTGA	2	2154
959325	1608	1623	25371	25386	TGACTCACAGACTCTT	60	2155
959335	1649	1664	25412	25427	TTAGCACCTCTGAAAG	54	1971
959345	1804	1819	25567	25582	ATGTATTAGAGTTAAG	79	1972
959355	1927	1942	25690	25705	CATTCTAAGAACCTCA	68	1973
959365	2183	2198	25946	25961	TTAGTTAGGTGAAAAA	69	1974
959375	2261	2276	26024	26039	CACTGATTCACATAAT	73	1975
959385	N/A	N/A	4305	4320	AGAAACAAACCCTCCG	70	2156
959395	N/A	N/A	4737	4752	TGCAAATGATCATGTG	69	1976
959405	N/A	N/A	4769	4784	GCTGACTTTTATTGTT	83	1977
959415	N/A	N/A	4872	4887	AGTGAGTGTACTTTAG	94	1978
959425	N/A	N/A	5395	5410	TTAATGCTATCAGGTG	82	1979
959435	N/A	N/A	5580	5595	ATGCACAATGACATCA	84	1980
959445	N/A	N/A	5624	5639	ATTCTACCTGTGTCTT	95	1981
959455	N/A	N/A	5651	5666	TTGGATATATTGGGCT	97	1982
959465	N/A	N/A	5800	5815	ACTATGGGAGCCACAT	26	2157
959475	N/A	N/A	5868	5883	TACTTTTGGCAAGGCC	69	1983
959485	N/A	N/A	7697	7712	GCACAGAGTAGGTTAA	70	1984
959655	N/A	N/A	12146	12161	TGGTAGCTCCTGGCAA	50	1985
959665	N/A	N/A	12162	12177	TTGTGCAGACAGTAAC	87	2158

959675	N/A	N/A	12201	12216	TTTCCGTTAACCATCA	95	1986
959685	N/A	N/A	12284	12299	TTTTAGGTCTGGGTAT	81	2159
959695	N/A	N/A	12667	12682	CATCTTAGTGGCTGGG	91	1987
959705	N/A	N/A	12695	12710	GTTCTTGACCGTGTTT	97	1988
959715	N/A	N/A	12717	12732	GGTTAAACTACCGAAC	26	1989
959725	N/A	N/A	12783	12798	CATGGTCTGCAAATTT	89	1990
959735	N/A	N/A	12837	12852	ATTGCATTGCATAGCC	95	2160
959745	N/A	N/A	12887	12902	AAACCAATCCTGTTAG	54	1991
959755	N/A	N/A	12910	12925	ATCTGCTTATAAAGCA	43	1992
959765	N/A	N/A	12964	12979	CTTTGCAGCCTATCCC	95	2161
959775	N/A	N/A	13374	13389	ACTTTGCAGGCACCCC	86	1993
959785	N/A	N/A	13399	13414	GCTTGAATGTCACCCT	95	1994
959795	N/A	N/A	13534	13549	TCAGCATCATTGGAAG	60	2162
959805	N/A	N/A	13716	13731	TTTACCAAGACCGCTA	82	1995
959815	N/A	N/A	13745	13760	AGTGTTAGAACTAAGG	93	2163
959825	N/A	N/A	14230	14245	CAACTTTTAGTATTAA	9	1996
959835	N/A	N/A	14417	14432	TCGACACAGCATCACC	59	1997
959845	N/A	N/A	14556	14571	CAAAGTCTTAATGTGG	84	2164
959855	N/A	N/A	14671	14686	TCTTAGTGTCCCCATC	78	1998
959865	N/A	N/A	15209	15224	TTAGGAATATTGCCAG	91	1999
959875	N/A	N/A	15769	15784	GGGTTAGTGTTGGTTT	93	2000
959885	N/A	N/A	17200	17215	TGTTTGACTTAGTCCG	96	2165
959895	N/A	N/A	17288	17303	TAGGGCACCTCAAGAA	0	2001
959905	N/A	N/A	17734	17749	AATCTGGTCATATGGT	42	2166
959915	N/A	N/A	18400	18415	CAAATATGTTTGGAAG	55	2002
959925	N/A	N/A	20293	20308	CCCAATCAGAGGAAGC	57	2003
959935	N/A	N/A	20599	20614	TCATCATTATTACCTG	93	2004
959945	N/A	N/A	20651	20666	TGCAGTGCCCTGGCCT	40	2167
959955	N/A	N/A	20803	20818	TTCAGACCAGGGTAAT	93	2005
959965	N/A	N/A	20845	20860	GCCTGTATTAGCTCAA	88	2006
959975	N/A	N/A	20941	20956	GCCTCTTTGTAGCAGA	0	2007
959985	N/A	N/A	21007	21022	AGGTTATTTTAACAGC	94	2168
959995	N/A	N/A	21434	21449	TACTGAGAGGAAATGA	65	2008
960005	N/A	N/A	21538	21553	TGACATTTCAGAGTAT	87	2169

960015	N/A	N/A	22714	22729	TGTAAAGATGTGAGTG	75	2009
960025	N/A	N/A	22772	22787	TCAAAGGACATGACAG	78	2010
960037	N/A	N/A	22590	22605	GAGCAACGAGGAAGGA	64	2011

Example 2: Dose-dependent antisense inhibition of human PNPLA3 in A431 cells

Gapmers from Example 1 exhibiting significant *in vitro* inhibition of PNPLA3 mRNA were selected and tested at various doses in A431 cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cells were plated at a density of 10,000 cells per well and transfected free uptake with different concentrations of antisense oligonucleotide, as specified in the Tables below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and PNPLA3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS36070 was used to measure mRNA levels. PNPLA3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PNPLA3, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. PNPLA3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 37

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (µM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
912712	27	67	76	74	0.2
912732	54	78	88	87	< 0.1
912733	45	74	85	88	< 0.1
912734	33	64	80	83	0.1
912756	46	72	89	92	< 0.1
912757	31	62	78	86	0.2
912758	38	70	85	90	0.1
912759	66	92	97	98	< 0.1
912772	46	63	79	88	0.1
912795	40	64	83	84	0.1
912812	43	81	88	88	< 0.1
912822	81	83	92	86	< 0.1

912823	67	80	91	86	< 0.1
912825	58	80	86	88	< 0.1
912834	37	75	81	84	0.1
912841	17	62	79	69	0.3
912847	70	83	90	91	< 0.1
912848	80	89	90	90	< 0.1
912855	48	62	77	80	0.1

Table 38

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	68	94	94	98	< 0.1
912813	57	84	90	87	< 0.1
912856	60	81	91	88	< 0.1
912859	48	79	81	72	< 0.1
912864	60	88	90	90	< 0.1
912870	67	81	91	94	< 0.1
912871	21	67	84	89	0.2
912872	18	73	90	92	0.2
912876	43	70	87	92	0.1
912933	68	89	90	90	< 0.1
912940	86	91	95	96	< 0.1
912941	87	94	96	96	< 0.1
912952	68	85	90	91	< 0.1
912953	80	90	95	93	< 0.1
912964	59	78	88	91	< 0.1
912973	53	70	87	91	< 0.1
912980	54	77	84	88	< 0.1
912985	23	61	81	87	0.2
912988	65	83	86	89	< 0.1

Table 39

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (μM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
912759	72	95	97	99	< 0.1
912874	78	90	96	97	< 0.1
912875	64	83	92	94	< 0.1
912886	49	78	85	92	< 0.1
912931	68	88	94	95	< 0.1
912934	57	83	90	92	< 0.1
912936	50	78	89	89	< 0.1
912938	57	73	85	87	< 0.1
912943	64	84	90	93	< 0.1
912954	80	92	93	94	< 0.1
912970	44	73	86	90	< 0.1
912986	56	78	91	92	< 0.1
912987	79	90	92	88	< 0.1
912992	21	59	74	81	0.3
915603	50	88	96	98	< 0.1
915623	81	96	98	98	< 0.1
915643	67	89	94	96	< 0.1
916602	79	92	95	96	< 0.1
916642	44	83	91	93	< 0.1

Table 40

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (μM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
912759	73	94	98	99	< 0.1
915484	67	87	93	95	< 0.1
915543	34	69	87	90	0.1
915604	54	78	91	95	< 0.1
915763	63	80	87	87	< 0.1
915904	50	83	92	94	< 0.1

915923	63	74	82	87	< 0.1
916183	33	78	89	91	0.1
916303	58	73	84	91	< 0.1
916343	15	72	76	87	0.2
916563	46	74	90	95	< 0.1
916582	48	74	89	91	< 0.1
916623	64	81	91	94	< 0.1
916702	45	70	78	79	< 0.1
916761	46	75	85	88	< 0.1
916781	55	79	86	87	< 0.1
916782	62	87	91	93	< 0.1
916802	66	88	94	91	< 0.1
916822	29	72	83	87	0.1

Table 41

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	72	95	98	99	< 0.1
915525	51	76	88	84	< 0.1
915546	39	79	90	94	0.1
915605	59	84	96	96	< 0.1
915606	74	94	99	98	< 0.1
915625	72	82	91	95	< 0.1
915944	36	71	75	83	0.1
916065	36	62	78	79	0.1
916144	71	86	90	92	< 0.1
916163	36	67	81	74	0.1
916164	82	88	89	92	< 0.1
916184	60	79	87	89	< 0.1
916304	46	65	80	84	0.1
916324	57	77	87	92	< 0.1
916344	41	70	83	88	0.1

916564	38	66	88	94	0.1
916604	67	87	95	96	< 0.1
916624	43	59	79	87	0.1
916803	67	84	93	92	< 0.1

Table 42

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	70	94	98	99	< 0.1
915486	35	64	82	90	0.1
915487	62	89	94	95	< 0.1
915626	67	83	92	94	< 0.1
915786	65	84	88	88	< 0.1
916145	53	66	85	87	< 0.1
916146	62	77	86	86	< 0.1
916165	71	86	89	88	< 0.1
916166	71	83	87	88	< 0.1
916305	57	86	90	92	< 0.1
916306	86	96	98	98	< 0.1
916325	59	78	83	86	< 0.1
916345	21	47	67	73	0.4
916545	63	88	95	94	< 0.1
916546	66	85	92	95	< 0.1
916625	47	71	84	92	< 0.1
916706	22	65	80	85	0.2
916765	67	85	92	93	< 0.1
916845	38	71	80	87	0.1

Table 43

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

	PNPLA3 % Inhibition	

Compound Number	62.5 nM	250 nM	1,000 nM	4,000 nM	IC_{50} (μM)
912759	97	99	100	100	< 0.1
915608	66	91	97	97	< 0.1
915609	71	97	99	99	< 0.1
915627	0	26	53	62	1.3
915768	39	69	86	91	0.1
915908	49	70	80	85	< 0.1
915987	47	60	75	78	0.1
916008	45	69	84	83	< 0.1
916187	71	82	88	92	< 0.1
916247	34	72	83	84	0.1
916287	31	70	90	91	0.1
916547	79	93	97	97	< 0.1
916566	8	45	73	81	0.5
916586	47	67	89	91	< 0.1
916587	48	81	90	94	< 0.1
916606	18	64	87	90	0.2
916607	72	94	94	95	< 0.1
916627	18	51	82	79	0.3
916805	18	65	78	81	0.2

Table 44

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (μM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
912759	64	92	97	99	< 0.1
915610	74	94	98	99	< 0.1
915789	35	72	82	85	0.1
915909	52	69	82	86	< 0.1
915929	13	32	60	59	1.0
915969	39	54	74	74	0.2
915989	46	67	81	86	0.1

916069	24	59	75	56	0.3
916148	42	71	85	80	< 0.1
916168	28	54	74	68	0.3
916188	22	42	72	70	0.4
916309	30	77	91	96	0.1
916348	41	57	65	73	0.1
916549	64	85	94	96	< 0.1
916568	54	66	81	87	< 0.1
916569	60	86	92	95	< 0.1
916728	22	50	68	73	0.4
916788	60	89	94	96	< 0.1
916848	49	75	89	95	< 0.1

Table 45

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	81	93	98	100	< 0.1
915390	9	40	67	77	0.6
915611	46	80	93	88	< 0.1
915630	52	69	81	82	< 0.1
915910	44	64	79	80	0.1
915931	83	88	89	86	< 0.1
916149	73	87	89	83	< 0.1
916150	51	68	77	84	< 0.1
916189	60	73	77	79	< 0.1
916310	45	77	88	95	< 0.1
916330	48	67	84	86	< 0.1
916550	62	85	94	97	< 0.1
916570	89	96	98	98	< 0.1
916629	26	53	73	86	0.3
916630	52	68	87	91	< 0.1
916670	43	77	78	85	< 0.1

916730	61	74	82	86	< 0.1
916768	35	57	67	72	0.2
916789	79	92	96	96	< 0.1

Table 46

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	76	94	96	99	< 0.1
915532	31	66	82	92	0.1
915612	54	77	86	90	< 0.1
915732	42	63	80	84	0.1
915932	45	71	88	89	< 0.1
915951	26	58	71	74	0.3
915991	67	84	85	85	< 0.1
915992	54	78	86	87	< 0.1
916112	35	67	76	78	0.1
916151	51	79	87	90	< 0.1
916311	36	70	81	87	0.1
916331	56	85	93	95	< 0.1
916332	82	91	94	96	< 0.1
916390	30	41	68	64	0.5
916552	79	93	96	97	< 0.1
916571	53	78	90	94	< 0.1
916631	48	77	86	90	< 0.1
916651	81	89	94	95	< 0.1
916711	37	66	85	91	0.1

Table 47

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	

912759	58	90	98	99	< 0.1
915474	51	79	90	93	< 0.1
915493	48	58	83	80	0.1
915494	46	73	86	90	< 0.1
915674	49	72	89	93	< 0.1
915933	40	63	75	79	0.1
916153	68	86	89	91	< 0.1
916172	85	89	87	87	< 0.1
916173	81	90	91	88	< 0.1
916292	64	83	92	92	< 0.1
916312	60	84	91	92	< 0.1
916333	75	92	96	96	< 0.1
916572	29	62	79	88	0.2
916592	52	74	89	90	< 0.1
916593	25	67	83	93	0.2
916613	46	75	89	92	< 0.1
916652	65	83	91	88	< 0.1
916672	73	89	93	90	< 0.1
916772	50	61	83	89	< 0.1

Table 48

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	50	89	96	99	< 0.1
915534	0	33	66	57	1.1
915535	51	81	92	96	< 0.1
915634	18	67	79	84	0.2
915635	44	72	86	91	0.1
915675	36	68	82	90	0.1
915735	45	68	73	84	0.1
915936	36	67	78	83	0.1
915995	78	87	90	89	< 0.1

915996	83	91	93	92	< 0.1
916174	80	84	86	81	< 0.1
916175	55	82	86	89	< 0.1
916334	50	82	92	94	< 0.1
916335	52	76	89	93	< 0.1
916575	62	88	93	93	< 0.1
916753	49	69	76	74	< 0.1
916774	49	72	86	91	< 0.1
916794	26	64	85	85	0.2
916873	16	49	72	82	0.4

Table 49

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	57	90	97	99	< 0.1
915477	47	65	88	93	0.1
915478	47	78	90	95	< 0.1
915497	63	68	79	86	< 0.1
915637	67	91	97	98	< 0.1
916037	15	47	70	61	0.6
916236	80	87	90	88	< 0.1
916336	52	67	81	87	< 0.1
916576	50	76	89	93	< 0.1
916596	55	82	93	94	< 0.1
916636	42	71	87	90	0.1
916637	56	85	90	93	< 0.1
916715	27	38	68	68	0.5
916716	35	77	89	93	0.1
916796	14	62	84	89	0.3
916814	22	44	70	79	0.4
916815	56	79	87	89	< 0.1
916816	33	72	83	93	0.1

916874	5	34	61	70	0.8

Table 50

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (µM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
912759	56	91	97	100	< 0.1
915479	38	70	89	94	0.1
915618	42	63	75	85	0.1
915619	65	87	96	97	< 0.1
915638	31	64	80	82	0.2
915639	33	78	88	93	0.1
915778	41	50	78	87	0.2
916058	26	34	73	81	0.4
916177	38	55	83	82	0.1
916238	84	91	93	93	< 0.1
916298	79	87	92	94	< 0.1
916318	59	71	91	94	< 0.1
916338	71	91	94	92	< 0.1
916558	73	89	94	94	< 0.1
916577	41	66	78	82	0.1
916578	69	85	91	93	< 0.1
916638	46	84	90	92	< 0.1
916757	33	60	82	88	0.2
916817	31	67	82	87	0.1

Table 51

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (µM)
	62.5 nM	250 nM	1,000 nM	4,000 nM	
841947	50	78	89	92	< 0.1
912759	75	50	85	99	< 0.1

