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(54) Title: BIOMARKER OF PNPLA3 EXPRESSION

(57) Abstract: The present disclosure relates to a novel biomarker for measuring PNPLA3 gene expression and methods of using the novel biomarker for treating, preventing, or ameliorating a disease associated with PNPLA3.

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BIOMARKER 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 200834-US-PSP SEQUENCE LISTING_ST25.txt created December 19, 2018, which is 1 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Description

The present disclosure relates to a novel biomarker for measuring gene expression and methods of using the novel biomarker. At least one embodiment of the present disclosure includes a novel biomarker for measuring PNPLA3 (patatin like phospholipase domain containing 3; hypothetical protein dJ796I17.1; adiponutrin; DJ796I17.1) expression and methods of using the biomarker, which can be useful for treating, preventing, or ameliorating a disease associated with PNPLA3.

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 liver hepatocytes, whereas adipose tissue expression is five-fold less (Huang et al, Proc. Natl. Acad. Sci. USA 2010. 107: 7892-7). PNPLA3 is not a circulating protein and because it targets hepatocytes and there is no suitable surrogate tissue to monitor target engagement, there remains a lack of mechanistically derived target engagement biomarker suitable for determining protein expression and efficacy. Thus, there is a clear need for a biomarker that will measure target engagement, and for monitoring the response to treatment with a compound targeting a PNPLA3 nucleic acid.

Applicants have found that the ratio of cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) in the plasma or liver of a patient ("the CE 16:1/16:0 ratio") represents a novel biomarker that may be used for predicting, diagnosing and/or prognosticating a disease associated with PNPLA3. The CE 16:1/16:0 ratio is modulated by stearoyl-CoA desaturase-1 ("SCD1") activity. SCD1 is an enzyme that actively regulates *de novo* lipogenesis, PARα activity,

polyunsaturated fatty acids and cholesterol levels. (Lee et al, Am J Clin Nutr. 2015, 101(1): 34-43; Chong et al, Am J Clin Nutr. 2008, 87(4):817-23; Miller et al, PNAS 1996, 93 (18) 9443-48; Oosterveer et al. J. Biol. Chem. 2009;284:34036-44; Ntambi, J. Lipid Res. 1999. 40: 1549–58; Kim et al, J. Lipid Res. 2002, 43, 1750-57). In particular, SCD1 is the enzyme that converts the fatty acid cholesteryl palmitate 16:0 to the fatty acid 16:1 cholesteryl palmitate, so a shift in the CE 16:1/16:0 ratio is likely due to change in SCD1 activity. Moreover, a reduction in PNPLA3 reduces SCD1 activity which subsequently leads to a reduction in the CE 16:1/16:0 ratio incorporated in cholesteryl esters. It is therefore likely that PNPLA3 liver levels regulates SCD1 activity via one or several of these pathways.

Accordingly, the present disclosure relates to the biomarker CE 16:1/16:0 ratio for measuring expression of PNPLA3 and to methods of using the CE 16:1/16:0 ratio in the treatment of a disease associated with PNPLA3. Moreover, the novel biomarker will allow for dose adjustment of a compound targeting a PNPLA3 nucleic acid used in the treatment of a disease associated with PNPLA3, which may allow for optimal treatment of patients being treated for a disease associated with PNPLA3.

A biomarker can be described as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." (Wagner et al., Clin. Pharmacol. Ther. 2015; 98(1): 2-5). A biomarker is any identifiable and measurable indicator associated with a particular condition or disease where there is a correlation between the presence or level of the biomarker and some aspect of the condition or disease (including the presence of, the level or changing level of, the type of, the stage of, the susceptibility to the condition or disease, or the responsiveness to a drug used for treating the condition or disease). The correlation may be qualitative, quantitative, or both qualitative and quantitative. In some instances, biomarkers may be used to predict or detect the presence, level, type or stage of conditions or diseases, the susceptibility to conditions or diseases, or the response to treatments. It is thought that biomarkers will play an increasingly important role in the future of drug discovery and development, by improving the efficiency of research and development programs. Biomarkers can be used as diagnostic agents, monitors of disease progression, monitors of treatment and predictors of clinical outcome. For example, various biomarker research projects are attempting to identify markers of specific cancers and of specific cardiovascular and immunological diseases. It

is believed that the development of new validated biomarkers will lead both to significant reductions in healthcare and drug development costs and to significant improvements in treatment for a wide variety of diseases and conditions.

In at least one embodiment of the present disclosure, the CE 16:1/16:0 ratio is measured in the plasma of the patient as a biomarker for the expression of PNPLA3. For example, a population of individuals having type 2 diabetes and NAFLD has been identified as having an average CE 16:1/16:0 ratio of 0.3. (Eriksson et al. Diabetologia 2018; 61: 1923-1934). In at least one embodiment, a reduction in average CE 16:1/16:0 ratio below 0.3 indicates a corresponding reduction in PNPLA3 expression.

In other embodiments, the ratio of cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) can be used for the screening and/or diagnosis of a disease associated with PNPLA3. For example, a measurement of average CE 16:1/16:0 ratio above 0.3 indicates a patient in need of therapy with a compound associated with the treatment of a disease associated with PNPLA3. In yet another example, a reduction in average CE 16:1/16:0 ratio below 0.3 indicates a patient who has responded to a compound associated with the treatment of a disease associated with PNPLA3. In at least one embodiment, the disease is chosen from liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in a patient having, or at risk of having, a liver 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 other embodiments, the ratio of cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) can be used for monitoring the activity and/or progression of a disease associated with PNPLA3. For example, a measurement of average CE 16:1/16:0 ratio above 0.3 indicates a patient in need of further therapy with a compound associated with the treatment of a disease associated with PNPLA3. In yet another example, a reduction in average CE 16:1/16:0 ratio below 0.3

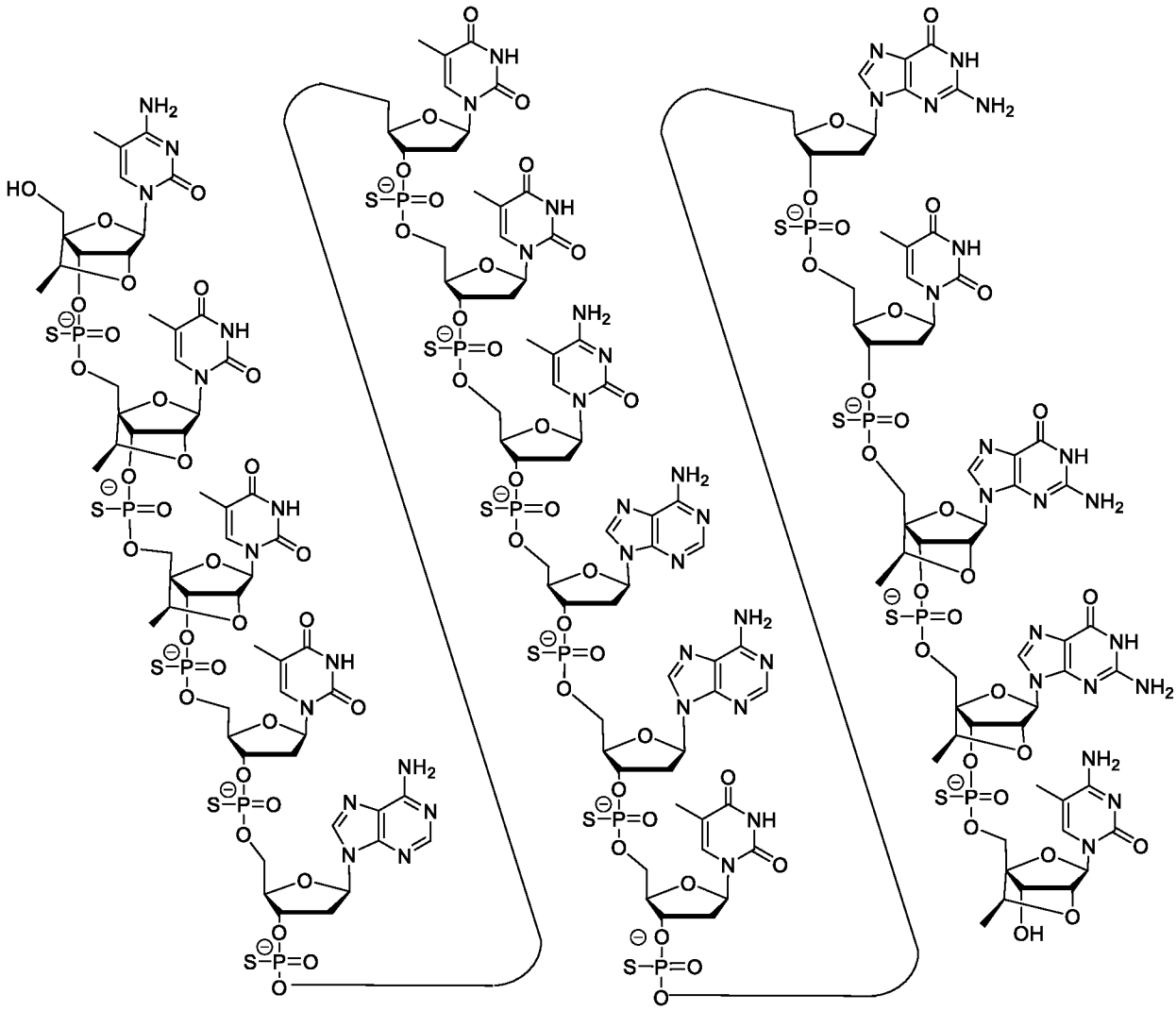
indicates a patient who has responded to a compound associated with the treatment of a disease associated with PNPLA3. In at least one embodiment, the disease is chosen from liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in a patient having, or at risk of having, a liver 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 other embodiments, the ratio can be used for the prediction of the efficacy of a compound in a method for the treatment of a disease associated with PNPLA3. In at least one embodiment, the disease is chosen from liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in a patient having, or at risk of having, a liver 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 at least one embodiment, a reduction in average CE 16:1/16:0 ratio below 0.3 indicates a corresponding reduction in PNPLA3 expression.

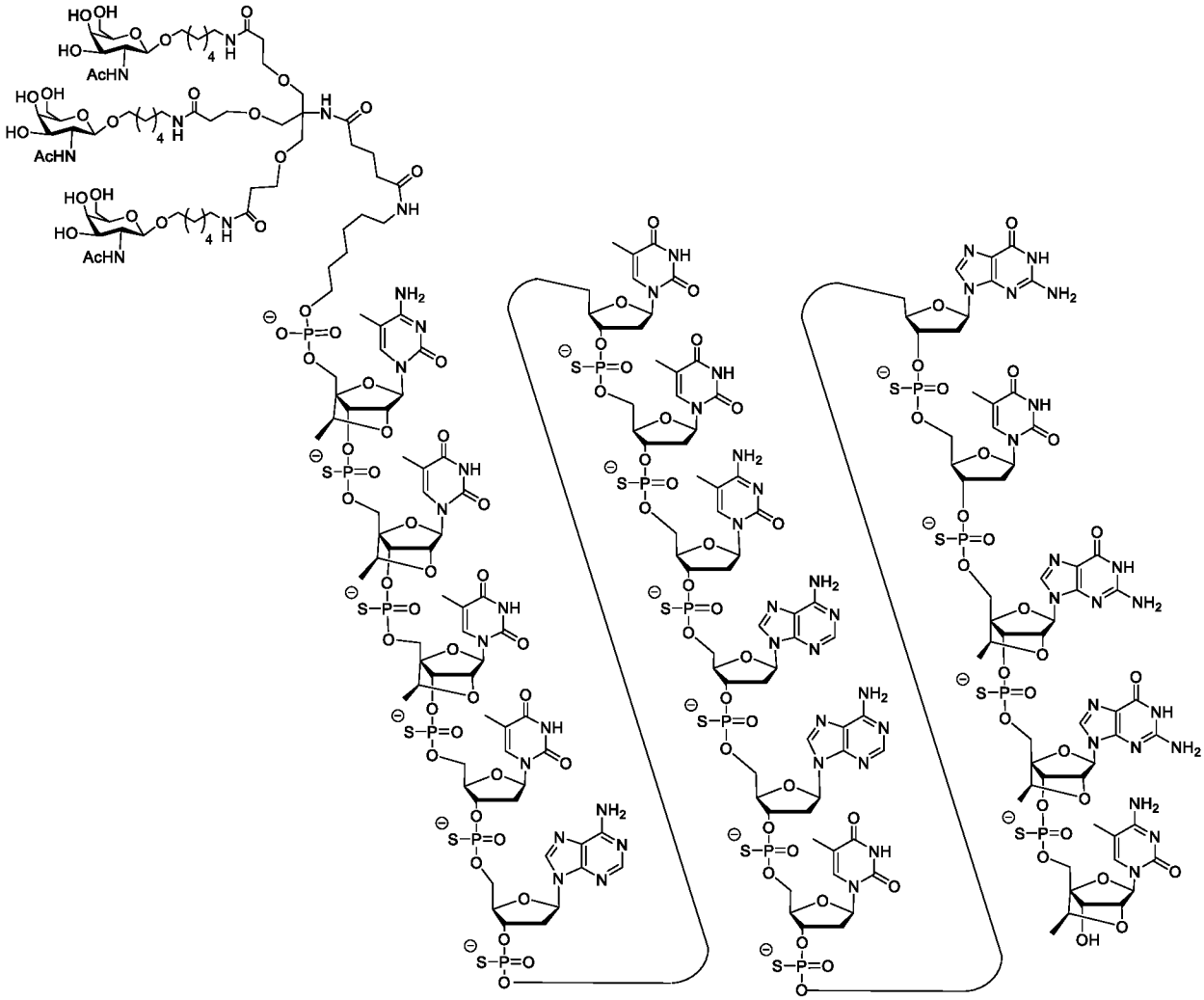
In yet another embodiment, the ratio of cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) can be used for adjusting the dose of a compound targeting a PNPLA3 nucleic acid used in the treatment of a disease associated with PNPLA3. In at least one embodiment, the disease is chosen from liver damage, steatosis, liver fibrosis, liver inflammation, liver scarring or cirrhosis, liver failure, liver enlargement, elevated transaminases, or hepatic fat accumulation in a patient having, or at risk of having, a liver 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 at least one embodiment of the present disclosure, the dose of the compound targeting a PNPLA3 nucleic acid will be increased. In another embodiment, the dose of the compound targeting a PNPLA3 nucleic acid will be decreased.

The diagnostic methods of the specification can be undertaken using a sample previously taken from the patient or patient. Such samples may be preserved by freezing or fixed and embedded in formalin-paraffin or other media. Alternatively, a fresh sample containing sample may be obtained and used.