912986	54	78	90	95	< 0.1
915480	61	87	94	97	< 0.1
915519	47	77	86	85	< 0.1
915620	46	75	88	91	< 0.1
915780	24	76	92	94	0.1
915920	23	65	79	82	0.2
916020	45	80	85	81	< 0.1
916299	59	87	92	93	< 0.1
916339	88	95	97	97	< 0.1
916340	83	96	97	98	< 0.1
916559	41	68	83	89	0.1
916579	71	86	96	96	< 0.1
916580	59	90	95	96	< 0.1
916618	10	47	70	78	0.5
916639	38	58	80	86	0.1
916778	68	87	92	94	< 0.1
916818	60	77	90	91	< 0.1

Table 52

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	62.5 nM	**250 nM**	**1,000 nM**	**4,000 nM**	
912759	60	0	85	99	0.3
915541	48	71	89	91	< 0.1
915542	50	72	86	93	< 0.1
915601	8	53	84	84	0.3
915602	1	56	77	91	0.4
915621	21	54	75	80	0.3
915622	0	44	73	84	0.5
915922	27	64	79	85	0.2
916042	6	57	89	88	0.3
916140	43	82	90	89	< 0.1
916141	72	88	93	91	< 0.1

916180	33	62	69	83	0.2
916181	53	80	89	92	< 0.1
916341	0	78	94	94	0.3
916560	72	91	95	94	< 0.1
916581	38	76	91	91	0.1
916601	44	80	88	90	< 0.1
916701	61	83	91	93	< 0.1
916780	75	91	93	94	< 0.1

Table 53

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (µM)
	15.6 nM	62.5 nM	250 nM	1,000 nM	
915609	52	86	96	99	< 0.01
959430	42	76	90	95	< 0.01
959440	39	73	92	98	0.02
959470	46	73	89	94	< 0.01
959670	52	90	96	98	< 0.01
959680	50	75	91	96	< 0.01
959730	83	96	98	98	< 0.01
959740	50	70	90	96	< 0.01
959820	40	69	85	92	0.02
959830	46	69	93	97	0.02
959880	34	62	85	93	0.03
960010	48	78	92	95	< 0.01

Table 54

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (µM)
	15.6 nM	62.5 nM	250 nM	1,000 nM	
915609	53	87	98	99	< 0.01
959271	55	74	91	91	< 0.01

959360	7	43	79	85	0.1
959361	60	87	93	94	< 0.01
959411	56	76	91	94	< 0.01
959441	50	81	93	97	< 0.01
959460	0	29	75	90	0.2
959701	62	91	97	98	< 0.01
959721	80	94	97	97	< 0.01
959731	25	64	82	91	0.05
959741	41	65	83	91	0.02
959750	0	26	65	87	0.2
959761	28	60	84	91	0.05
959781	39	58	75	87	0.04
959911	20	54	78	90	0.1
959921	37	61	83	91	0.03
959931	48	72	89	92	< 0.01
959960	11	51	79	90	0.1
959961	38	64	85	92	0.03

Table 55

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	15.6 nM	**62.5 nM**	**250 nM**	**1,000 nM**	
915609	11	71	93	98	0.1
959412	52	77	90	94	< 0.01
959413	34	82	95	97	0.02
959422	15	50	80	87	0.1
959432	33	60	86	95	0.04
959662	0	53	84	92	0.1
959672	54	85	95	97	< 0.01
959673	18	62	88	95	0.1
959682	46	77	90	91	< 0.01
959702	39	71	91	96	0.02
959703	81	96	99	99	< 0.01

959712	4	30	75	92	0.1
959713	0	53	86	96	0.1
959722	33	80	90	94	0.02
959733	31	68	92	96	0.03
959782	35	63	86	94	0.03
959872	29	64	77	89	0.04
959912	25	69	89	92	0.04
959982	21	61	83	91	0.1

Table 56

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	15.6 nM	**62.5 nM**	**250 nM**	**1,000 nM**	
915609	2	73	93	98	0.1
959363	49	82	91	91	< 0.01
959393	38	71	87	95	0.02
959394	27	73	91	97	0.03
959414	69	94	98	99	< 0.01
959664	51	77	95	98	< 0.01
959674	43	74	95	98	0.02
959683	14	71	90	96	0.05
959704	57	92	98	99	< 0.01
959724	0	68	90	95	0.1
959734	71	93	98	98	< 0.01
959814	24	76	90	95	0.03
959873	38	53	83	90	0.04
959874	51	82	95	97	< 0.01
959884	44	77	94	97	< 0.01
959913	18	50	85	92	0.1
959953	6	51	85	92	0.1
959983	22	54	81	92	0.1
960004	10	71	92	96	0.1

Table 57

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (μM)
	15.6 nM	62.5 nM	250 nM	1,000 nM	
915609	12	69	93	98	0.1
959364	32	72	89	91	0.03
959415	21	70	91	96	0.04
959444	6	47	82	91	0.1
959445	32	70	92	97	0.03
959455	61	87	95	97	< 0.01
959675	20	56	80	94	0.1
959684	8	47	83	86	0.1
959705	77	95	98	99	< 0.01
959735	12	67	90	95	0.1
959764	4	32	80	92	0.1
959765	1	59	88	93	0.1
959784	3	35	75	90	0.1
959785	27	72	92	96	0.03
959794	0	0	53	83	0.3
959864	26	61	84	91	0.05
959885	49	81	95	96	< 0.01
959914	7	43	76	89	0.1
959964	17	55	83	91	0.1

Table 58

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	PNPLA3 % Inhibition				IC_{50} (μM)
	15.6 nM	62.5 nM	250 nM	1,000 nM	
915609	0	73	95	97	0.1
959456	66	90	97	98	< 0.01
959666	29	60	89	97	0.04
959676	15	44	81	93	0.1
959686	71	92	97	97	< 0.01

959695	40	75	91	93	0.02
959696	21	81	90	92	0.03
959706	81	95	98	98	< 0.01
959725	8	55	76	84	0.1
959726	0	59	88	91	0.1
959736	46	84	94	98	< 0.01
959766	22	57	83	94	0.1
959776	1	53	87	93	0.1
959815	31	67	89	91	0.03
959865	6	49	84	91	0.1
959875	34	74	91	92	0.02
959935	22	55	84	94	0.1
959955	0	55	83	89	0.1
959985	29	71	88	93	0.03

Table 59

Multi-dose assay of 3-10-3 cEt gapmers in A431 cells

Compound Number	**PNPLA3 % Inhibition**				**IC_{50} (μM)**
	15.6 nM	**62.5 nM**	**250 nM**	**1,000 nM**	
915609	37	80	96	99	0.02
959356	40	71	87	88	0.02
959417	25	58	83	92	0.1
959437	65	88	94	95	< 0.01
959667	37	69	90	95	0.02
959677	29	56	82	92	0.05
959687	51	79	93	97	< 0.01
959697	51	75	93	95	< 0.01
959707	81	94	98	98	< 0.01
959727	71	92	96	96	< 0.01
959737	45	75	89	94	< 0.01
959767	47	76	93	96	< 0.01
959797	32	59	87	94	0.04
959856	13	35	67	80	0.1

959876	38	75	89	90	0.02
959877	40	81	89	94	< 0.01
959956	25	25	66	85	0.1
960006	13	40	68	83	0.1
960007	24	59	88	91	0.05

Example 3: Tolerability of modified oligonucleotides targeting human PNPLA3 in BALB/c mice

BALB/c mice are a multipurpose mouse model frequently utilized for safety and efficacy testing. The mice were treated with antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Ionis oligonucleotides selected from the studies above were conjugated with 3'-THA-C_6-$GalNAc_3$-(3R,5S)-5-(hydroxymethyl) pyrrolidin-3-ol phosphate endcap (henceforth referred to as 3'-THA).

Treatment

Groups of 6- to 7-week-old male mice were injected subcutaneously once with 200 mg/kg of modified oligonucleotides. One group of male BALB/c mice was injected with PBS. Mice were euthanized 72-96 hours after the single dose and plasma was harvested for further analysis.

To evaluate the effect of modified oligonucleotides on liver function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Beckman Coulter AU480, Brea, CA). Modified oligonucleotides that caused changes in the levels of transaminases outside the expected range for antisense oligonucleotides were excluded in further studies. The oligonucleotides which were considered tolerable in this study and were selected for further evaluation are presented in the Table below. 'Parent Oligo' indicates the Ionis oligonucleotide that has been described in the studies above and that was conjugated with 3'-THA and tested in this study.

Table 60

Antisense oligonucleotides in BALB/c mouse study

Compound ID	**Parent oligo ID**
975746	916339
975747	912941
975748	916306
975755	916332
975760	912848

975764	916298
975766	916552
975767	916789
975768	916602
975770	912874
975771	916333
975772	916780
975775	916672
975777	916558
975780	916607
975783	916338
975788	912847
975790	916778
975792	912870
975794	916802
975797	916637
975799	912732
975800	912733
975803	912813
975804	912823
975805	912834
975806	912855
975807	912856
975808	912864
975809	912871
975810	912872
975811	912875
975813	912931
975814	912934
975815	912936
975816	912938
975817	912943
975820	912988
975822	915486

975829	915619
975836	915780
975840	915989
975844	916151
975849	916292
975850	916299
975851	916303
975852	916309
975853	916310
975854	916312
975855	916318
975856	916324
975857	916331
975858	916334
975859	916335
975860	916336
975861	916549
975862	916550
975864	916563
975865	916564
975866	916568
975868	916571
975869	916575
975870	916580
975871	916581
975873	916586
975875	916601
975878	916624
975879	916625
975880	916636
975881	916638
975883	916670
975886	916711
975887	916716

975888	916774
975889	916781
975890	916782
975891	916788
975893	916815
975894	916816
975895	916817
975896	916818
975897	916822
975898	916845
994288	959455
994289	960010
994290	959361
994291	959271

Example 4: Effect of antisense inhibition of PNPLA3 in transgenic mouse model

A PNPLA3 transgenic mouse model from wild-type C57BL/6 generated by the University of California, Irvine was used. The mouse model comprises a genomic construct containing the entire PNPLA3 gene fosmid, generously provided by the University of Washington. The efficacy of Ionis oligonucleotides was evaluated in this model.

Treatment

Transgenic mice were maintained on a 12-hour light/dark cycle and were fed *ad libitum* normal Purina mouse chow. Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. Antisense oligonucleotides (ASOs) were prepared in buffered saline (PBS) and sterilized by filtering through a 0.2 micron filter. Oligonucleotides were dissolved in 0.9% PBS for injection.

The hPNPLA3 Tg mice were divided into groups of 2 mice each. Groups received subcutaneous injections of Ionis oligonucleotide at a dose of 2.5 mg/kg on days 1 and 8. One group of 4 mice received subcutaneous injections of PBS on days 1 and 8. The saline-injected group served as the control group to which oligonucleotide-treated groups were compared.

RNA analysis

On day 10, RNA was extracted from liver for real-time PCR analysis of measurement of mRNA expression of PNPLA3. Primer probe sets RTS36070 and RTS36075 were both used to measure PNPLA3

mRNA levels. Results are presented as percent change of mRNA, relative to PBS control, normalized with RIBOGREEN®. As presented in the Table below, treatment with Ionis antisense oligonucleotides resulted in significant reduction of PNPLA3 mRNA in comparison to the PBS control. '0' indicates that the oligonucleotides did not inhibit mRNA expression.

Table 61

Percent inhibition of PNPLA3 mRNA in the transgenic mice liver relative to the PBS control

Compound ID	Inhibition (%) measured with RTS36070	Inhibition (%) measured with RTS36075
975746	99	99
975747	99	99
975748	98	98
975755	99	99
975760	96	97
975764	75	83
975766	99	99
975767	98	98
975768	98	98
975770	97	97
975771	98	99
975772	96	96
975775	90	91
975777	85	89
975780	44	63
975783	87	90
975788	0	26
975790	0	0
975792	9	34
975794	44	50
975797	0	0
975799	0	0
975800	0	5

975803	68	68
975804	11	38
975805	0	0
975806	0	0
975807	0	0
975808	47	58
975809	0	19
975810	12	22
975811	19	32
975813	36	39
975814	48	54
975815	78	77
975816	56	56
975817	84	86
975820	35	45
975822	0	0
975829	98	98
975836	85	91
975840	19	44
975844	21	31
975849	88	89
975850	41	48
975851	5	18
975852	24	41
975853	0	0
975854	0	0
975855	0	0
975856	45	31
975857	73	67
975858	58	40
975860	92	92
975861	66	49
975862	46	36
975864	16	21

975865	0	0
975866	40	41
975868	56	48
975869	30	19
975870	0	14
975871	0	0
975875	75	73
975878	18	12
975879	7	0
975880	0	0
975881	54	54
975883	77	80
975886	18	28
975887	49	57
975888	10	9
975889	90	91
975890	96	98
975891	97	98
975893	95	95
975894	85	87
975895	89	89
975896	91	89
975898	94	95
975897	96	97
975873	99	99
994288	99	99
994289	98	99
994290	98	99
994291	95	95
975859	95	96

Example 5: Tolerability of modified oligonucleotides targeting human PNPLA3 in CD1 mice

CD1® mice (Charles River, MA) are a multipurpose mice model, frequently utilized for safety and efficacy testing. The mice were treated with Ionis antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Ionis oligonucleotides selected from the studies above were conjugated with 5'-Trishexylamino-(THA)-C_6GalNAC3 endcap (henceforth referred to as 5'-THA). The Ionis oligonucleotides tested are presented in the Table below. 'Unconjugated parent ION No.' refers to the Ionis oligonucleotide described in the in vitro studies above with the same sequence. '3'-THA counterpart ION No.' refers to the 3'-THA conjugated oligonucleotide with the same sequence and evaluated in the mice studies above.

Table 62

5'-THA oligonucleotides tested in CD1 mice tolerability study

Compound ID	Unconjugated parent ION No.	3'-THA counterpart ION No	Sequence	SEQ ID NO
975591	916339	975746	GGATATATTGGGCTCA	1512
975592	912941	975747	TTGCATTGCATAGCCT	182
975593	916306	975748	GTGTACTTTAGGCTCC	598
975600	916332	975755	CACAATGACATCATGG	1020
975605	912848	975760	CGTTTTTAGTAGTCAA	141
975611	916552	975766	CCTTTTATTTCCGTTA	1024
975612	916789	975767	GTAATATTCAGACCAG	899
975613	916602	975768	CTAGTAAATGCTTGTC	330
975615	912874	975770	ATACTTTTGGCAAGGC	217
975616	916333	975771	CTTTATTCAATGTGGC	1089
975617	916780	975772	AGAAATTGCAGTGCCC	1665
975674	915619	975829	GACTTTAGGGCAGATG	1400
975704	916335	975859	TAATTCTACCTGTGTC	1227
975718	916586	975873	AACTTTGCAGCCTATC	605
975735	916782	975890	CTTAGAAATTGCAGTG	408
975736	916788	975891	TAATATTCAGACCAGG	830
975738	916815	975893	CAATTCTAGACATGGC	1313
975742	916822	975897	TATGACATTTCAGAGT	410
975743	916845	975898	GTAAAGATGTGAGTGA	618

994282	959455	994288	TTGGATATATTGGGCT	1982
994283	960010	994289	AGACATATGACATTTC	1745
994284	959361	994290	TTTTTAGTAGTCAAGG	1757
994285	959271	994291	GTTGAAGGATGGATGG	1748

Treatment

Groups of four CD1 mice each were weekly injected subcutaneously with 15 mg/kg of Ionis oligonucleotides for 6 weeks, with one loading dose at day 4 (total 8 doses). One group of male CD1 mice was injected subcutaneously for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of Ionis oligonucleotides on liver and kidney function, plasma levels of transaminases (ALT and AST), albumin, total bilirubin, and creatinine were measured at week 3 using an automated clinical chemistry analyzer (Beckman Coulter AU480, Brea, CA). The results are presented in the Table below. Ionis oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 63

Plasma chemistry marker levels in CD1 mice at week 3

	Albumin (g/dL)	**ALT (IU/L)**	**AST (IU/L)**	**Total bilirubin (mg/dL)**	**Creatinine (mg/dL)**
PBS	2.9	31	64	0.4	0.1
975611	2.7	640	385	0.3	0.1
994282	2.4	76	83	0.3	0.1
975592	3.0	786	942	0.5	0.1
975600	2.7	334	431	0.3	0.1
975591	2.6	62	115	0.4	0.1
975718	2.4	1717	2183	1.2	0.1
994284	2.7	41	97	0.3	0.1
994283	2.8	216	154	0.3	0.1
975616	3.0	69	137	0.3	0.1