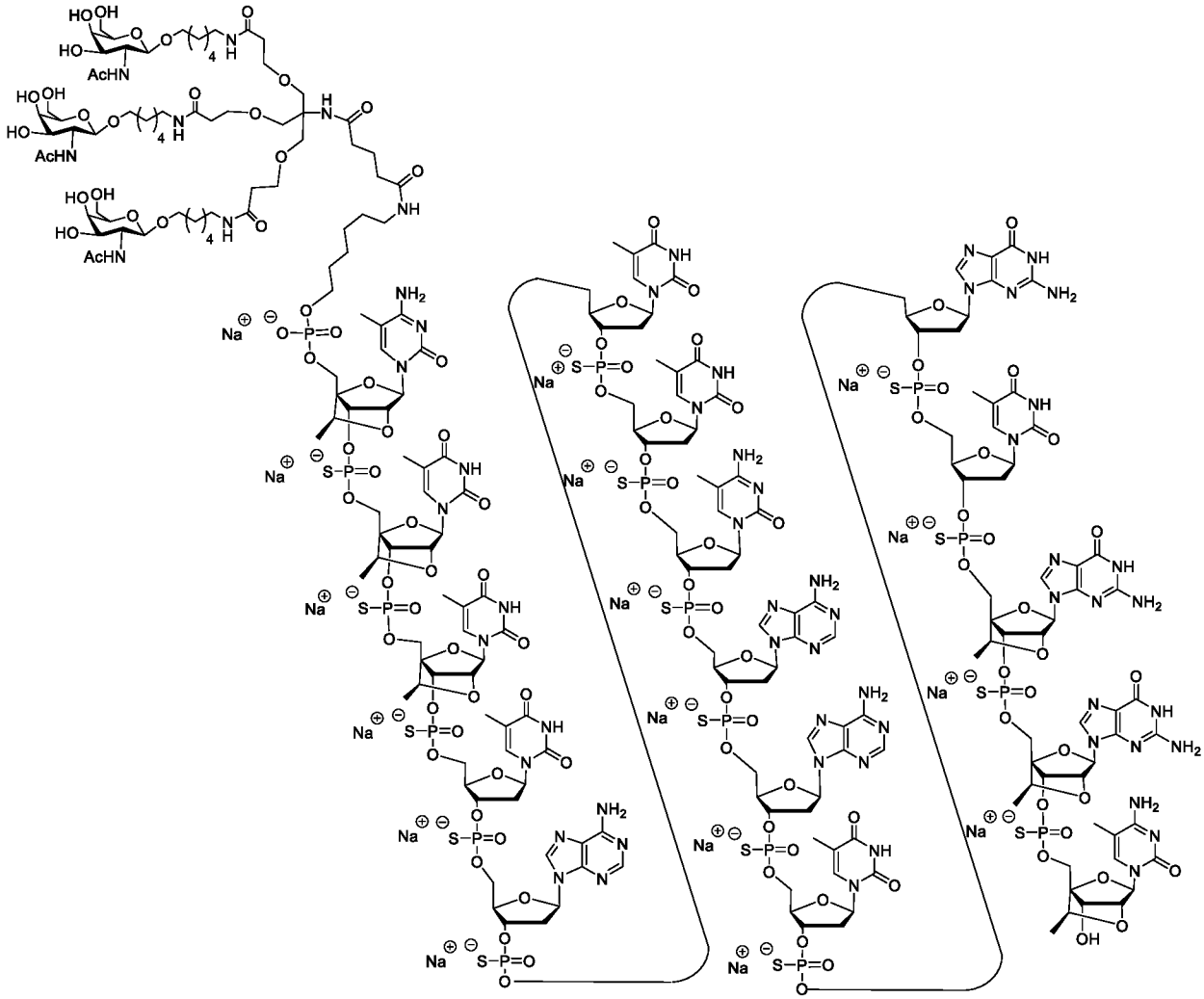
Certain embodiments provide methods of monitoring activity and/or progression of a disease associated with PNPLA3 where the patient is being treated with a compound targeted to a PNPLA3 nucleic acid. In certain embodiments, a compound comprises or consists of ION 916333 or salt thereof, having the following chemical structure:



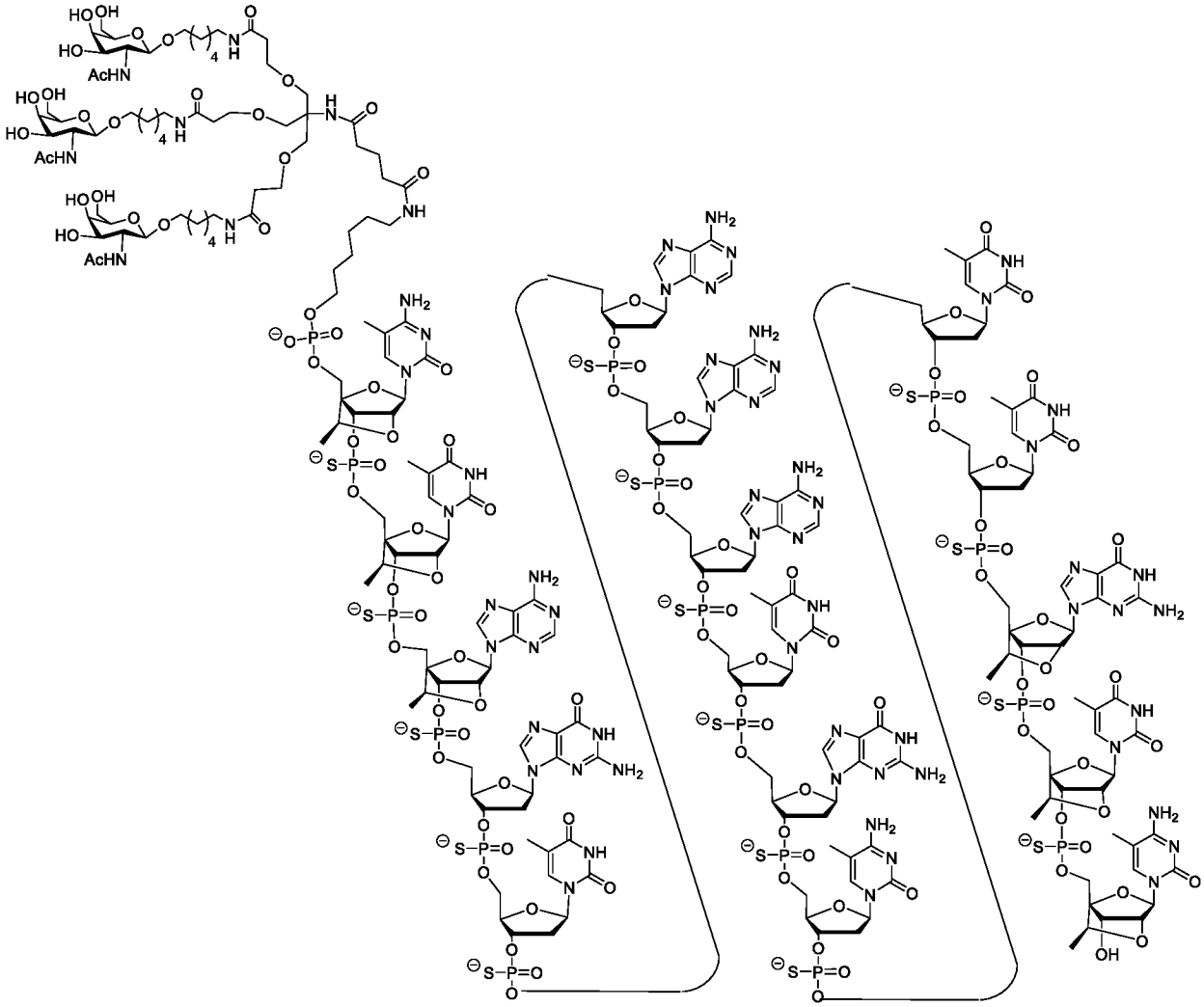
In certain embodiments, a compound comprises or consists of ION 975616 or salt thereof, having the following chemical structure:



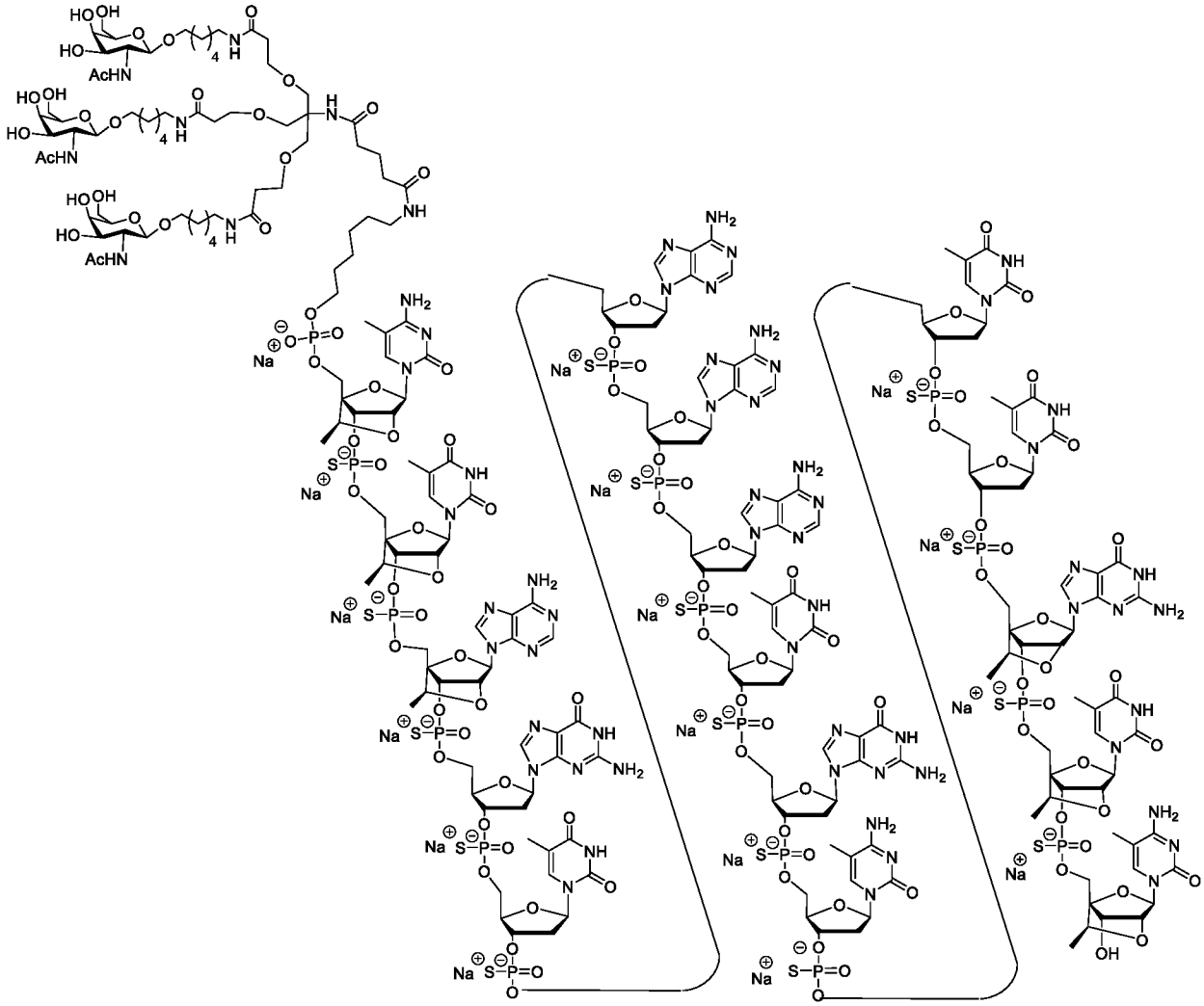
In certain embodiments, a compound comprises or consists of the sodium salt of 975616, having the following chemical structure:



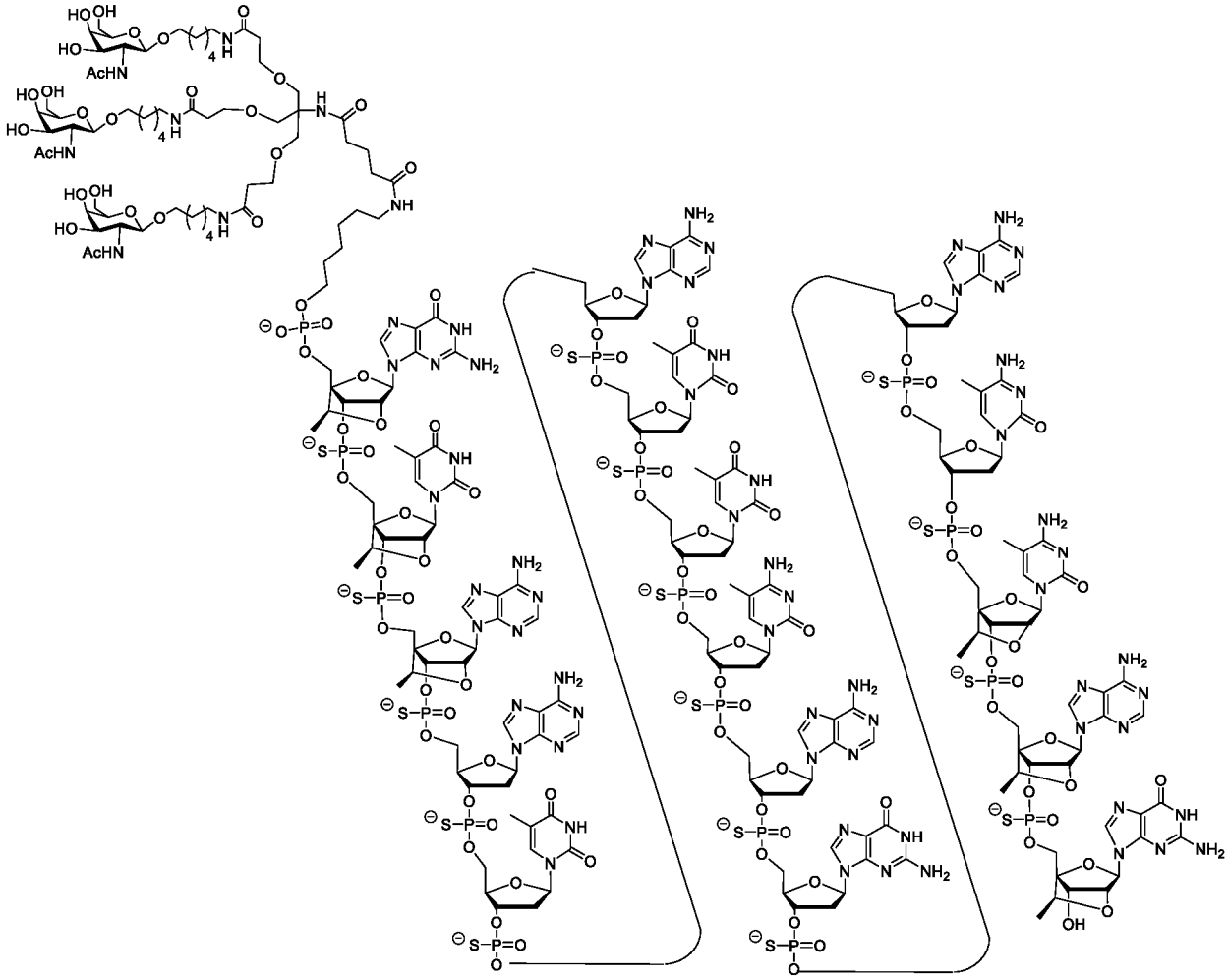
In certain embodiments, a compound comprises or consists of ION 975613 or salt thereof, having the following chemical structure:



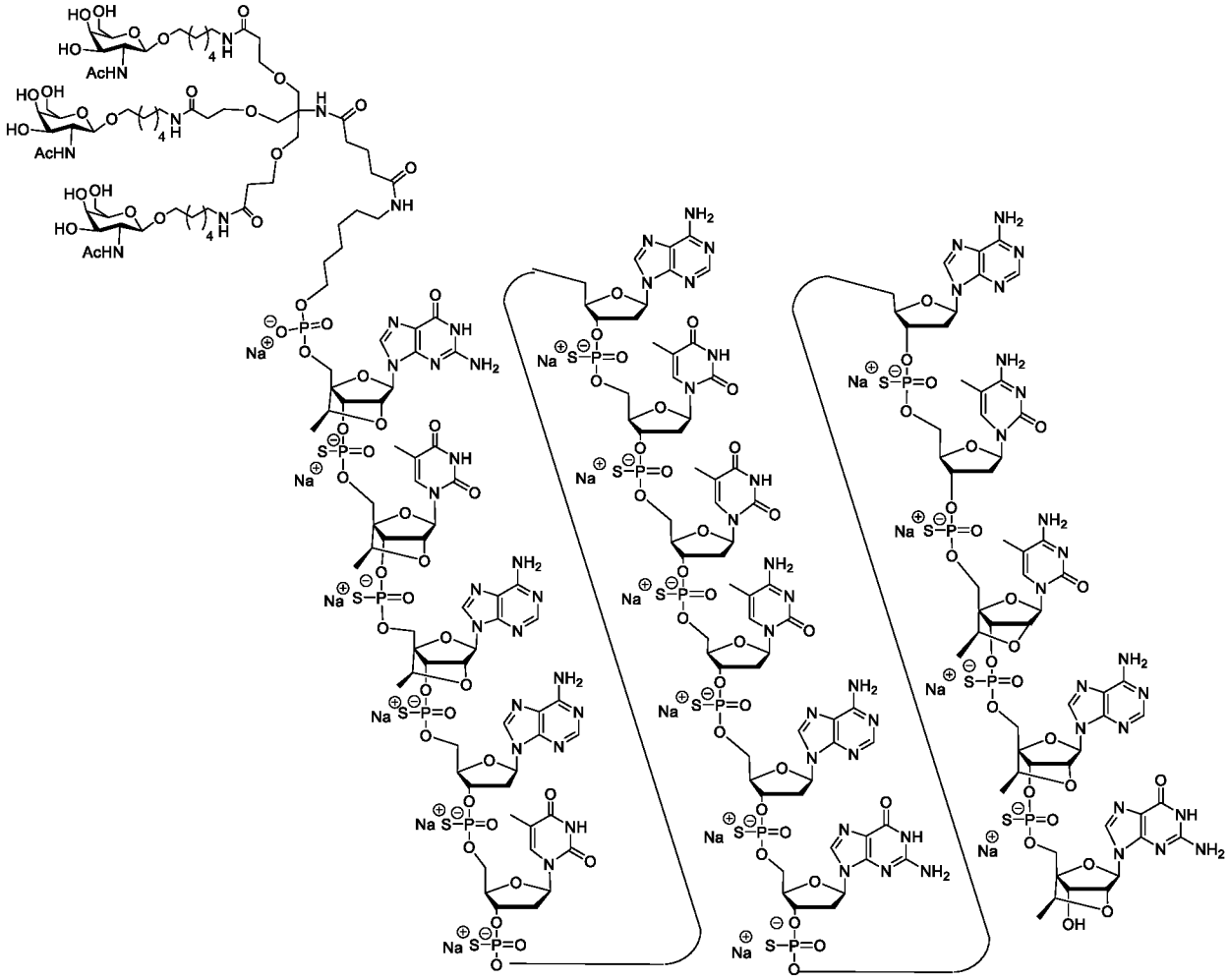
In certain embodiments, a compound comprises or consists of the sodium salt of 975613, having the following chemical structure:



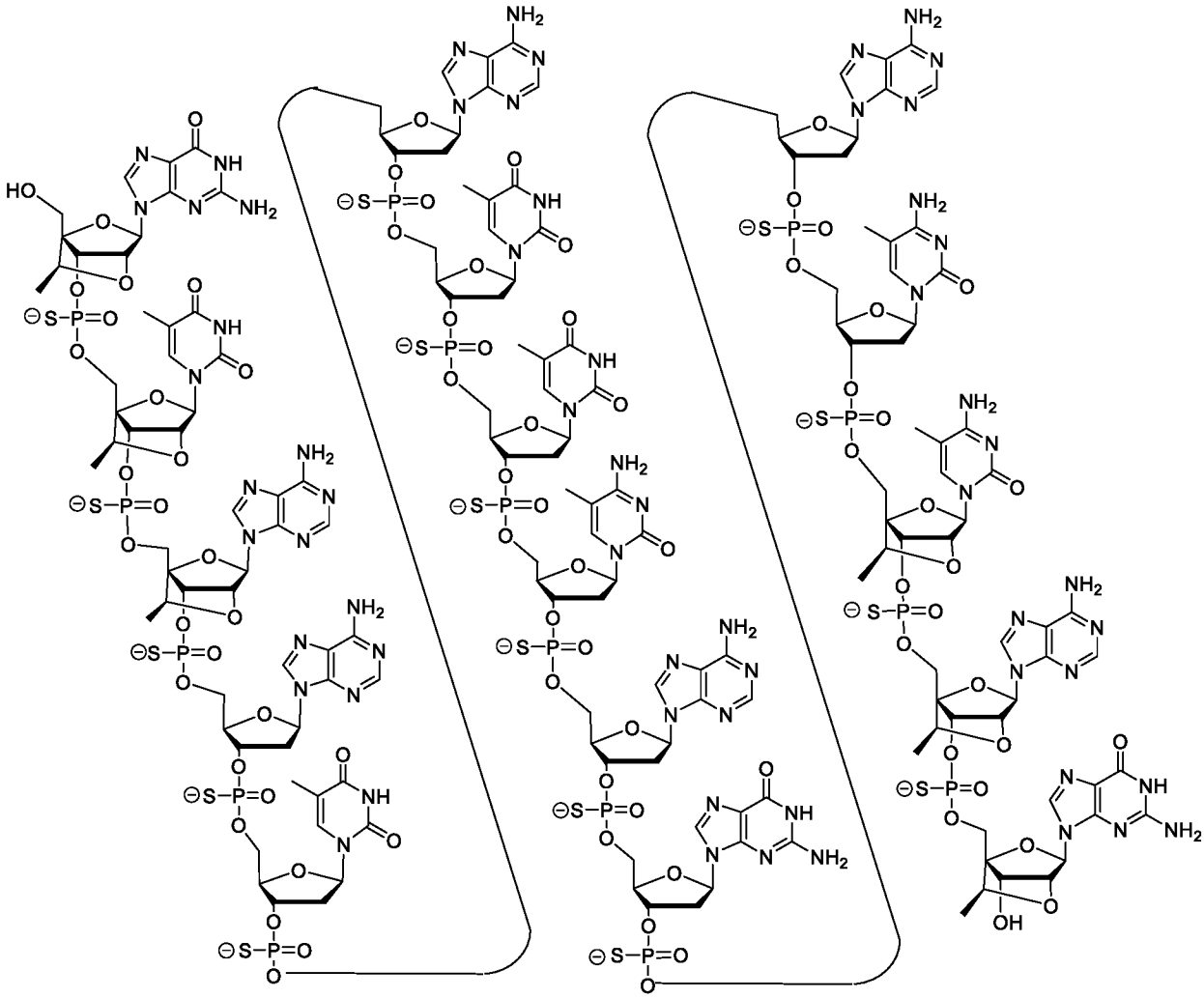
In certain embodiments, a compound comprises or consists of ION 975612 or salt thereof, having the following chemical structure:



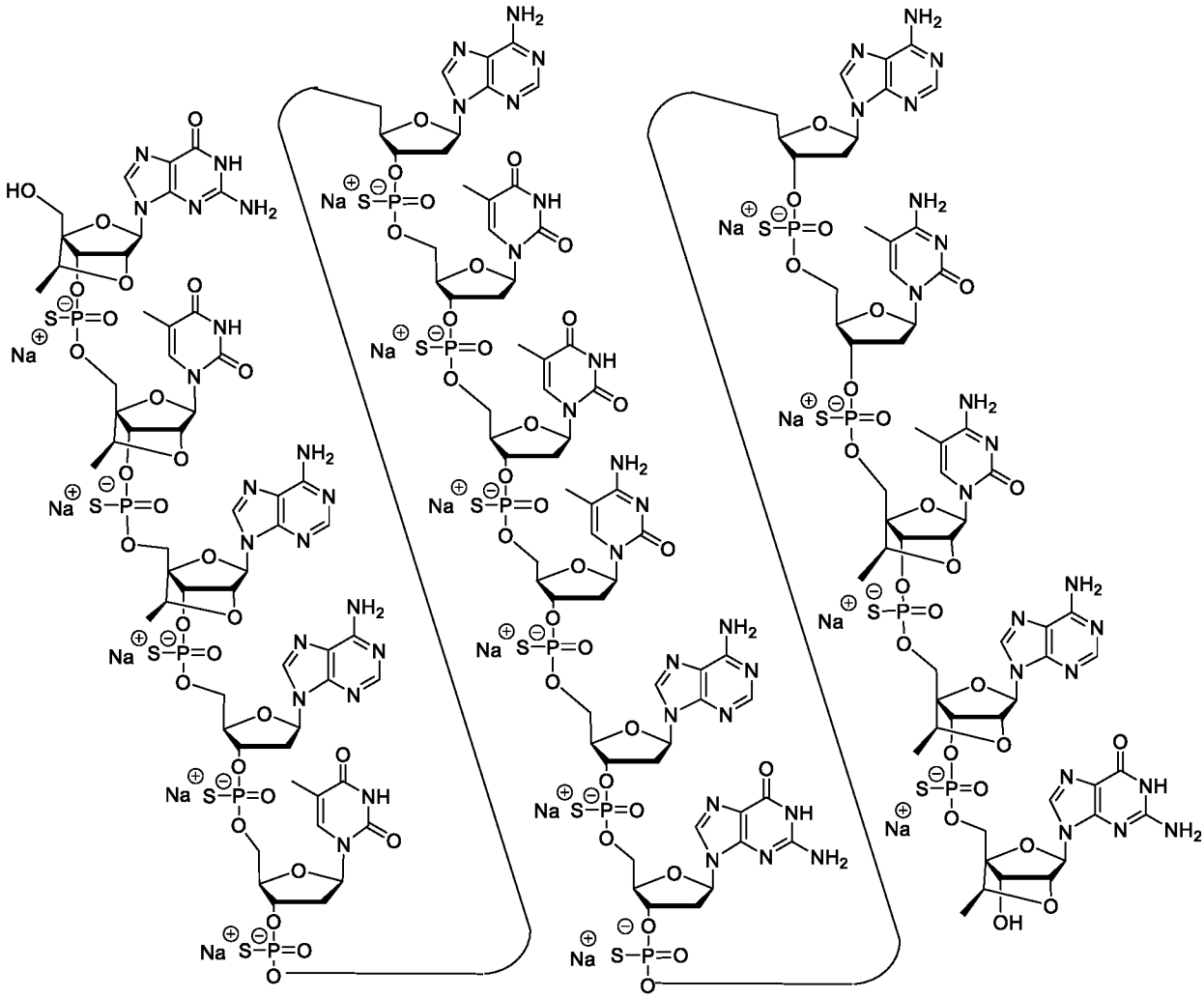
In certain embodiments, a compound comprises or consists of the sodium salt of 975612, having the following chemical structure:



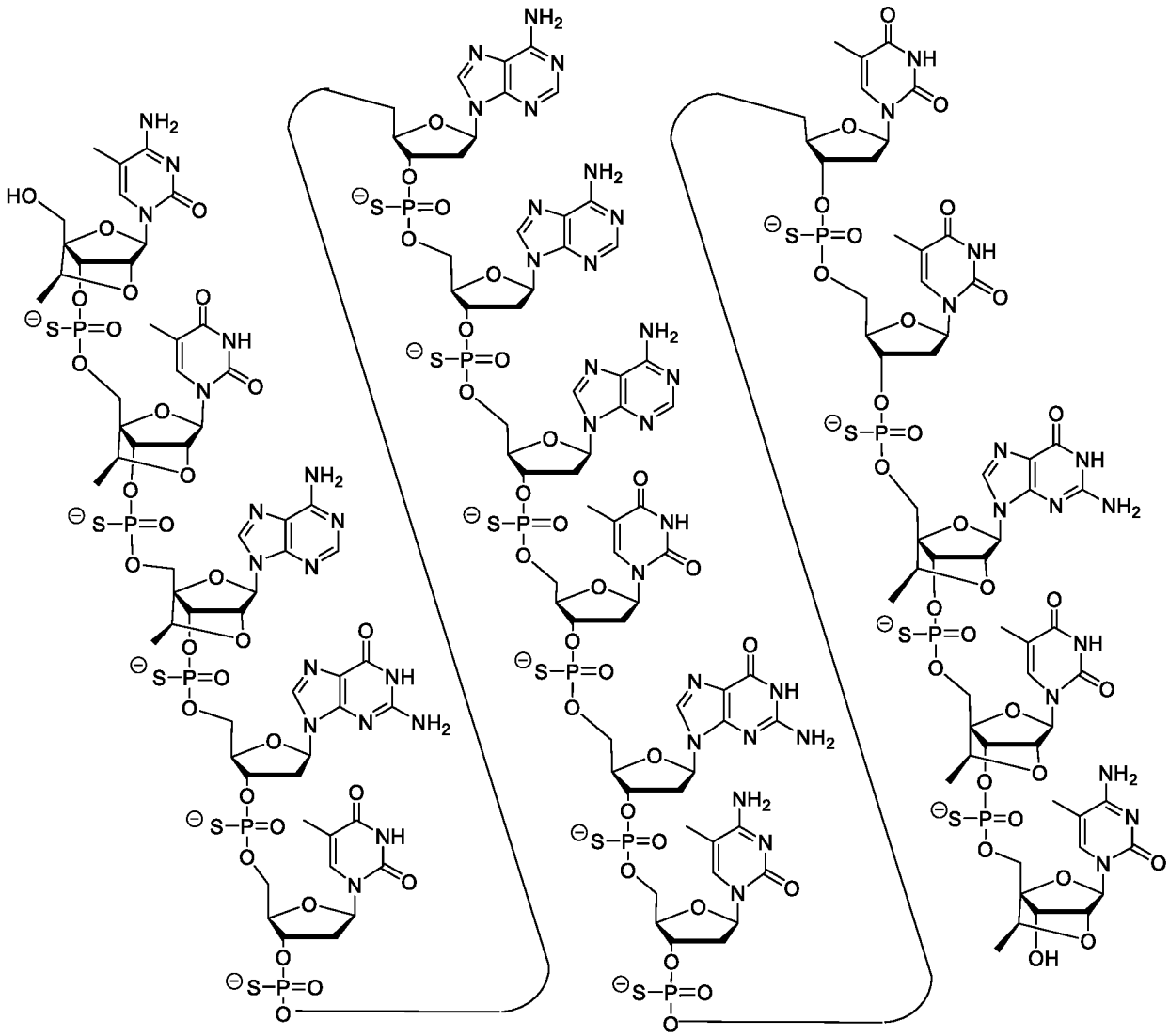
In certain embodiments, a compound comprises or consists of ION 916789 or salt thereof, having the following chemical structure:



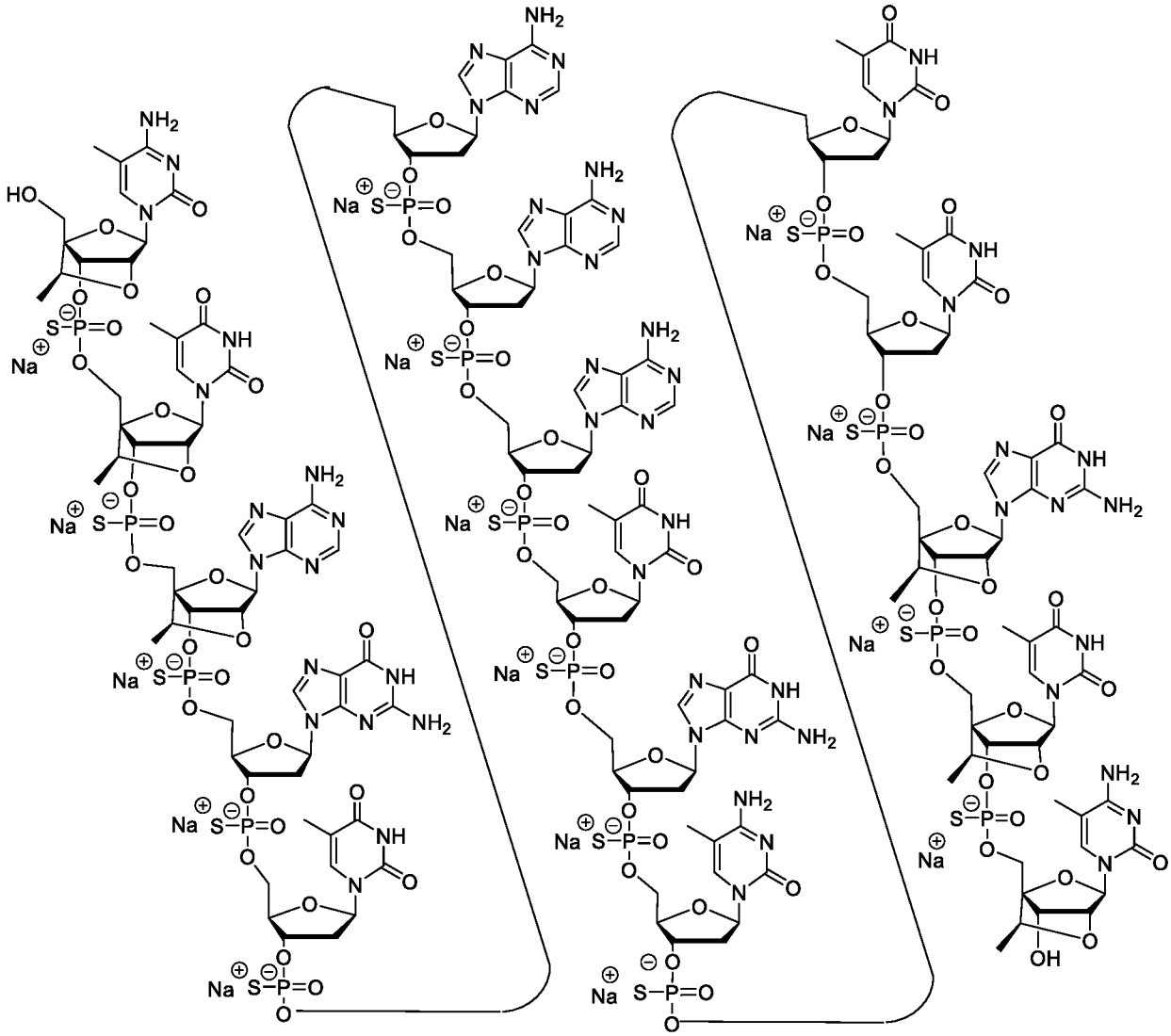
In certain embodiments, a compound comprises or consists of the sodium salt of 916789, having the following chemical structure:



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 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 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.

The following examples and figures are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the various aspects of the invention, and are not intended to limit the scope of what the inventors regard as their invention

Figure Legend

Fig. 1 The CE 16:1/16:0 ratio measured in both plasma and liver of wild-type and 148M knock-in mice after PNPLA3 silencing

Fig. 2 The correlation between plasma CE 16:1/16:0 and liver PNPLA3 mRNA after a single dose of PNPLA3 ASO

EXAMPLES

Effects of PNPLA3 silencing with a potent liver-targeted GalNAc-conjugated antisense oligonucleotide (ASO), ION 975616, in both wild-type and 148M knock-in mice, on plasma and liver lipidomics were investigated. Cohorts of mice were fed either a steatogenic high-sucrose diet or a NASH diet high in fructose, trans-fatty acids and cholesterol to induce marked liver steatosis, inflammation and fibrosis. Plasma and liver lipid composition were investigated using a combination of UPLC-MS/MS and direct infusion (shotgun). The ratio between cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) was reduced in both plasma and liver after PNPLA3 silencing (Fig 1). This effect was independent of PNPLA3 genotype or diet. In addition, there were a correlation between plasma CE 16:1/16:0 and liver PNPLA3 mRNA after a single dose of PNPLA3 ASO (Fig 2). The ratio between cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) in human plasma or serum can be measured by mass spectrometric detection after separation using either liquid or gas chromatography.

WHAT IS CLAIMED:

1. A method for the screening and/or diagnosis of a disease associated with PNPLA3 comprising measuring the CE 16:1/16:0 ratio in a patient in need of therapy of a disease associated with PNPLA3.