975612	2.7	47	218	0.4	0.1
975674	2.9	134	114	0.4	0.1
975613	2.8	60	277	0.3	0.1
975593	2.7	429	405	0.4	0.1
975736	2.9	46	63	0.2	0.2
975735	2.5	46	79	0.2	0.1
975742	2.6	152	96	0.2	0.1
975615	2.9	207	189	0.4	0.1
975617	2.9	65	70	0.3	0.1
975605	2.9	67	92	0.3	0.1
975704	2.4	33	61	0.2	0.1
975738	2.6	43	67	0.2	0.1
975743	2.9	119	126	0.4	0.1
994285	2.8	400	353	0.2	0.1

Hematology assays

Blood obtained from selected mouse groups at week 6 were sent to IDEXX BioResearch for measurement of platelet count. The results are presented in the tables below. Ionis oligonucleotides that caused changes in the platelet count outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 64

Platelet count in CD1 mice

	Platelet ($x10^3/\mu L$)
PBS	1067
975605	1202
975612	1200
975613	1417
975616	1178
975617	922
975674	618
975591	941
975743	1127

994282	1384
994284	1255
975704	939
975735	1039
975736	1116
975738	1126
975742	808

Example 6: Tolerability of modified oligonucleotides targeting human PNPLA3 in Sprague-Dawley rats

Sprague-Dawley rats are a multipurpose model used for safety and efficacy evaluations. The rats were treated with Ionis antisense oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed *ad libitum* with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were weekly injected subcutaneously with 15 mg/kg of Ionis oligonucleotide for 6 weeks, with one loading dose on day 4 (total 8 doses). Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of Ionis oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Beckman Coulter AU480, Brea, CA). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. Plasma levels of bilirubin, creatinine, albumin, and BUN were also measured using the same clinical chemistry analyzer and the results are also presented in the Table below expressed in mg/dL. Ionis oligonucleotides that caused changes in the levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 65

Plasma chemistry markers in Sprague-Dawley rats

	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	Total bilirubin (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)
PBS	3	35	81	0.2	0.2	12
975591	3	57	161	0.2	0.3	14

975605	4	62	176	0.3	0.2	14
975612	3	106	153	0.2	0.3	13
975613	3	32	94	0.2	0.2	12
975616	4	31	106	0.2	0.3	13
975617	3	49	263	0.2	0.2	12
975735	3	44	128	0.2	0.2	14
975736	3	73	293	0.3	0.3	14
994282	3	41	135	0.1	0.3	12
994284	3	32	95	0.1	0.2	13

Kidney function

To evaluate the effect of Ionis oligonucleotides on kidney function, urinary levels of protein and creatinine were measured using an automated clinical chemistry analyzer (Beckman Coulter AU480, Brea, CA). The ratios of total protein to creatinine are presented in the Table below. Ionis oligonucleotides that caused changes in the levels of the ratio outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 66

Total protein to creatinine ratio in Sprague-Dawley rats

PBS	1.5
975591	2.0
975605	1.6
975612	1.9
975613	2.3
975616	2.0
975617	1.4
975735	2.2
975736	1.1
994282	2.1
994284	2.1

Organ weights

Liver, heart, spleen and kidney weights were measured at the end of the study, and are presented in the Table below. Ionis oligonucleotides that caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 67

Organ weights (g)

	Liver	Kidney	Spleen
Saline	16	3	1
975591	16	4	1
975605	21	3	1
975612	12	3	1
975613	16	3	1
975616	15	3	1
975617	19	4	2
975735	14	4	1
975736	15	3	1
994282	14	3	1
994284	15	3	1

Example 7: Effect of antisense inhibition of PNPLA3 in transgenic mouse model

Ionis oligonucleotides were tested in a multi-dose assay in the hPNPLA3 Tg model.

Treatment

Transgenic mice were maintained on a 12-hour light/dark cycle and were fed *ad libitum* normal Purina mouse chow. Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. Antisense oligonucleotides (ASOs) were prepared in buffered saline (PBS) and sterilized by filtering through a 0.2 micron filter. Oligonucleotides were dissolved in 0.9% PBS for injection.

Study 1

The hPNPLA3 Tg mice were divided into groups of 4 mice each. Groups received subcutaneous injections of Ionis oligonucleotide at a weekly dose of 5 mg/kg, 1 mg/kg, or 0.25 mg/kg administered on days 1, 5, 8, 15, and 23. One group of 4 mice received subcutaneous injections of PBS on days 1, 5, 8, 15, and 23. The saline-injected group served as the control group to which oligonucleotide-treated groups were compared.

RNA analysis

On day 26, RNA was extracted from liver for real-time PCR analysis of measurement of mRNA expression of PNPLA3. Primer probe sets RTS36070 and RTS36075 were both used to measure PNPLA3 mRNA levels. Results are presented as percent change of mRNA, relative to PBS control, normalized with RIBOGREEN®. As presented in the Table below, treatment with Ionis antisense oligonucleotides resulted in significant dose-dependent reduction of PNPLA3 mRNA in comparison to the PBS control.

Table 68

Percent inhibition of PNPLA3 mRNA in the transgenic mice liver relative to the PBS control

	mg/kg/wk	Inhibition measured by RTS36070	Inhibition measured by RTS36075	EC_{50} (µg/g)
975605	5	93	90	2.2
	1	66	57	
	0.25	45	46	
975612	5	98	99	3.1
	1	89	88	
	0.25	34	44	
975613	5	98	97	1.0
	1	87	85	
	0.25	58	56	
975616	5	93	93	0.5
	1	85	87	
	0.25	60	63	
975617	5	97	97	0.3
	1	76	78	
	0.25	55	53	
975735	5	97	98	1.5
	1	74	75	
	0.25	29	33	
975736	5	98	98	0.9
	1	73	71	
	0.25	44	45	
994282	5	98	98	0.2
	1	91	80	

	0.25	62	58	
994284	5	99	100	0.3
	1	89	88	
	0.25	53	47	

Study 2

The hPNPLA3 Tg mice were divided into groups of 4 mice each. Groups received subcutaneous injections of Ionis oligonucleotide at a weekly dose of 5 mg/kg, 2.5 mg/kg, 1 mg/kg, 0.5 mg/kg, or 0.25 mg/kg administered on days 1, 5, 8, 15, and 23. One group of 4 mice received subcutaneous injections of PBS on days 1, 5, 8, 15, and 23. The saline-injected group served as the control group to which oligonucleotide-treated groups were compared.

RNA analysis

On day 26, RNA was extracted from liver for real-time PCR analysis of measurement of mRNA expression of PNPLA3. Primer probe sets RTS36070 and RTS36075 were both used to measure PNPLA3 mRNA levels. Results are presented as percent change of mRNA, relative to PBS control, normalized with RIBOGREEN®. As presented in the Table below, treatment with Ionis antisense oligonucleotides resulted in significant dose-dependent reduction of PNPLA3 mRNA in comparison to the PBS control.

Table 69

Percent inhibition of PNPLA3 mRNA in the transgenic mice liver relative to the PBS control

	mg/kg/wk	Inhibition measured by RTS36070	Inhibition measured by RTS36075	EC_{50} (µg/g)	EC_{90} (µg/g)
975612	5	96	97	1.0	8.6
	2.5	98	98		
	1	95	96		
	0.5	82	83		
	0.25	43	44		
975613	5	99	99	0.9	7.7
	2.5	99	99		
	1	91	91		
	0.5	82	83		
	0.25	69	74		

975616	5	96	96	1.0	9.4
	2.5	94	93		
	1	89	89		
	0.5	81	81		
	0.25	73	60		

Example 8: Effect of modified oligonucleotides targeting human PNPLA3 in cynomolgus monkeys

Cynomolgus monkeys were treated with Ionis antisense oligonucleotides selected from studies described in the Examples above. Antisense oligonucleotide tolerability was evaluated.

Treatment

Prior to the study, the monkeys were kept in quarantine during which the animals were observed daily for general health. The monkeys were 2-4 years old and weighed 2-4 kg. Nine groups of 5 randomly assigned male cynomolgus monkeys each were injected subcutaneously with Ionis oligonucleotide or PBS in a clock-wise rotation between four different sites on the back. The monkeys were dosed twice per week (days 1, 5, 9, and 14) for the first 2 weeks, and then subsequently once a week for 10 weeks with 10 mg/kg of Ionis oligonucleotide on days 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84. A control group of 5 cynomolgus monkeys was injected with PBS in a similar manner and served as the control group.

During the study period, the monkeys were observed twice daily for signs of illness or distress. Any animal experiencing more than momentary or slight pain or distress due to the treatment, injury or illness was treated by the veterinary staff with approved analgesics or agents to relieve the pain after consultation with the Study Director. Any animal in poor health or in a possible moribund condition was identified for further monitoring and possible euthanasia. Scheduled euthanasia of the animals was conducted on day 86 approximately 48 hours after the last dose by exsanguination while under deep anesthesia. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Body and organ weight measurements

To evaluate the effect of Ionis oligonucleotides on the overall health of the animals, body and organ weights were measured. Body weights and organ weights were measured on day 86 and the data is presented in the Table below. The results indicate that effect of treatment with antisense oligonucleotides on body and organ weights was within the expected range for antisense oligonucleotides. Specifically, treatment with ION 945616 was well tolerated in terms of the body and organ weights of the monkeys.

Table 70

Final body and organ weights in cynomolgus monkey

	Body Wt (kg)	Spleen (g)	Kidney (g)	Liver with gallbladder (g)
PBS Control	2797	2.6	13.1	53
994284	2789	3.3	14.7	69
975605	2685	4.1	12.2	58
975616	2868	3.1	12.9	63
994282	2782	4.4	12.1	62
975613	2704	3.0	13.5	60
975617	2761	3.8	14.1	61
975735	2765	4.1	15.5	67
975736	2844	3.0	14.1	66
975612	2711	2.8	13.2	60

Liver function

To evaluate the effect of Ionis oligonucleotides on hepatic function, blood samples were collected from all the study groups on day 86. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Levels of various liver function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma levels of ALT and AST were measured and the results are presented in the Table below, expressed in IU/L. Bilirubin, a liver function marker, was similarly measured and is presented in the Table below, expressed in mg/dL. The results indicate that antisense oligonucleotides had no effect on liver function outside the expected range for antisense oligonucleotides.

Table 71

Liver function markers in cynomolgus monkey plasma

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (g/dL)
PBS Control	38	55	0.2	4.3
994284	64	48	0.2	3.7
975605	48	54	0.3	4.0

975616	54	57	0.3	3.9
994282	89	53	0.3	4.0
975613	60	71	0.4	4.0
975617	65	61	0.3	4.0
975735	59	79	0.3	4.1
975736	70	56	0.3	3.9
975612	61	66	0.3	3.9

Kidney function

To evaluate the effect of Ionis oligonucleotides on kidney function, blood samples were collected from all the study groups on day 86. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Levels of BUN and creatinine were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in the Table below, expressed in mg/dL.

The plasma chemistry data indicate that most of the Ionis oligonucleotides did not have any effect on the kidney function outside the expected range for antisense oligonucleotides.

Table 72

Plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys

	BUN	Creatinine
PBS Control	23	0.8
994284	24	0.8
975605	27	0.7
975616	21	0.8
994282	24	0.8
975613	23	0.9
975617	21	0.7
975735	20	0.8
975736	23	0.8
975612	20	0.8

Hematology

To evaluate any effect of Ionis oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 0.5 mL of blood was collected from each of the available study animals on day 86. The samples were collected in tubes containing K_2-EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, individual white blood cell counts, such as that of monocytes, neutrophils, lymphocytes, as well as for platelet count, hemoglobin content and hematocrit, using an ADVIA2120i hematology analyzer (Siemens, USA).

The data indicate the oligonucleotides did not cause any changes in hematologic parameters outside the expected range for antisense oligonucleotides at this dose.

Table 73

Blood cell counts in cynomolgus monkeys

	RBC (x 10^6/μL)	Platelets (x 10^3/μL)	WBC (x 10^3/μL)	Neutrophils (x 10^3/μL)	Lymphocytes (x 10^3/μL)	Monocytes (x 10^3/μL)
PBS Control	6.0	342	12	3.2	7.8	0.3
994284	6.0	410	10	2.7	6.7	0.3
975605	5.8	326	10	4.8	4.5	0.4
975616	6.0	362	10	3.4	5.8	0.3
994282	5.8	359	10	3.9	5.5	0.3
975613	5.5	327	8	2.6	5.5	0.2
975617	6.1	358	10	3.1	6.4	0.3
975735	5.9	241	13	5.4	6.6	0.4
975736	5.8	360	10	3.5	6.4	0.2
975612	6.2	421	11	5.1	5.7	0.2

Table 74

Hematologic parameters in cynomolgus monkeys

	Hemoglobin (g/dL)	HCT (%)
PBS Control	14	49
994284	14	48
975605	14	46
975616	14	49

994282	14	47
975613	13	46
975617	14	49
975735	14	48
975736	14	48
975612	14	49

Pro-inflammatory proteins analysis

To evaluate any inflammatory effect of Ionis oligonucleotides in cynomolgus monkeys, blood samples were taken for analysis. The monkeys were fasted overnight prior to blood collection. Approximately 1.5 mL of blood was collected from each animal and put into tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min at room temperature to obtain serum. C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, and complement C3 were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan).

Example 9: Measurement of viscosity of antisense oligonucleotides targeting human PNPLA3

The viscosity of select antisense oligonucleotides from the studies described above was measured with the aim of screening out antisense oligonucleotides which have a viscosity of more than 40 centipoise (cP). Oligonucleotides having a viscosity greater than 40 cP would have less than optimal viscosity.

Oligonucleotides (32-35 mg) were weighed into a glass vial, 120 µL of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50^0C. Part (75 µL) of the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometer was set to 25^0C and the viscosity of the sample was measured. Another part (20 µL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85^0C (Cary UV instrument). The results are presented in the Table below, where the concentration of each antisense oligonucleotide was 200 mg/ml, and indicate that most of the antisense oligonucleotides solutions are optimal in their viscosity under the criterion stated above.

Table 75

Viscosity of antisense oligonucleotides at 200 mg/mL

	Viscosity (cP)
994284	21
975605	19
975616	20

994282	30
975613	24
975617	22
975735	15
975736	49
975612	25

Example 10: Design of oligonucleotides at the site of ION 975616

Additional antisense oligonucleotides were designed targeting a PNPLA3 nucleic acid that overlap the target site of ION 916333, which is the unconjugated version of ION 975616, and with different chemical modifications and motifs.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers or deoxy, MOE, and cEt oligonucleotides. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; the number after the 'd' indicates the number of deoxyribose; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence (SEQ ID NO: 2).

Table 76

Modified oligonucleotides targeting human PNPLA3

Start Site	Stop Site	Sequence	Compound Number	Chemistry	SEQ ID NO
5599	5614	TCAATGTGGCTTCTAG	995553	kkk-d10-kkk	2170
5600	5615	TTCAATGTGGCTTCTA	959437	kkk-d10-kkk	2089
5601	5616	ATTCAATGTGGCTTCT	959438	kkk-d10-kkk	2171
5602	5617	TATTCAATGTGGCTTC	959439	kkk-d10-kkk	2172
5603	5618	TTATTCAATGTGGCTT	959440	kkk-d10-kkk	1705

5603	5618	TTATTCAATGTGGCTT	995696	k-d10-kekek	1705
5603	5618	TTATTCAATGTGGCTT	995906	kk-d9-eeekk	1705
5603	5618	TTATTCAATGTGGCTT	996116	kk-d9-ekeke	1705
5604	5619	TTTATTCAATGTGGCT	959441	kkk-d10-kkk	1765
5604	5619	TTTATTCAATGTGGCT	995697	k-d10-kekek	1765
5604	5619	TTTATTCAATGTGGCT	995907	kk-d9-eeekk	1765
5604	5619	TTTATTCAATGTGGCT	996117	kk-d9-ekeke	1765
5605	5620	CTTTATTCAATGTGGC	916333	kkk-d10-kkk	1089
5605	5620	CTTTATTCAATGTGGC	995698	k-d10-kekek	1089
5605	5620	CTTTATTCAATGTGGC	995908	kk-d9-eeekk	1089
5605	5620	CTTTATTCAATGTGGC	996118	kk-d9-ekeke	1089
5605	5620	CTTTATTCAATGTGGC	996277	kek-d9-eekk	1089
5606	5621	ACTTTATTCAATGTGG	916334	kkk-d10-kkk	1158
5606	5621	ACTTTATTCAATGTGG	995699	k-d10-kekek	1158
5606	5621	ACTTTATTCAATGTGG	995909	kk-d9-eeekk	1158
5606	5621	ACTTTATTCAATGTGG	996119	kk-d9-ekeke	1158
5607	5622	TACTTTATTCAATGTG	959442	kkk-d10-kkk	1825
5608	5623	TTACTTTATTCAATGT	959443	kkk-d10-kkk	1885

The oligonucleotides were tested in a series of experiments. Cultured A-431 cells at a density of 10,000 cells per well were treated using free uptake with modified oligonucleotides diluted to different concentrations. After a treatment period of approximately 48 hours, PNPLA3 mRNA levels were measured as previously described using the Human PNPLA3 primer-probe set RTS36070. PNPLA3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. The IC_{50} ratios of the assays are presented in the tables below, which is the ratio of the IC_{50} of a benchmark oligonucleotide to the IC_{50} of the oligonucleotide. Hence, a bigger value of the ratio indicates that the oligonucleotide is more active than the benchmark.