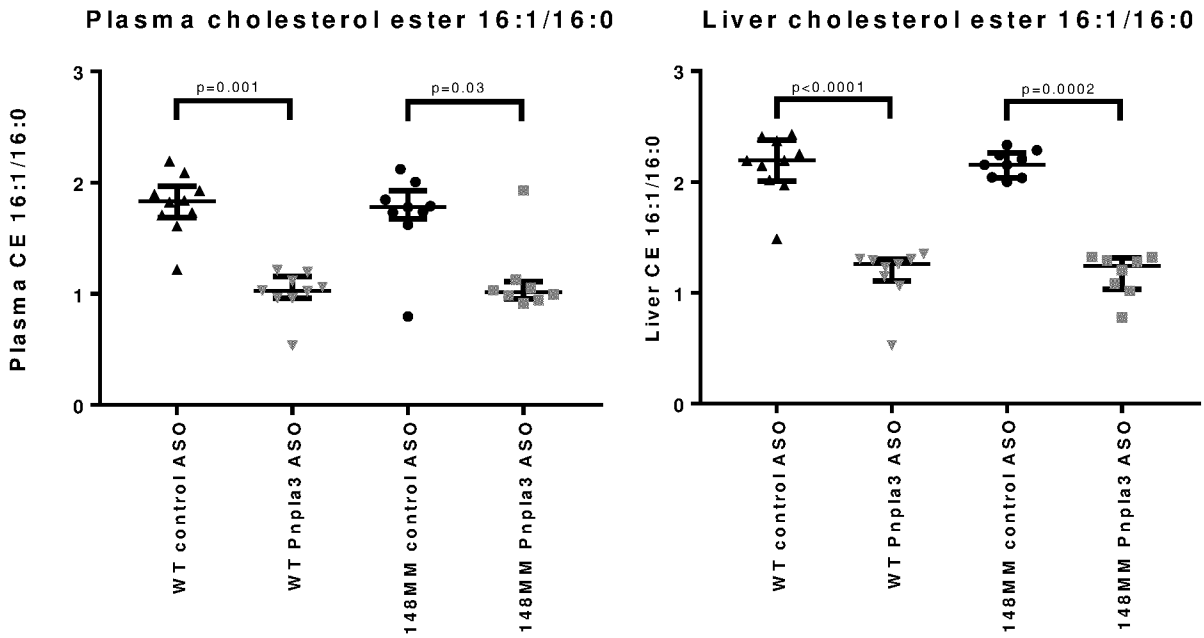

Plasma cholesterol ester 16:1/16:0
Plasma CE 16:1/16:0
p=0.001
p=0.03
WT control ASO
WT Pnpla3 ASO
148MM control ASO
148MM Pnpla3 ASO
Liver cholesterol ester 16:1/16:0
Liver CE 16:1/16:0
p<0.0001
p=0.0002
WT control ASO
WT Pnpla3 ASO
148MM control ASO
148MM Pnpla3 ASO

FIG. 1

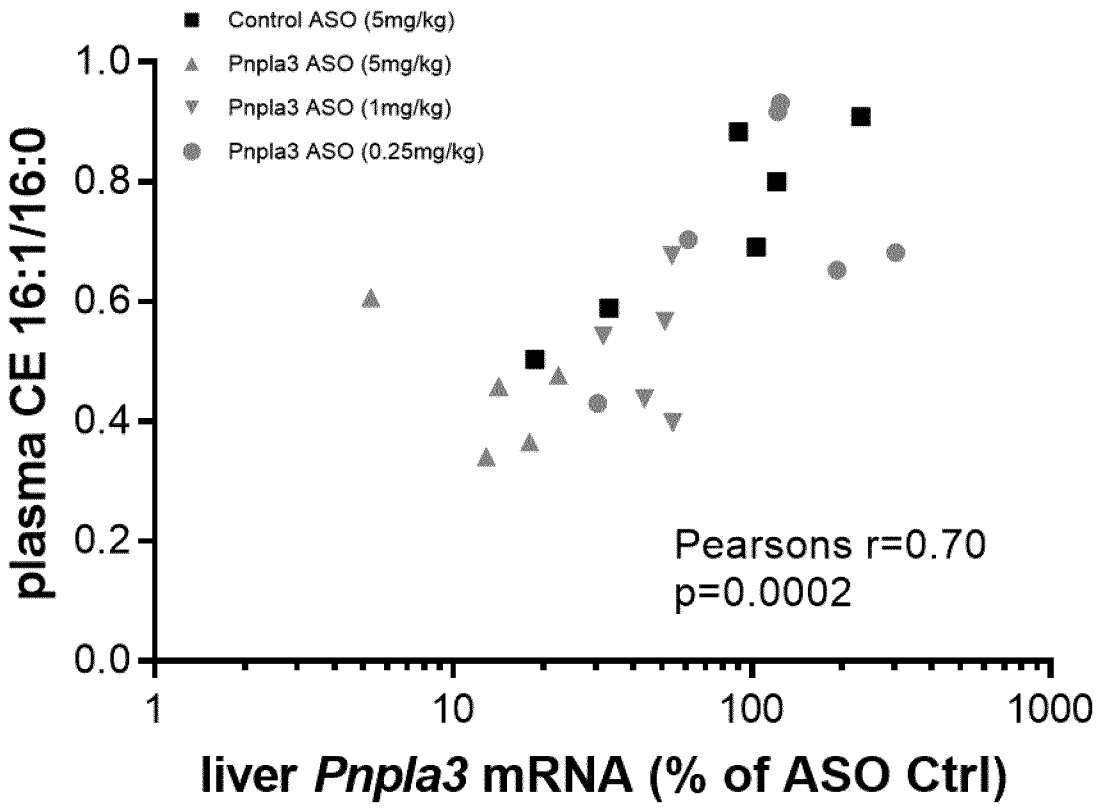

Control ASO (5mg/kg)
Pnpla3 ASO (5mg/kg)
Pnpla3 ASO (1mg/kg)
Pnpla3 ASO (0.25mg/kg)
plasma CE 16:1/16:0
1.0
0.8
0.6
0.4
0.2
0.0
Pearsons r=0.70
p=0.0002
1
10
100
1000
liver *Pnpla3* mRNA (% of ASO Ctrl)

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/084791

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/60 G01N33/68 G01N33/92 C12Q1/44
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)
G01N C12Q

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, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/028110 A2 (UNIV TEXAS [US]; HOBBS HELEN H [US]; COHEN JONATHAN C [US]) 11 March 2010 (2010-03-11) the whole document ----- -/--	1

[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
17 March 2020	30/03/2020

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	Stricker, J

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOEKSTRA M ET AL: "The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status", JOURNAL OF HEPATOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 52, no. 2, 1 February 2010 (2010-02-01), pages 244-251, XP026885417, ISSN: 0168-8278, DOI: 10.1016/J.JHEP.2009.11.004 [retrieved on 2009-11-24] the whole document -----	1
A	JOSEPH J LEE ET AL: "Palmitoleic acid is elevated in fatty liver disease and reflects hepatic lipogenesis", THE AMERICAN JOURNAL OF CLINICAL NUTRITION, vol. 101, no. 1, 19 November 2014 (2014-11-19), pages 34-43, XP055677129, US ISSN: 0002-9165, DOI: 10.3945/ajcn.114.092262 cited in the application abstract -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/084791

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010028110 A2	11-03-2010	US 2010056384 A1	04-03-2010
		US 2014179536 A1	26-06-2014
		WO 2010028110 A2	11-03-2010