Table 77

Efficacy of modified oligonucleotides targeting human PNPLA3

Start Site	Stop Site	Compound Number	Chemistry	IC_{50} ratio
5600	5615	959437	kkk-d10-kkk	1.42
5601	5616	959438	kkk-d10-kkk	0.49
5602	5617	959439	kkk-d10-kkk	0.36

5603	5618	959440	kkk-d10-kkk	0.55
5603	5618	995906	kk-d9-eeekk	1.42
5604	5619	959441	kkk-d10-kkk	1.66
5605	5620	916333	kkk-d10-kkk	1.96
5605	5620	995908	kk-d9-eeekk	0.70
5606	5621	916334	kkk-d10-kkk	0.95
5606	5621	995909	kk-d9-eeekk	1.47

Example 11: Screen and selection of antisense oligonucleotide to human PNPLA3

S-constrained ethyl (cEt)-modified 16-mer antisense oligonucleotides (ASOs) targeting the human *PNPLA3* gene were screened and tested for potency in human HepG2 cells, delivered by electroporation. A human cEt ASO (5′-GAGTTAAGTGCTGGAC-3′; SEQ ID NO: 115) was selected for all subsequent pharmacology studies. Specificity of the target knockdown was demonstrated using a chemistry-matched scrambled control ASO (5′-GGCCAATACGCCGTCA-3′; SEQ ID NO: 2173).

HepG2 cells were purchased from ATCC® (Manassas, VA). After thawing, cells were plated in T-75 flasks and grown in Minimum Essential Medium (MEM) containing 10% Fetal Bovine Serum (FBS) (HyClone Laboratories, Logan, UT).

Cells were seeded in 6-well plates or in 24-well plates with cover slips for Oil Red O (ORO) staining. Then cells were incubated with MEM (FBS 2%) and transfected with control or PNPLA3 ASOs (1 μM) or with control siRNA or PNPLA3 siRNA (10 nM) for 24 h using lipofectamine 3000 (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. Thereafter, cells were incubated with MEM (no FBS) for 24 h and a new transfection was performed. Control siRNA was a mixture of two negative control siRNA molecules (Ambion by Thermo Scientific, Waltham, MA). PNPLA3 siRNA was a mixture of three siRNA molecules (5′-GGUCCUCUCAGAUCUUGUGtt-3′ (SEQ ID NO: 2170); 5′-GGAGUGAGUGACAACGUACtt-3′ (SEQ ID NO: 2171); 5′-GGUUCUUGGAAGAGAAGGGtt-3′ (SEQ ID NO: 2172) (Ambion by Thermo Scientific, Waltham, MA).

For Oil Red O (ORO) staining, pictures were obtained using Axio KS 400 Imaging System and Axio Vision 4.8 Software (Zeiss, Oberkochen, Germany) at 100X magnification. ORO-stained area was quantified by BioPix iQ 2.1.4 software (BioPix AB, Gothenburg, Sweden).

Total RNA was isolated from the cells using RNeasy Mini Kit (Qiagen, Valencia, CA). First-strand complementary DNAs were synthesized from RNA using a reverse-transcription kit (Applied Biosystems, Foster City, CA). PNPLA3 and β-ACTIN mRNA expression were assessed in HepG2 cells transfected with control ASO, PNPLA3 ASO, control siRNA or PNPLA3 siRNA by real-time quantitative polymerase chain

reaction (PCR). TaqMan probes (PNPLA3 probe: Hs00228747_m1; β-ACTIN probe: Hs01060665_g1) and master mix (Life Technologies, Carlsbad, CA) were used according to the manufacturer's protocol. Real-time quantitative PCR assay was performed on a CFX Real-Time PCR Detection System (Bio-Rad, Hercules, CA).

To test whether knock-down of PNPLA3 148M mutant protein affects the intracellular neutral fat content, *PNPLA3* was suppressed in HepG2 cells (homozygous for the PNPLA3 148M/M mutation) by using ASO and siRNA as described herein. An approximately 70% reduction of the endogenous PNPLA3 mRNA expression was obtained using a *PNPLA3* ASO (Figure 1A). When intracellular neutral fat was examined by ORO staining, a 40% reduction of the intracellular lipid content with the PNPLA3 ASO was observed (Figure 1B,C). To confirm these data by an independent approach, PNPLA3 was suppressed in these cells by using siRNA and observed consistent results (Figure 1D-F).

Example 12: Antisense oligonucleotide treatment of wild type and PNPLA3 I148M knock-in mice

The materials and methods used in the Examples herein are further described in Lindén et al., *Molecular Metabolism* 22:49-61, 2019, incorporated by reference herein in its entirety.

S-constrained ethyl (cEt)-modified 16-mer ASOs targeting the mouse *Pnpla3* gene were screened and tested for potency in primary mouse embryonic cortical neurons via free uptake. A potent mouse (5'-TATTTTTGGTGTATCC-3'; SEQ ID NO: 2174) cEt ASO lead was selected for all subsequent pharmacological studies. This mouse Pnpla3 ASO was modified by 5'-conjugation with triantennary N-acetylgalactosamine ($GalNAc_3$) to further enhance the liver cell targeting in vivo following subcutaneous administration. The specificity of target knockdown was demonstrated using a chemistry-matched scrambled control GalNAc3- conjugated ASO (5'-GGCCAATACGCCGTCA-3'; SEQ ID NO: 2175). The control $GalNAc_3$-conjugated ASO did not affect body weight-gain, liver weight, plasma alanine aminotransferase (ALT) or liver triglyceride content when dosed at 10 mg/kg/week for six weeks in mice fed a NASH-inducing diet (D09100301, Research Diets, New Brunswick, NJ) as compared to saline vehicle controls.

All animal experiments were performed with humane care and were approved by the Gothenburg Ethics Committee for Experimental Animals in Sweden. The holding facility has received full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

The human PNPLA3 I148M mutation was introduced into the mouse *Pnpla3* gene by replacing the isoleucine codon with a methionine codon in amino acid position 148 of the mouse *Pnpla3* gene using homologous recombination. Founders were backcrossed with C57BL/6N females to generate heterozygous Pnpla3 148I/M mice. Sequence-verified heterozygous *Pnpla3* 148I/M mice were intercrossed to generate experimental homozygous Pnpla3 148M/M and wild-type littermates (Pnpla3 148I/I) as control mice for the dietary challenge and ASO pharmacology studies. All experimental animals were verified to have the correct

genotype using PCR before the study began and verified again using PCR after termination. A few experimental animals were also verified by cDNA sequencing. All animals were housed in transparent Makrolon cages with aspen wood chip bedding and nesting material, and the temperature- (21 ± 1 °C) and humidity (50 ± 10%) of the holding facility were controlled. The mice had free access to tap water and food and were on a 12-h day/night cycle.

Female *Pnpla3* 148M/M (n = 21) and wild-type littermates (n = 19) (6–8 weeks of age) were fed a high-sucrose diet (70% sucrose diet; TD98090, Envigo, Huntingdon, UK) for 15 weeks. Female mice were used in this experiment to replicate the established model by Smagris et al. *Hepatology* 61(1):108-118, 2015, who used female animals in their high-sucrose diet experiments. In addition, in a pilot experiment, female mice accumulated more triglycerides in the liver compared to male mice when fed this diet. In addition, female mice fed the high-sucrose diet accumulated more liver triglycerides compared to mice fed a regular chow diet containing (energy percentage) 12% fat, 62% carbohydrates, and 26% protein, with a total energy content of 3 kcal/g (R3; Lactamin, Kimstad, Sweden). After 5 weeks of feeding on the high-sucrose diet, liver lipid levels were assessed using a magnetic resonance imaging (MRI)–derived marker, proton-density fat fraction (PDFF). The mice were then assigned to $GalNAc_3$-conjugated ASO study groups (n = 9–12 animals/group) based on randomized stratification for body weight and liver lipid contents before the treatment was initiated. During the last 8 weeks of the study, groups of mice were dosed with either the control ASO or *Pnpla3* ASO (5 mg/kg/week administered by two subcutaneous injections per week with saline as vehicle). After 6 weeks of ASO dosing, liver lipid levels were again assessed using MRI. Before unfasted mice were euthanized at 8:00–10:00 AM, the mice were metabolically synchronized for 24 h by withdrawing the food from 8:00 AM to 8:00 PM and then allowed free access to the food again from 8:00 PM to 8:00 AM. Mice were euthanized with isoflurane (Forene, Abbot Scandinavia AB, Sweden), blood was collected and plasma isolated, livers were collected, and pieces (same position in the left lateral lobe for all mice) were fixed with 4% formaldehyde in PBS for histology or snap-frozen in liquid N_2 and stored at −80 °C.

Male *Pnpla3148* M/M mice (n = 17) and wild-type littermates (n = 17) (6–8 weeks of age) were fed a diet high in fat (40%, containing 18% trans-fat), carbohydrates (40%, containing 20% fructose) and cholesterol (2%) (NASH diet; D09100301, Research Diets, New Brunswick, NJ) for 26 weeks. In a separate experiment, liver *Pnpla3* mRNA, triglyceride content as well as plasma ALT levels were found to be elevated in wild-type male mice fed the NASH-inducing diet as compared to mice fed a regular chow diet. The mice were assigned to $GalNAc_3$-conjugated ASO study groups (n = 8–9 mice/group) based on body weight, and during the last 14 weeks, the mice were dosed with either the control ASO or *Pnpla3* ASO (5 mg/kg/week administered by two subcutaneous injections per week with saline as vehicle). Before unfasted mice were euthanized at 8:00–10:00 AM, the mice were metabolically synchronized for 24 h as described above. Mice were euthanized with isoflurane (Forene, Abbot Scandinavia AB, Sweden), blood was collected and plasma isolated, livers were

collected, and pieces (same position in the left lateral lobe for all mice) were fixed with 4% formaldehyde in PBS for histology or snap-frozen in liquid N2 and stored at −80 °C.

Example 13: Effect of *Pnpla3* ASO on liver steatosis in wild-type and I148M mice fed a high-sucrose diet

To drive hepatic lipogenesis and to evaluate the impact of Pnpla3 silencing on hepatic fat accumulation, homozygous *Pnpla3* 148M/M (mutant) knock-in female mice and wild-type littermates were fed a high-sucrose diet (70%) for 15 weeks as described in Example 11. During the last 8 weeks of the experiment, mice of the two genotypes were treated with $GalNAc_3$-conjugated *Pnpla3* or $GalNAc_3$-conjugated control ASOs. No differences in body weight gain, food intake (Figure 2A and B) or in ovarian white adipose tissue weight were observed between the groups. In addition, the *Pnpla3* ASO treatment did not affect plasma glucose or insulin levels. Compared to the control ASO, the *Pnpla3* ASO treatment markedly reduced the hepatic expression of *Pnpla3* mRNA (98% reduction, $p < 0.0001$) and levels of PNPLA3 protein on lipid droplets in both *Pnpla3* mutant knock-in and wild-type mice (Figure 2C and D). The *Pnpla3* ASO treatment did not affect the white adipose tissue expression of *Pnpla3* mRNA levels.

Six weeks of the *Pnpla3* ASO treatment reduced liver lipid levels by 20% in *Pnpla3* mutant knock-in mice, as measured by MRI (Figure 2F, $p = 0.025$). After 8 weeks of treatment, the *Pnpla3* mutant knock-in mice treated with the *Pnpla3* ASO showed reduced liver weights, lower liver Oil Red O staining of neutral lipids (Figure 2E), a 20% reduction ($p = 0.038$) in liver triglyceride content, as measured by biochemical analyses (Figure 2F), while no changes in circulating plasma triglyceride levels were observed (Figure 2G). Interestingly, the *Pnpla3* ASO treatment did not affect the liver weights, lipid level or liver triglyceride content in the wild-type mice (Figure 2H–J). *Pnpla3* mutant knock-in mice treated with the control ASO had 30% higher liver triglyceride content than the wild-type mice treated with the control ASO (*Pnpla3* mutant knock-in mice = 5.7 ± 0.4 g/100 g liver, wild-type mice = 4.4 ± 0.5 g/100 g liver, $p = 0.046$).

Example 14: Effect of *Pnpla3* ASO on liver inflammation and fibrosis in wild-type and I148M mice fed a NASH-inducing diet

Male *Pnpla3* mutant knock-in ($n = 17$) and wild-type littermate ($n = 17$) mice were fed a NASH-inducing diet for 26 weeks as described in Example 11. During the last 14 weeks of the experiment, mice of both genotypes were treated with a $GalNAc_3$-conjugated *Pnpla3* or $GalNAc_3$-conjugated control ASO. No differences in body weight gain, food intake (Figure 3A and B), or in epididymal white adipose tissue weight were observed between the groups. In addition, the *Pnpla3* ASO treatment did not affect plasma glucose or insulin levels. Compared to the control ASO, the *Pnpla3* ASO treatment markedly reduced (97%, $p < 0.0001$) hepatic expression of the *Pnpla3* mRNA and consistently reduced levels of the Pnpla3 protein on lipid droplets

in both *Pnpla3* mutant knock-in and wild-type mice (Figure 3C and D). Using the NASH diet with a longer treatment period than in the sucrose diet study (14 weeks vs 8 weeks, respectively), the *Pnpla3* ASO treatment also reduced the white adipose tissue expression of Pnpla3 mRNA levels.

The *Pnpla3* ASO treatment reduced plasma ALT levels in mice of both genotypes (*Pnpla3* mutant knock-in $p = 0.0006$, wild-type $p = 0.018$), while plasma AST was unchanged (Figure 3E and F). The *Pnpla3* ASO treatment reduced liver weights only in the *Pnpla3* mutant knock-in mice, triglyceride contents in both Pnpla3 mutant knock-in ($p = 0.002$) and wild-type mice ($p = 0.004$), while no changes in circulating plasma triglyceride levels were observed (Figure 3E and F).

The *Pnpla3* ASO treatment improved the liver steatosis score ($p = 0.007$), lobular inflammation score ($p = 0.018$), NAFLD activity score (NAS) ($p = 0.0003$), and fibrosis stage ($p = 0.031$) in *Pnpla3* mutant knock-in mice (Figure 4A) while only liver steatosis score ($p = 0.003$) and NAS ($p = 0.036$) were improved in the wild-type mice (Figure 4B). Hepatocellular ballooning degeneration was not found in any of the livers.

Example 15: Effect of *Pnpla3* ASO on *de novo* lipogenesis and palmitoleic acid in wild-type and I148M mice fed a NASH-inducing diet

The *Pnpla3* ASO treatment reduced the liver Oil Red O staining of neutral lipids in both *Pnpla3* mutant knock-in and wild-type mice (Figure 5A). The *Pnpla3* ASO treatment reduced the mRNA expression of lipogenic genes, such as acetyl-CoA carboxylase 1 (Acc1) and stearoyl-CoA desaturase 1 (Scd1) in both genotypes (Figure 5B and C), suggesting that hepatic lipogenesis was reduced. *Pnpla3* ASO treatment decreased the relative amount of monounsaturated fatty acids (MUFAs, $p = 6.1 \times 10^{-5}$ and 7.6×10^{-6} in the mutant and wild-type, respectively) and increased polyunsaturated fatty acids (PUFAs, $p = 1.2 \times 10^{-4}$ and 1.3×10^{-5} in the mutant and wild-type, respectively) irrespective of the genotype (Figures 5D-E and 6). Specifically, the reduction in MUFAs was stronger for the palmitoleic acid (16:1), 36% ($p = 2.4 \times 10^{-4}$) and 30% (1.0×10^{-9}) reduction for the mutant and wild-type, respectively as compared with the oleic acid where the reduction was only 2% ($p = 0.034$) and 5% ($p = 0.001$) for the mutant and wild-type, respectively.

Example 16: Effect of *Pnpla3* ASO on protein levels of haptoglobin, Mcp1 and Timp2 in wild-type and I148M mice fed a NASH-inducing diet

The *Pnpla3* ASO treatment reduced plasma haptoglobin levels ($p = 0.0005$) and the liver macrophage content ($p = 0.047$) in *Pnpla3* mutant knock-in mice but not in the wild-type littermates (Figure 7A–C), suggesting that *Pnpla3* suppression specifically reduced liver inflammation in the mutant mice. Pnpla3 ASO treatment reduced the liver Mcp1 (Figure 7D) protein levels in *Pnpla3* mutant knock-in mice. *Pnpla3* ASO treatment did not change the liver protein expression levels of Il1β (Figure 7E), Il6 (Figure 7F), Tnfα (Figure 7G), or αSma (Figure 7H) in either genotype. The *Pnpla3* ASO treatment reduced the hepatic expression of the

collagen type I alpha 1 (Col1a1) mRNA in both *Pnpla3* mutant knock-in and wild-type mice (Figure 8A and B). The *Pnpla3* ASO treatment reduced the liver collagen, as measured by immunohistochemistry ($p = 0.04$), in the Pnpla3 mutant knock-in mice (Figure 8A–C). Although ASO treatment tended to decrease liver hydroxyproline levels, no significant differences were observed (Figure 8D and E). *Pnpla3* ASO treatment reduced the liver Timp2 protein levels in *Pnpla3* mutant knock-in mice ($p = 0.007$) (Figure 9A). *Pnpla3* ASO treatment did not change the liver protein expression levels of Mmp2 (Figure 9B), Timp1 (Figure 9C), or Tgfβr2 (Figure 9D) in either genotype.

WHAT IS CLAIMED:

1. A method of treating an individual having or at risk of having liver disease, comprising administering a compound targeted to PNPLA3 to the individual,

wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

2. A method of reducing one or more of liver damage, hepatic steatosis, liver inflammation, liver fibrosis, and hepatic lipogenesis in an individual, comprising administering a compound targeted to PNPLA3 to the individual,

wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

3. A method of reducing protein levels of one or more of haptoglobin, MCP1, and TIMP2 in an individual, comprising administering a compound targeted to PNPLA3 to the individual,

wherein the individual has an I148M mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3).

4. The method of claim 1, wherein the liver disease is selected from non-alcoholic fatty liver disease (NAFLD), hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma, alcoholic liver disease, alcoholic steatohepatitis (ASH), HCV hepatitis, chronic hepatitis, hereditary hemochromatosis, or primary sclerosing cholangitis.

5. The method of claim 4, wherein the liver disease is hepatic steatosis.

6. The method of claim 2, wherein the method reduces or inhibits liver inflammation or liver fibrosis.

7. The method of claim 2 or 6, wherein reducing or inhibiting liver inflammation comprises reducing liver macrophage levels.

8. The method of claim 7, wherein the liver macrophage levels are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunohistochemical staining of a liver section of the individual.

9. The method of claim 3, wherein the protein levels of haptoglobin are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by a colorimetric assay of serum or plasma of the individual.

10. The method of claim 3, wherein the protein levels of MCP1 are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunoblotting of a liver sample of the individual.

11. The method of claim 3, wherein the protein levels of TIMP2 are reduced by at least 20% relative to an individual not administered the compound targeted to PNPLA3, as measured by immunoblotting of a liver sample of the individual.

12. The method of any of claims 1 to 11, wherein the individual has a homozygous I148M mutation in PNPLA3.

13. The method of any of claims 1 to 12, wherein the individual is a human individual.

14. The method of any of claims 1 to 13, wherein the compound targeted to PNPLA3 is an antisense compound targeted to PNPLA3.

15. The method of claim 14, wherein the antisense compound targeted to PNPLA3 is a short interfering RNA (siRNA).

16. The method of claim 14, wherein the antisense compound targeted to PNPLA3 is an antisense oligonucleotide (ASO).

17. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length having a having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-2169.

18. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-2169.

19. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 17-2169.

20. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731,

5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein the nucleobase sequence of the modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2.

21. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence complementary within nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2, and wherein said modified oligonucleotide is at least 85%, at least 90%, at least 95%, or 100% complementary to SEQ ID NO: 2.

22. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of a PNPLA3 nucleic acid having the nucleobase sequence of SEQ ID NO: 2, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 2.

23. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising a 16 nucleobase portion complementary to an equal length portion of nucleobases 5567-5642, 5644-5731, 5567-5731, 5567-5620, 13697-13733, 20553-20676, 20664-20824, 20553-20824, or 25844-25912 of SEQ ID NO: 2.

24. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 8 to 80 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899.

25. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899.

26. The method of any of claims 17 to 25, wherein the modified oligonucleotide has a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% complementary to SEQ ID NO: 2 over the entire length of the nucleobase sequence.

27. The method of any of claims 17 to 26, wherein the modified nucleotide comprises at least one modification selected from at least one modified internucleoside linkage, at least one modified sugar, and at least one modified nucleobase.

28. The method of claim 27, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

29. The method of claim 27 or 28, wherein the modified sugar is a bicyclic sugar.

30. The method of claim 29, wherein the bicyclic sugar is selected from the group consisting of: 4'-(CH2)-O-2' (LNA); 4'-(CH2)2-O-2' (ENA); and 4'-CH(CH3)-O-2' (cEt).

31. The method of claim 27 or 28, wherein the modified sugar is 2'-O-methoxyethyl.

32. The method of any of claims 27 to 31, wherein the modified nucleobase is a 5-methylcytosine.

33. The method of any one of claims 17 to 32, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

34. The method of any of claims 17 to 33, wherein the compound targeted to PNPLA3 is single-stranded.

35. The method of any of claims 17 to 33, wherein the compound targeted to PNPLA3 is double-stranded.

36. The method of any of claims 17 to 35, wherein the compound targeted to PNPLA3 comprises ribonucleotides.

37. The method of any of claims 17 to 29, wherein the compound targeted to PNPLA3 comprises deoxyribonucleotides.

38. The method of any of claims 17 to 37, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides.

39. The method of any of claims 17 to 37, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides.

40. The method of any of claims 17 to 37, wherein the modified oligonucleotide consists of 15 to 30 linked nucleosides.

41. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 16 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

42. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide 16 linked nucleosides in length, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 1089, 1757, 141, 1982, 330, 1665, 408, 830, and 899, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment and the 3' wing segment comprise cEt sugars; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

43. The method of any of claims 17 to 42, wherein the compound targeted to PNPLA3 comprises a conjugated moiety and a conjugate linker.

44. The method of claim 43, wherein the conjugate group comprises a GalNAc cluster comprising 1 to 3 GalNAc ligands.

45. The method of claim 43 or 44, wherein the conjugate linker consists of a single bond.

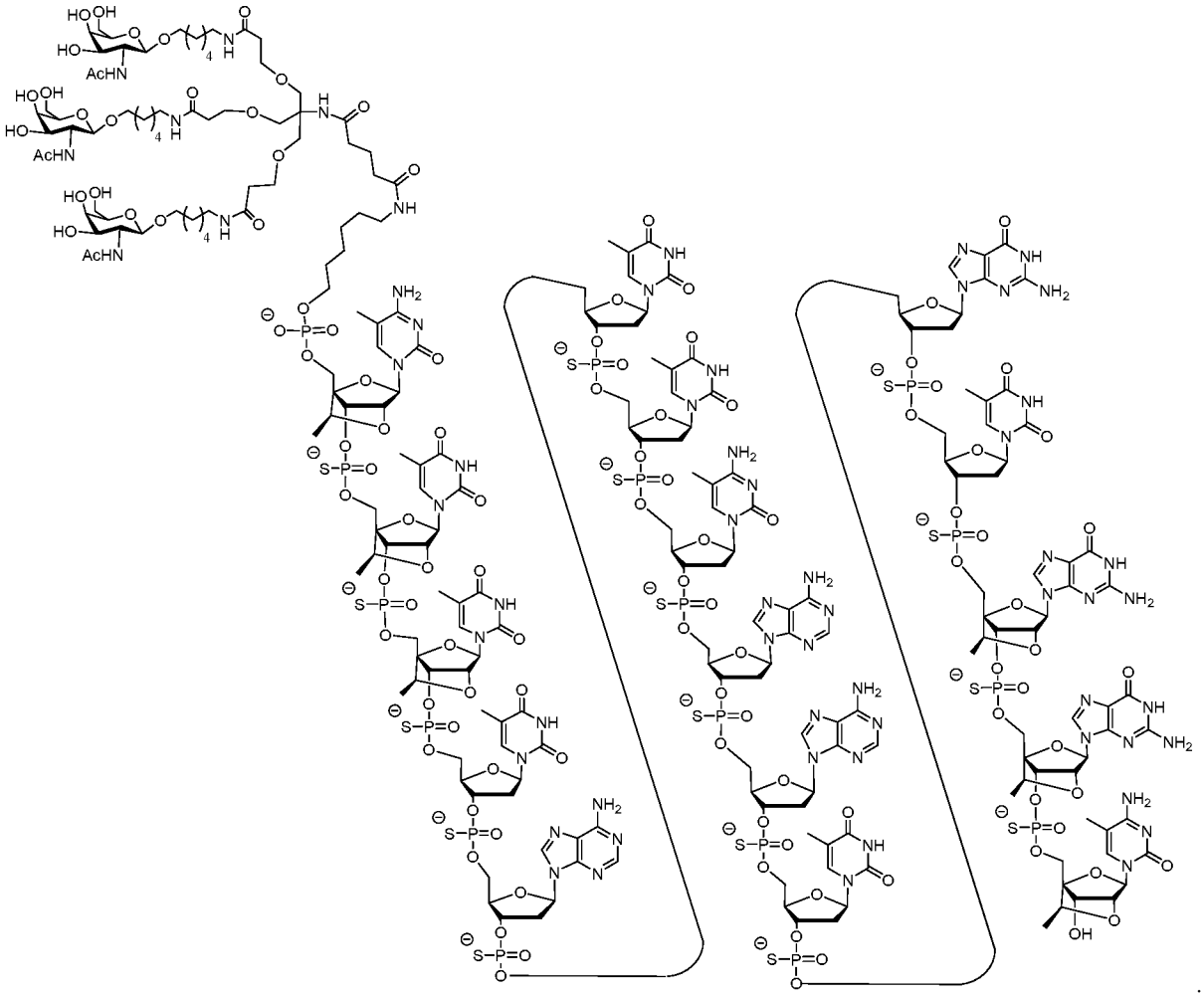
46. The method of claim 45, wherein the conjugate linker is cleavable.

47. The method of claim 45, wherein the conjugate linker comprises 1 to 3 linker-nucleosides.

48. The method of any of claims 43 to 47, wherein the conjugate group is attached to the modified oligonucleotide at the 5'-end of the modified oligonucleotide.

49. The method of any of claims 43 to 47, wherein the conjugate group is attached to the modified oligonucleotide at the 3'-end of the modified oligonucleotide.

50. The method of claim 14, wherein the compound targeted to PNPLA3 comprises the following formula or salt thereof:



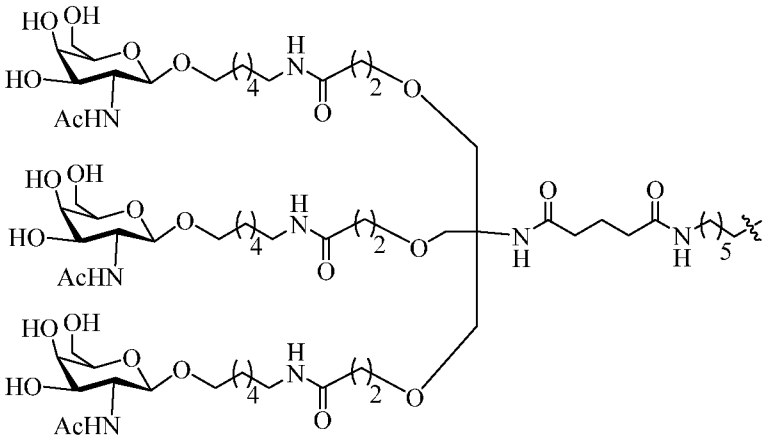
51. The method of claim 14, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide is 16 linked nucleosides in length and consists of the sequence of SEQ ID NO: 1089, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

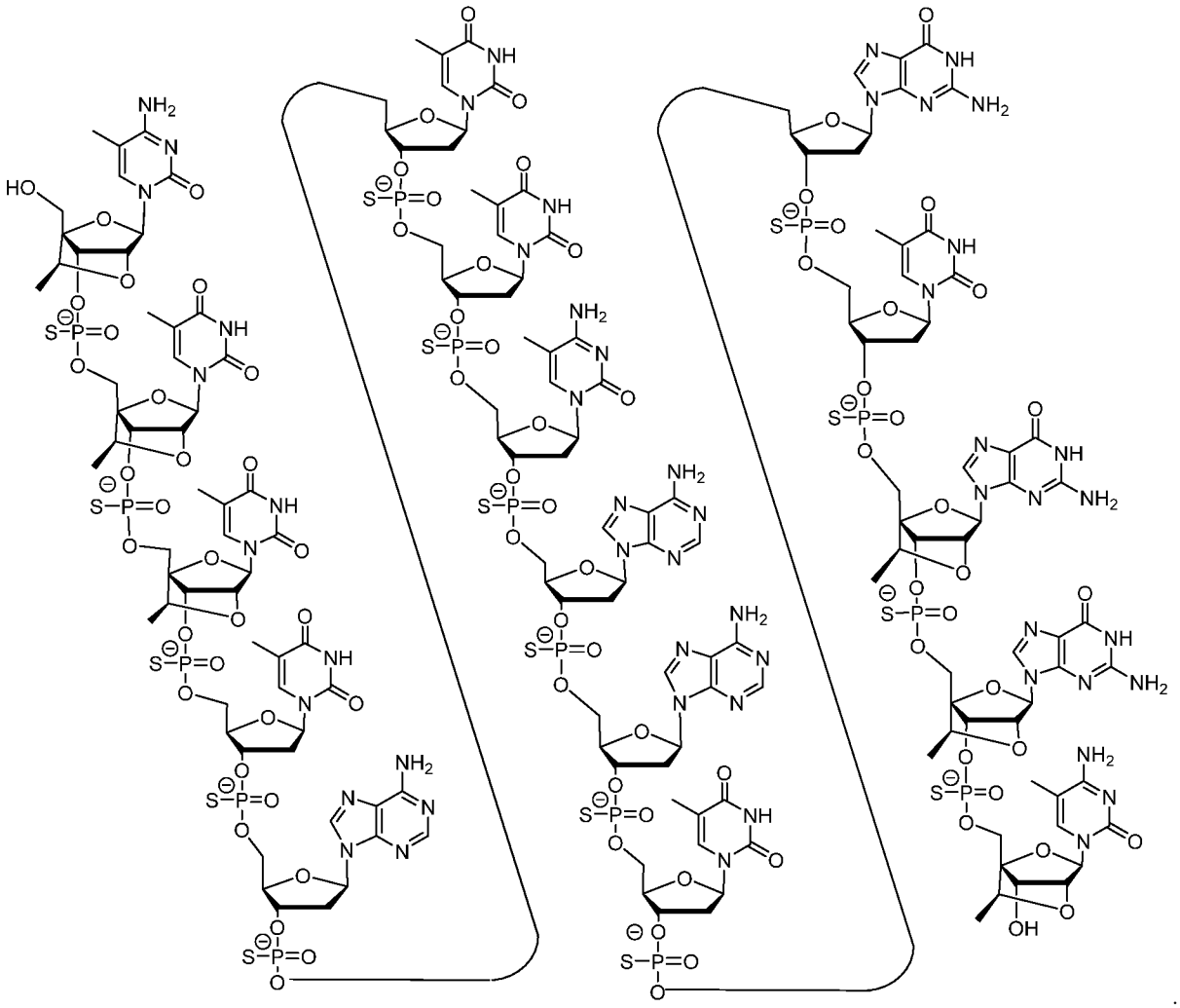
a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; wherein each cytosine is a 5-methylcytosine; and wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



52. The method of claim 14, wherein the compound targeted to PNPLA3 has the following formula or salt thereof:



53. The method of claim 52, wherein the compound targeted to PNPLA3 is a modified oligonucleotide in a pharmaceutically acceptable salt form.

54. The method of claim 53, wherein the pharmaceutically acceptable salt is a sodium salt.

55. The method of claim 53, wherein the pharmaceutically acceptable salt is a potassium salt.

56. The method of any of claims 1 to 55, wherein the compound targeted to PNPLA3 is administered to the individual as a composition comprising the compound targeted to PNPLA3 and a pharmaceutically acceptable carrier.

57. The method of any of claims 1 to 56, wherein the compound targeted to PNPLA3 is administered parenterally to the individual.

58. The method of any of claims 1 to 14 or 16 to 57, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to SEQ ID NO: 115.

59. The method of any of claims 1 to 15 or 17 to 57, wherein the compound targeted to PNPLA3 comprises a modified oligonucleotide, wherein the modified oligonucleotide has a nucleobase sequence at least 90% identical to any one of SEQ ID NOs: 2170-2172.

60. The method of claim 14, wherein the compound targeted to PNPLA3 consists of the sequence of SEQ ID NO: 115, wherein the modified oligonucleotide comprises:

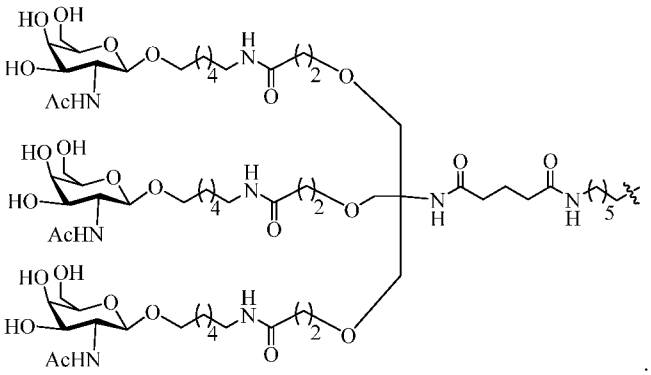
a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

61. The method of claim 60, wherein the compound targeted to PNPLA3 further comprises a conjugate group, wherein the conjugate group is positioned at the 5'end of the modified oligonucleotide and is



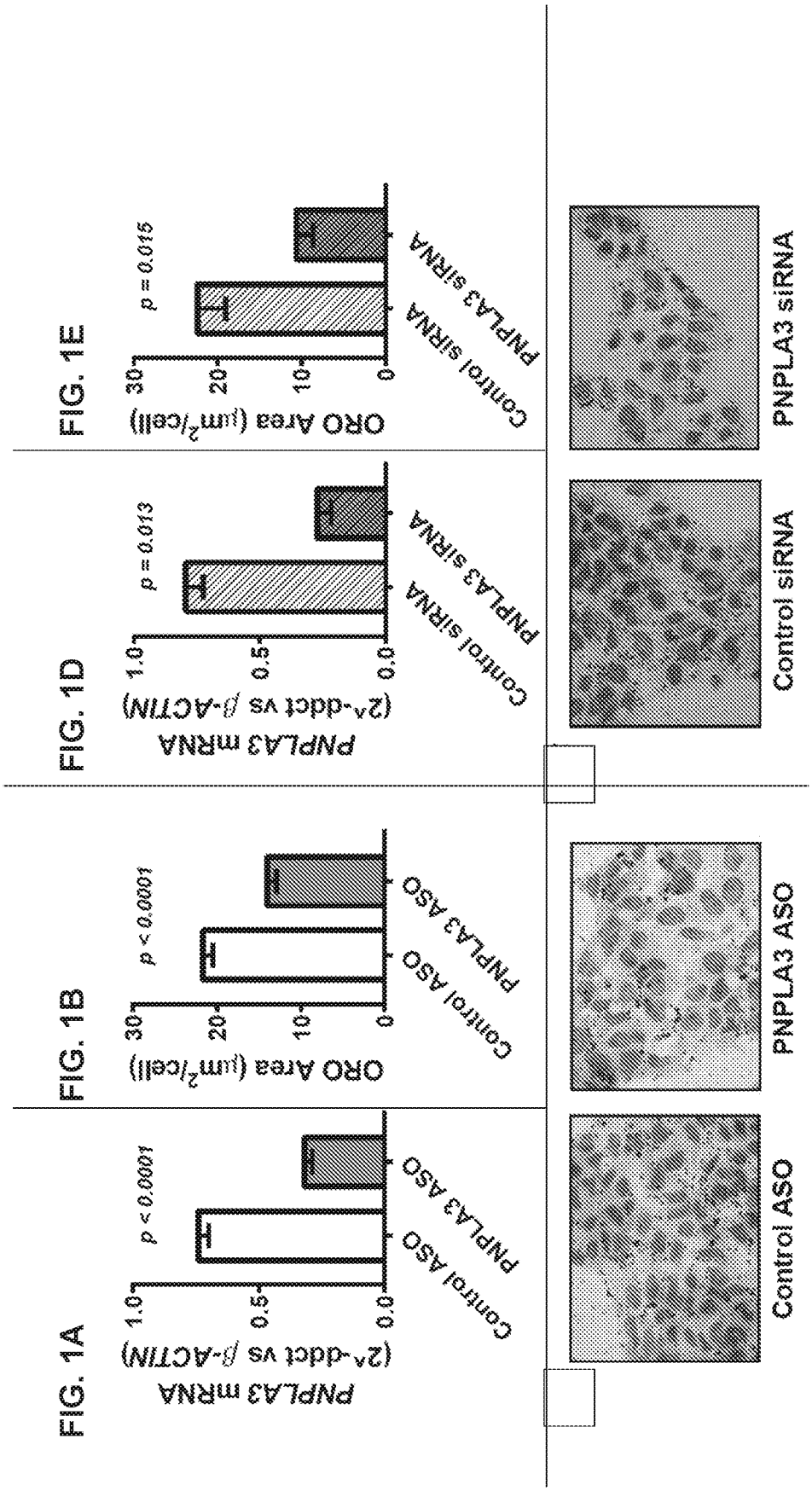

FIG. 1A
PNPLA3 mRNA (2^-ddct vs β-ACTIN)
p < 0.0001
1.0
0.5
0.0
Control ASO
PNPLA3 ASO
FIG. 1B
ORO Area (μm²/cell)
p < 0.0001
30
20
10
0
Control ASO
PNPLA3 ASO
FIG. 1D
PNPLA3 mRNA (2^-ddct vs β-ACTIN)
p = 0.013
1.0
0.5
0.0
Control siRNA
PNPLA3 siRNA
FIG. 1E
ORO Area (μm²/cell)
p = 0.016
30
20
10
0
Control siRNA
PNPLA3 siRNA
FIG. 1C
Control ASO
PNPLA3 ASO
FIG. 1F
Control siRNA
PNPLA3 siRNA

FIG. 1

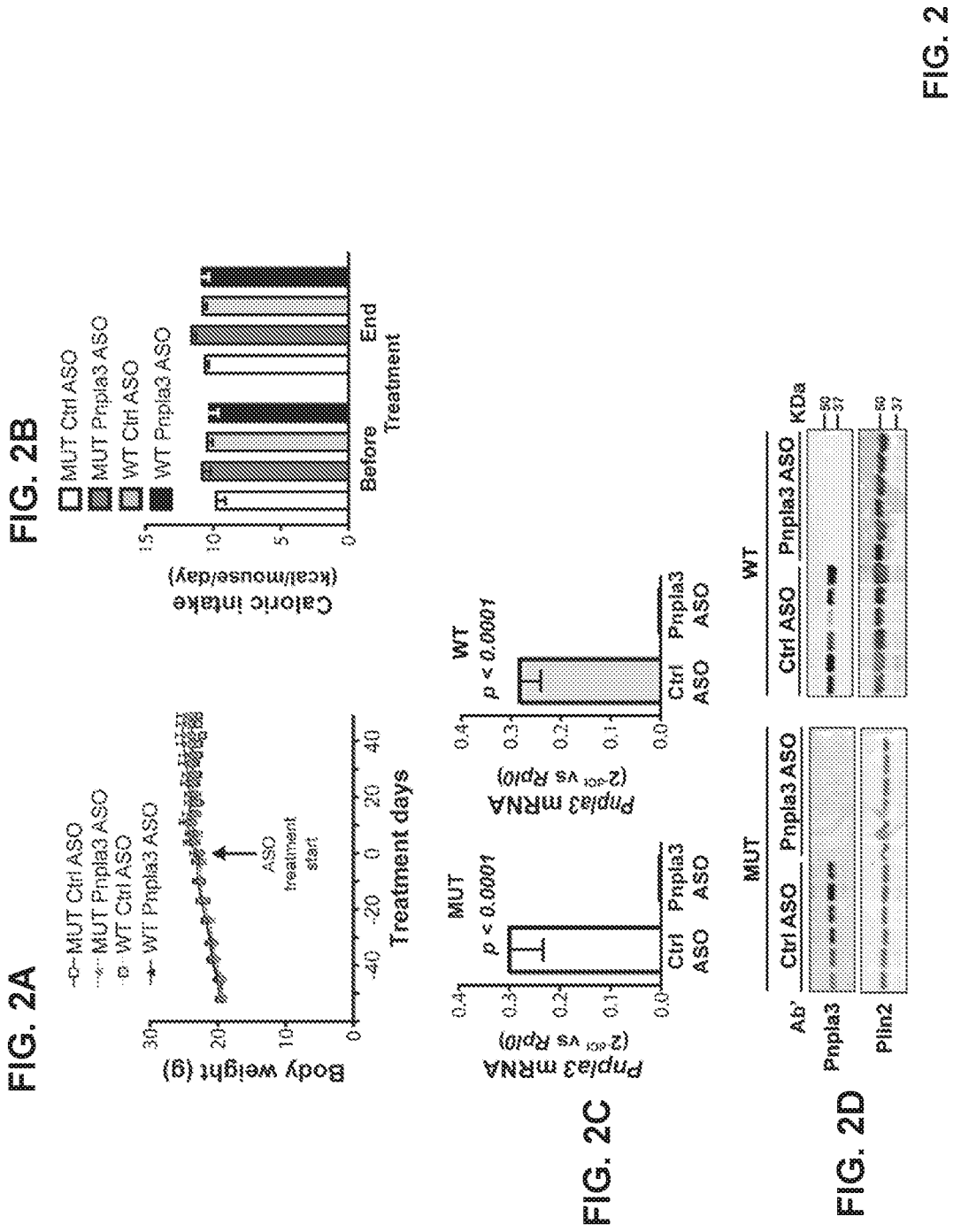

FIG. 2A
MUT Ctrl ASO
MUT Pnpla3 ASO
WT Ctrl ASO
WT Pnpla3 ASO
Body weight (g)
ASO treatment start
Treatment days
FIG. 2B
Caloric intake (kcal/mouse/day)
Before Treatment
End Treatment
FIG. 2C
Pnpla3 mRNA (2-dCt vs Rplp0)
MUT
p < 0.0001
Ctrl ASO
Pnpla3 ASO
WT
p < 0.0001
FIG. 2D
Ab'
MUT
WT
Ctrl ASO
Pnpla3 ASO
KDa
Pnpla3
Plin2

FIG. 2

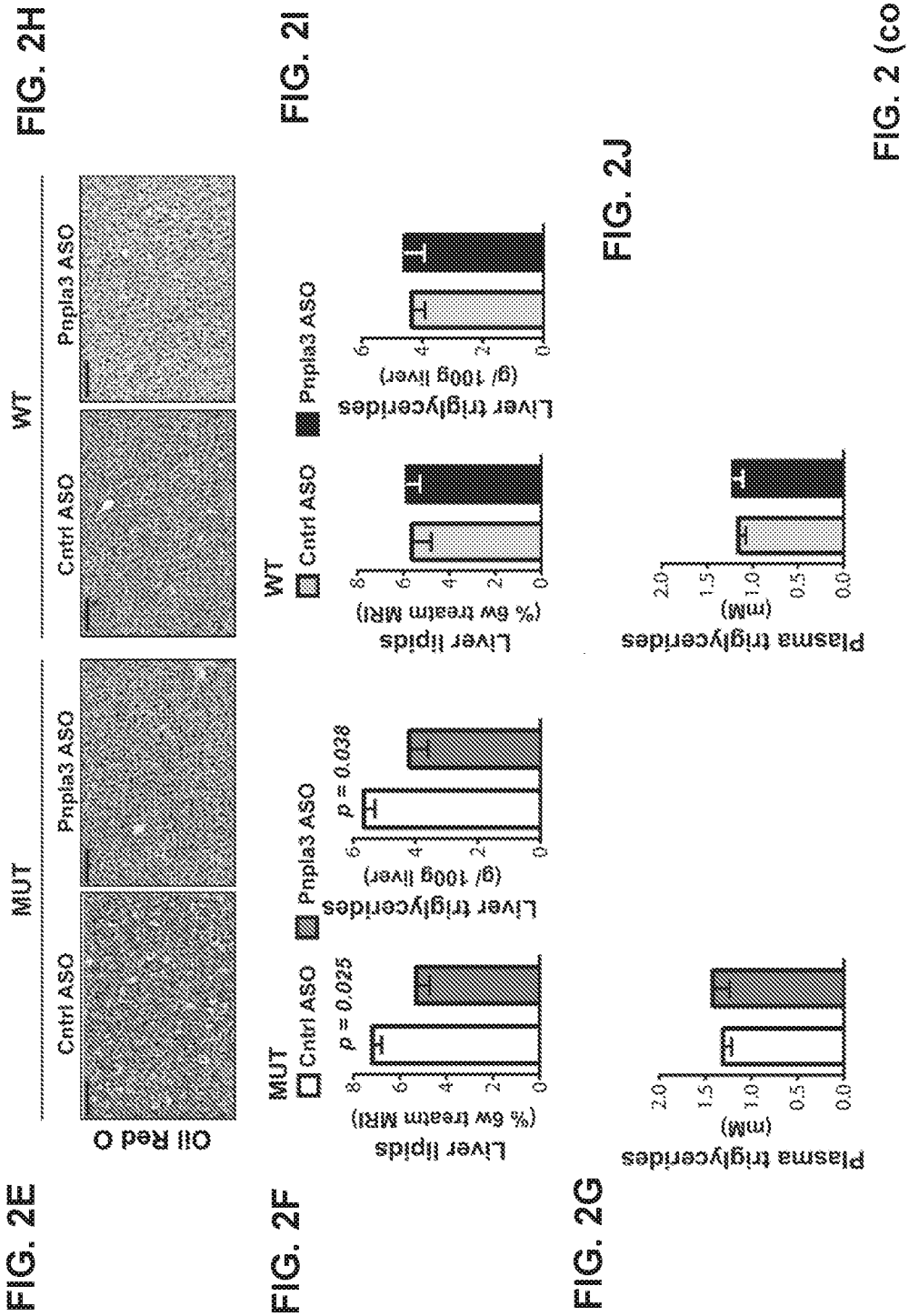


FIG. 2E

FIG. 2F

FIG. 2G

FIG. 2H

FIG. 2I

FIG. 2J

FIG. 2 (cont'd)

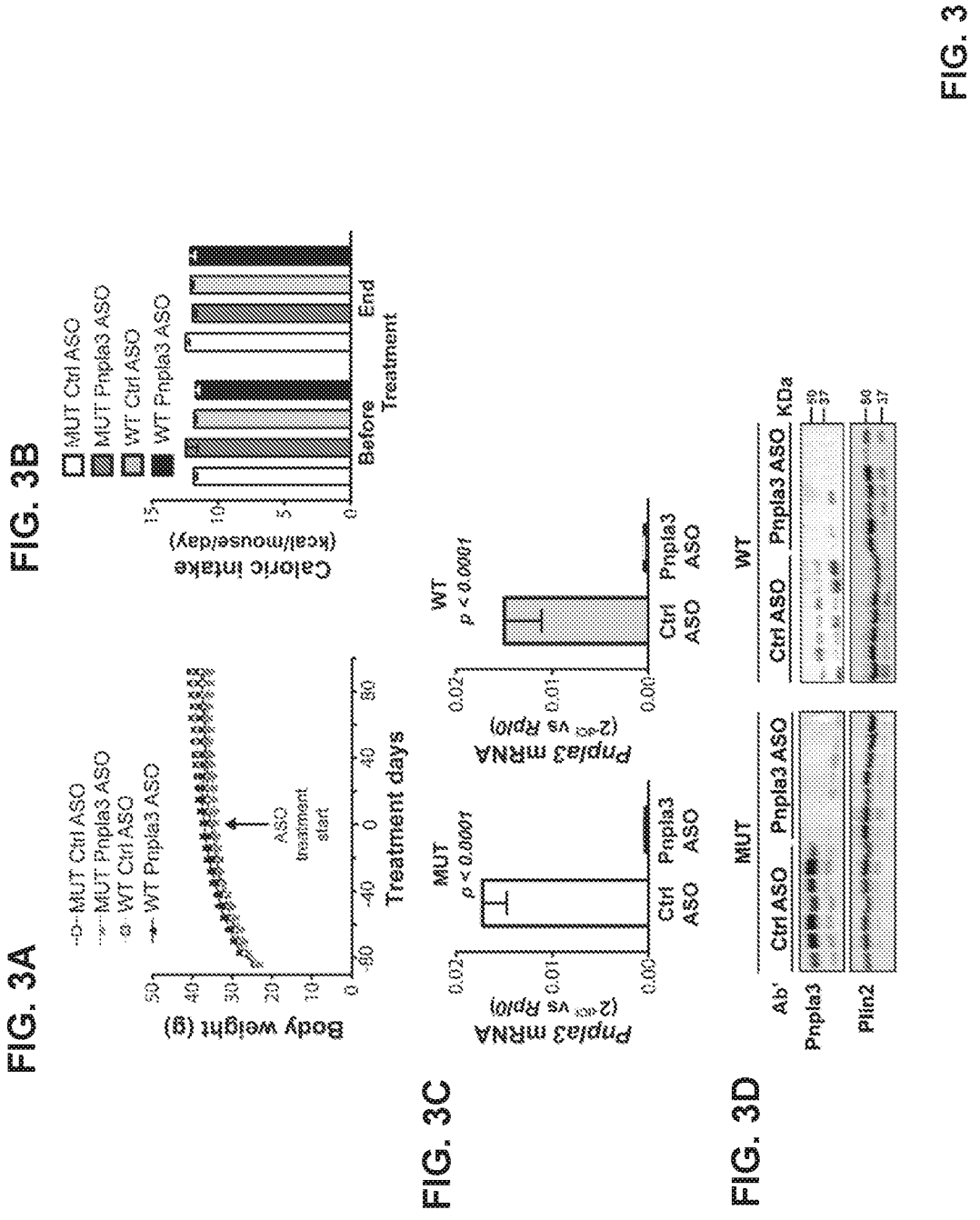

FIG. 3A
MUT Ctrl ASO
MUT Pnpla3 ASO
WT Ctrl ASO
WT Pnpla3 ASO
Body weight (g)
ASO treatment start
Treatment days
FIG. 3B
Caloric intake (kcal/mouse/day)
Before Treatment
End Treatment
FIG. 3C
MUT
p < 0.0001
WT
p < 0.0001
Pnpla3 mRNA (2^-dCt vs Rplp0)
Ctrl ASO
Pnpla3 ASO
FIG. 3D
MUT
WT
Ctrl ASO
Pnpla3 ASO
KDa
Ab'
Pnpla3
Plin2

FIG. 3

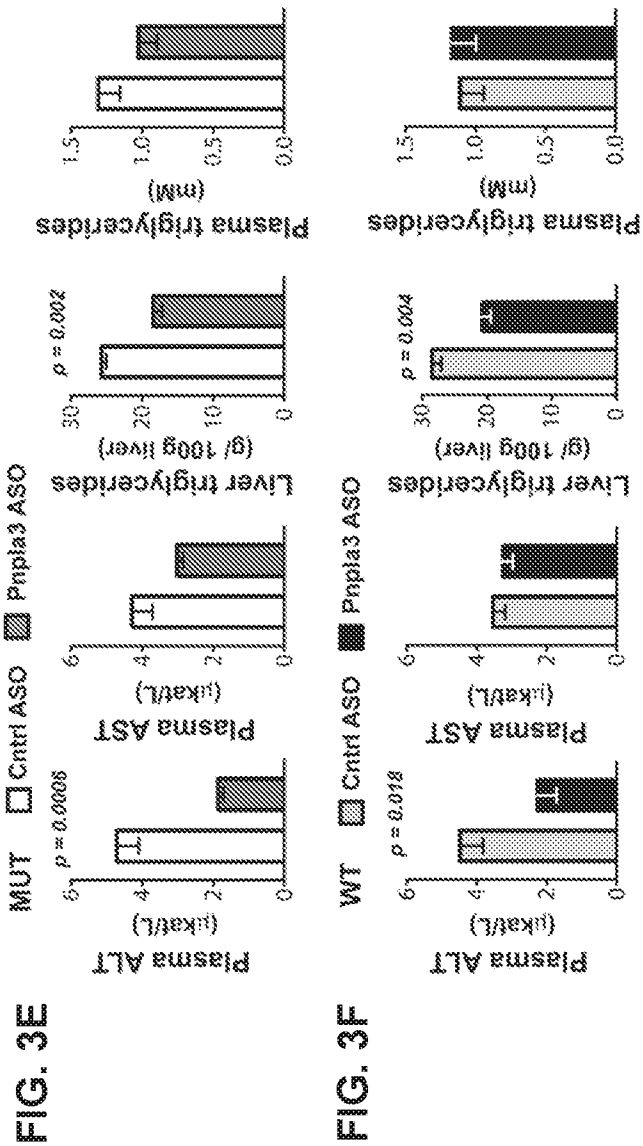


FIG. 3E

FIG. 3F

FIG. 3 (cont'd)

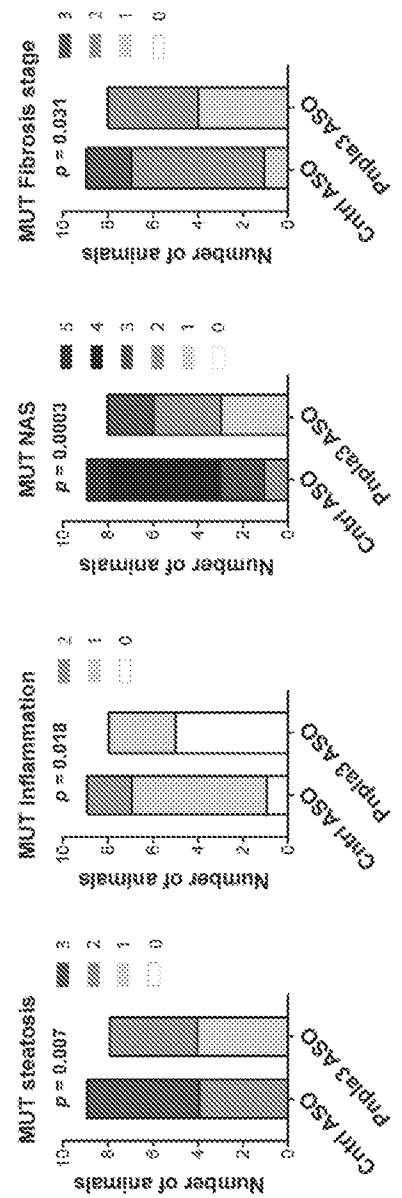


FIG. 4A

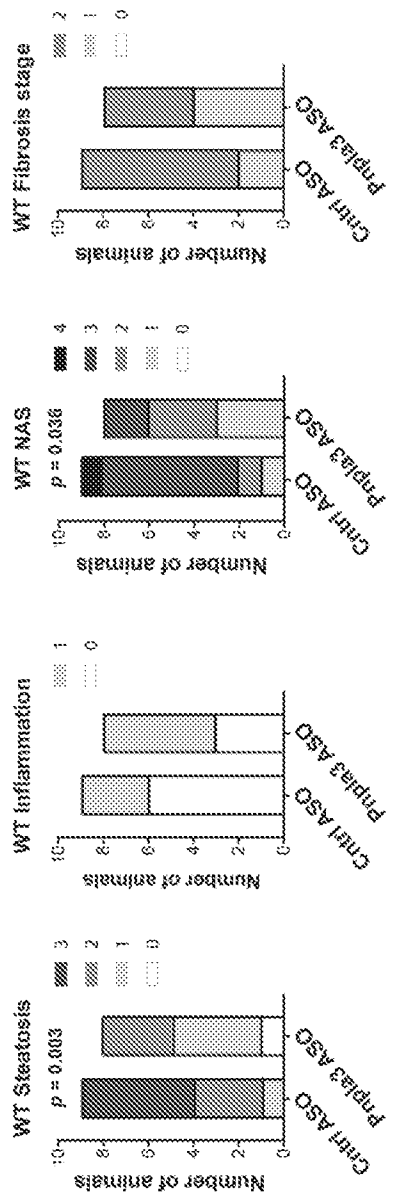


FIG. 4B

FIG. 4

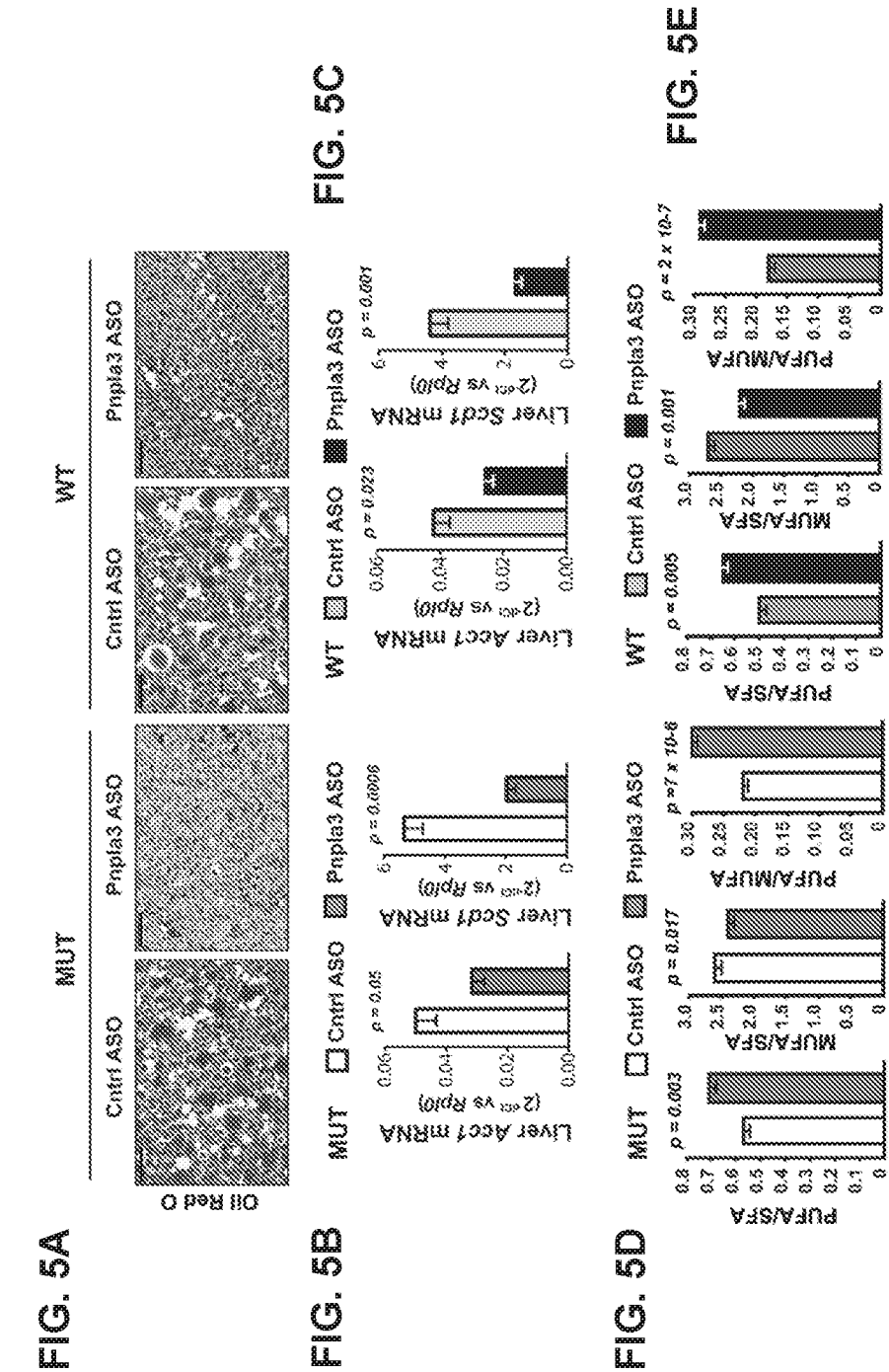

FIG. 5A
MUT
WT
Cntrl ASO
Pnpla3 ASO
Cntrl ASO
Pnpla3 ASO
Oil Red O
FIG. 5B
MUT
Cntrl ASO
Pnpla3 ASO
Liver Acc1 mRNA
p = 0.05
Liver Scd1 mRNA
p = 0.0006
FIG. 5C
WT
Cntrl ASO
Pnpla3 ASO
Liver Acc1 mRNA
p = 0.023
Liver Scd1 mRNA
p = 0.001
FIG. 5D
MUT
Cntrl ASO
Pnpla3 ASO
PUFA/SFA
p = 0.003
MUFA/SFA
p = 0.017
PUFA/MUFA
p = 7 x 10-8
FIG. 5E
WT
Cntrl ASO
Pnpla3 ASO
PUFA/SFA
p = 0.005
MUFA/SFA
p = 0.001
PUFA/MUFA
p = 2 x 10-7

FIG. 5

	MUT			WT		
	CTR	ASO	*P* value	CTR	ASO	*P* value
MUFA	63 (0.47)	58 (0.43)	6.1×10^{-5}	64 (0.39)	57 (0.58)	7.6×10^{-6}
16:1	10 (0.22)	7 (0.35)	2.4×10^{-4}	11 (0.17)	7 (0.13)	1.0×10^{-9}
18:1	53 (0.46)	52 (0.21)	0.034	54 (0.52)	51 (0.53)	0.001
PUFA	14 (0.29)	17 (0.50)	1.1×10^{-5}	12 (0.38)	17 (0.48)	1.3×10^{-6}
18:2	11 (0.42)	13 (0.48)	0.007	11 (0.35)	13 (0.49)	0.006
20:3	0.78 (0.03)	1.08 (0.05)	1.3×10^{-4}	0.53 (0.04)	1.01 (0.03)	3.3×10^{-5}
20:4	0.68 (0.02)	1.04 (0.03)	2.6×10^{-7}	0.31 (0.02)	1.09 (0.03)	1.2×10^{-7}
22:5	0.32 (0.05)	0.95 (0.05)	2.6×10^{-5}	0.07 (0.01)	0.90 (0.03)	9.1×10^{-9}
22:6	0.74 (0.17)	1.06 (0.09)	0.048	0.17 (0.01)	0.96 (0.03)	1.4×10^{-9}
SFA	24 (0.39)	25 (0.57)	0.999	24 (0.72)	26 (0.63)	0.141
14:0	0.47 (0.04)	0.41 (0.03)	0.275	0.46 (0.02)	0.40 (0.03)	0.122
16:0	22 (0.38)	22 (0.51)	0.693	22 (0.67)	24 (0.59)	0.093
18:0	1.3 (0.03)	1.8 (0.08)	4×10^{-4}	1.2 (0.06)	1.8 (0.06)	9×10^{-5}

Data are presented as mean (SEM). *P* values are calculated by *t* test and adjusted for multiple testing by Bonferroni. Individuals fatty acids are denoted as number of carbon and of double bonds.

Abbreviations: MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acid.

FIG. 6

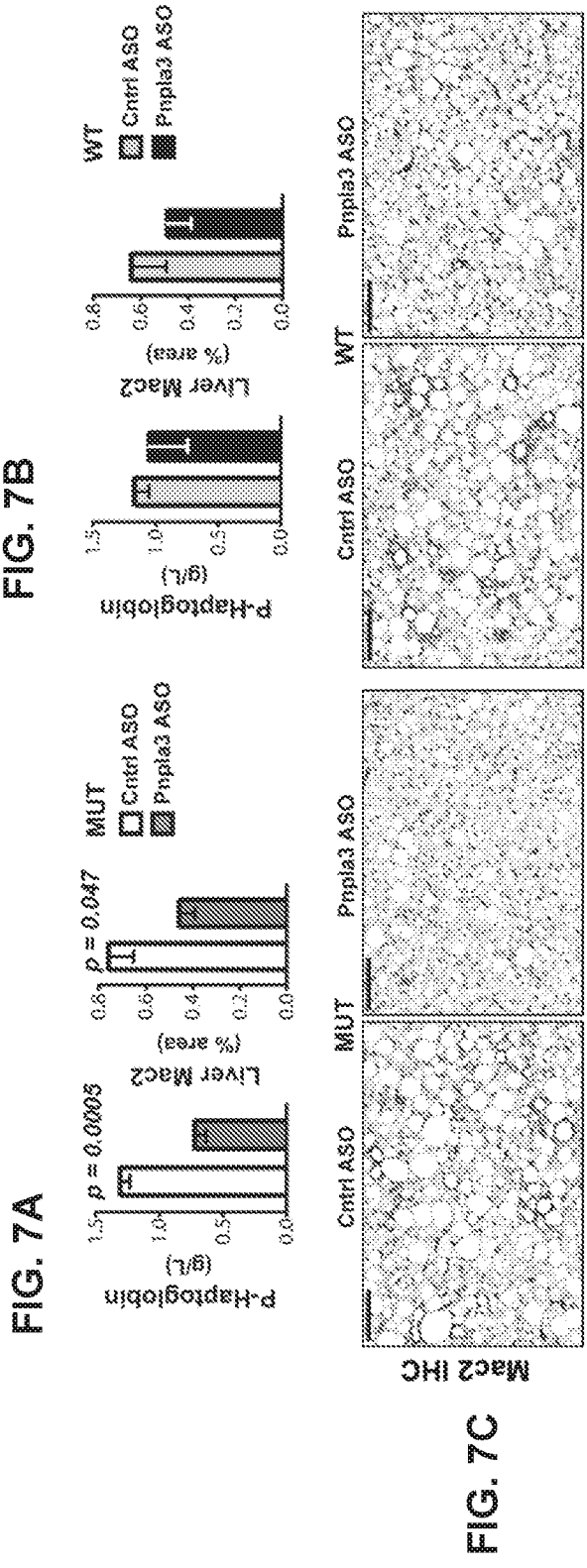
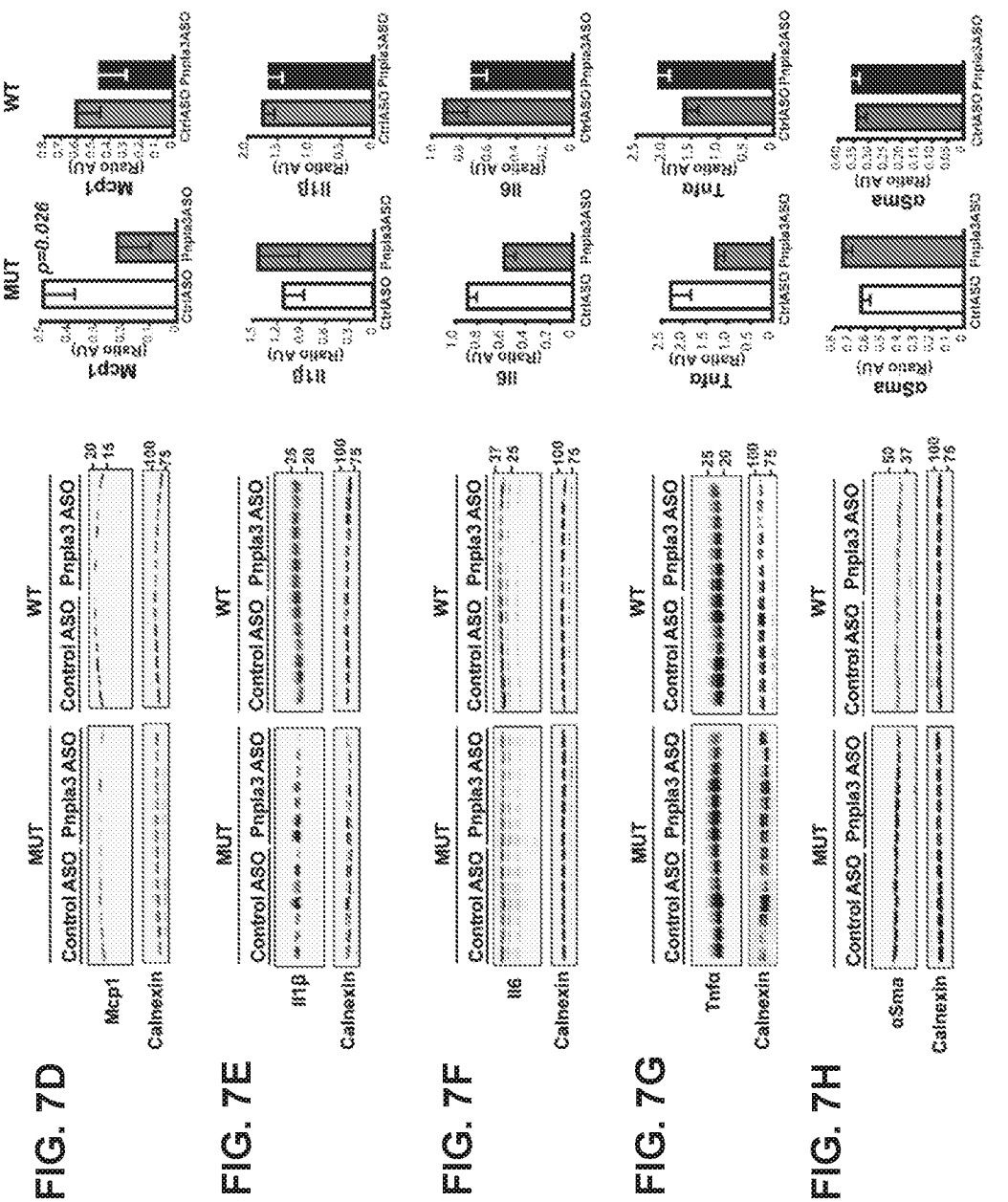


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 7


FIG. 7D
MUT
WT
Control ASO
Pnpla3 ASO
Mcp1
Calnexin
39
15
100
75
FIG. 7E
Il1β
Calnexin
25
28
100
75
FIG. 7F
Il6
Calnexin
37
25
100
75
FIG. 7G
Tnfa
Calnexin
25
28
100
75
FIG. 7H
αSma
Calnexin
50
37
100
76
p=0.026
(Ratio AU)
CtrlASO
Pnpla3ASO
FIG. 7 (cont'd)

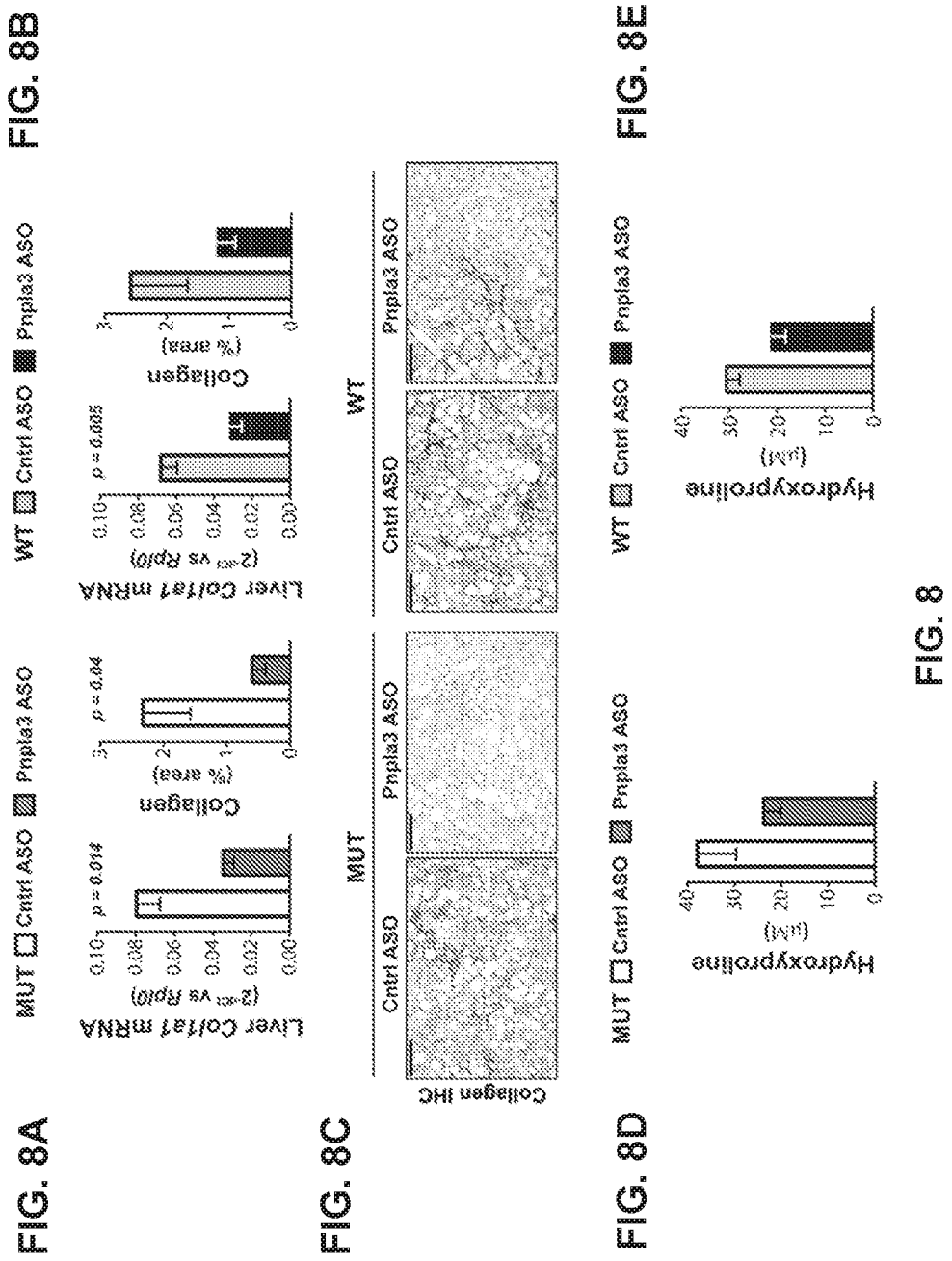

FIG. 8A
MUT
Cntrl ASO
Pnpla3 ASO
Liver Col1a1 mRNA
(2^-ΔCt vs Rplp0)
p = 0.014
Collagen
(% area)
p = 0.04
FIG. 8B
WT
Cntrl ASO
Pnpla3 ASO
Liver Col1a1 mRNA
(2^-ΔCt vs Rplp0)
p = 0.685
Collagen
(% area)
FIG. 8C
MUT
WT
Cntrl ASO
Pnpla3 ASO
Collagen IHC
FIG. 8D
MUT
Cntrl ASO
Pnpla3 ASO
Hydroxyproline
(μM)
FIG. 8E
WT
Cntrl ASO
Pnpla3 ASO
Hydroxyproline
(μM)

FIG. 8

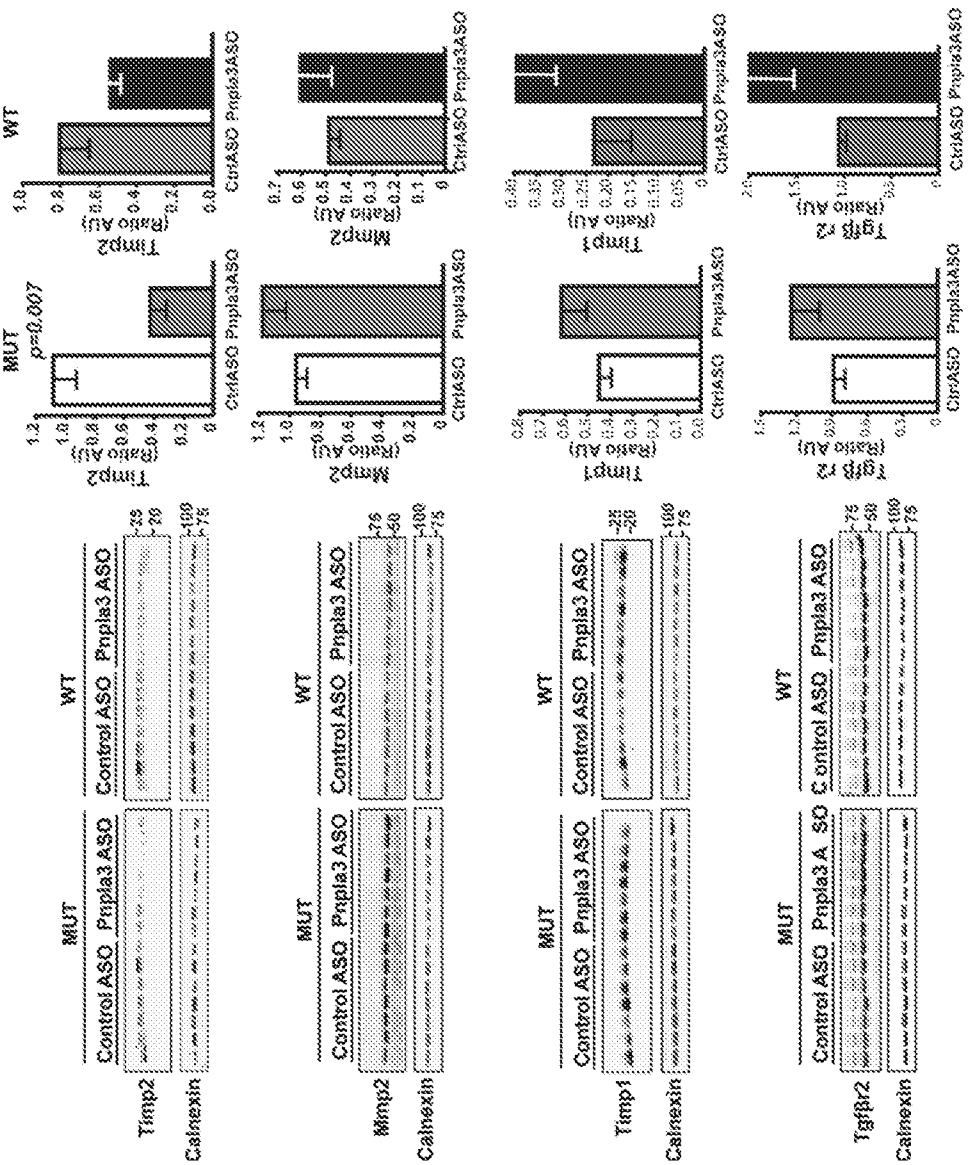


FIG. 9A

FIG. 9B

FIG. 9C

FIG. 9D

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/059566

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/713 C12N15/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DANIEL LINDÉN ET AL: "Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice", MOLECULAR METABOLISM, vol. 22, 1 April 2019 (2019-04-01), pages 49-61, XP055764085, ISSN: 2212-8778, DOI: 10.1016/j.molmet.2019.01.013 abstract, materials and methods, results section; figure 1	1-61

X	US 2017/349903 A1 (LIU WANQING [US] ET AL) 7 December 2017 (2017-12-07) paragraph [0018] - paragraph [0019]	1-61

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2021	15/03/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dolce, Luca

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/059566

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/118638 A2 (AMGEN INC [US]) 20 June 2019 (2019-06-20) paragraph [0020]	1-61
X	WO 2017/048620 A1 (ALNYLAM PHARMACEUTICALS INC [US]) 23 March 2017 (2017-03-23) page 5	1-61
A	WO 2017/106283 A1 (COLD SPRING HARBOR LABORATORY [US]; STOKE THERAPEUTICS INC [US] ET AL.) 22 June 2017 (2017-06-22) sequence 77380	1-61
A	US 2010/056384 A1 (HOBBS HELEN H [US] ET AL) 4 March 2010 (2010-03-04) the whole document	1-61

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2020/059566

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2020/059566

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-61(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-61(partially)

methods of treating liver diseases associated with the PNPLA3 I148M mutation, based on the ASO with SEQ ID NO: 17

2-2154. claims: 1-61(partially)

same as above for all remaining ASOs mentioned in claims 17 and 59

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2020/059566

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2017349903 A1	07-12-2017	US 2017349903 A1	07-12-2017
		US 2018327751 A1	15-11-2018
		US 2020270615 A1	27-08-2020
WO 2019118638 A2	20-06-2019	AR 113490 A1	06-05-2020
		AU 2018386089 A1	18-06-2020
		BR 112020011686 A2	24-11-2020
		CA 3084133 A1	20-06-2019
		CL 2020001543 A1	28-08-2020
		CN 111699257 A	22-09-2020
		CO 2020008485 A2	31-07-2020
		CR 20200304 A	04-09-2020
		EA 202091437 A1	29-12-2020
		EP 3724337 A2	21-10-2020
		JP 2021506238 A	22-02-2021
		KR 20200097299 A	18-08-2020
		SG 11202005257U A	29-07-2020
		TW 201938792 A	01-10-2019
		UY 38003 A	28-06-2019
		WO 2019118638 A2	20-06-2019
WO 2017048620 A1	23-03-2017	EP 3350328 A1	25-07-2018
		US 2018201936 A1	19-07-2018
		US 2020248188 A1	06-08-2020
		WO 2017048620 A1	23-03-2017
WO 2017106283 A1	22-06-2017	CA 3005090 A1	22-06-2017
		EP 3389672 A1	24-10-2018
		JP 2019500345 A	10-01-2019
		WO 2017106283 A1	22-06-2017
US 2010056384 A1	04-03-2010	US 2010056384 A1	04-03-2010
		US 2014179536 A1	26-06-2014
		WO 2010028110 A2	11-03-2010