



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2018/01/05
(87) Date publication PCT/PCT Publication Date: 2018/07/12
(85) Entrée phase nationale/National Entry: 2019/07/04
(86) N° demande PCT/PCT Application No.: US 2018/012672
(87) N° publication PCT/PCT Publication No.: 2018/129384
(30) Priorités/Priorities: 2017/01/06 (US62/443,514);
2017/09/22 (US62/561,939)

(51) Cl.Int./Int.Cl. *A61K 38/16* (2006.01),
A61K 47/64 (2017.01), *C07K 16/00* (2006.01),
C07K 7/06 (2006.01), *C07K 7/08* (2006.01)

(71) Demandeur/Applicant:
AVIDITY BIOSCIENCES LLC, US

(72) Inventeurs/Inventors:
LEVIN, ARTHUR A., US;
GEALL, ANDREW JOHN, US;
DOPPALAPUDI, VENKATA RAMANA, US;
COCHRAN, MICHAEL CARAMIAN, US;
HUANG, HANHUA, US;
BURKE, ROB, US

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : COMPOSITIONS D'ACIDE NUCLEIQUE-POLYPEPTIDE ET METHODES D'INDUCTION DE SAUT D'EXON
(54) Title: NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING

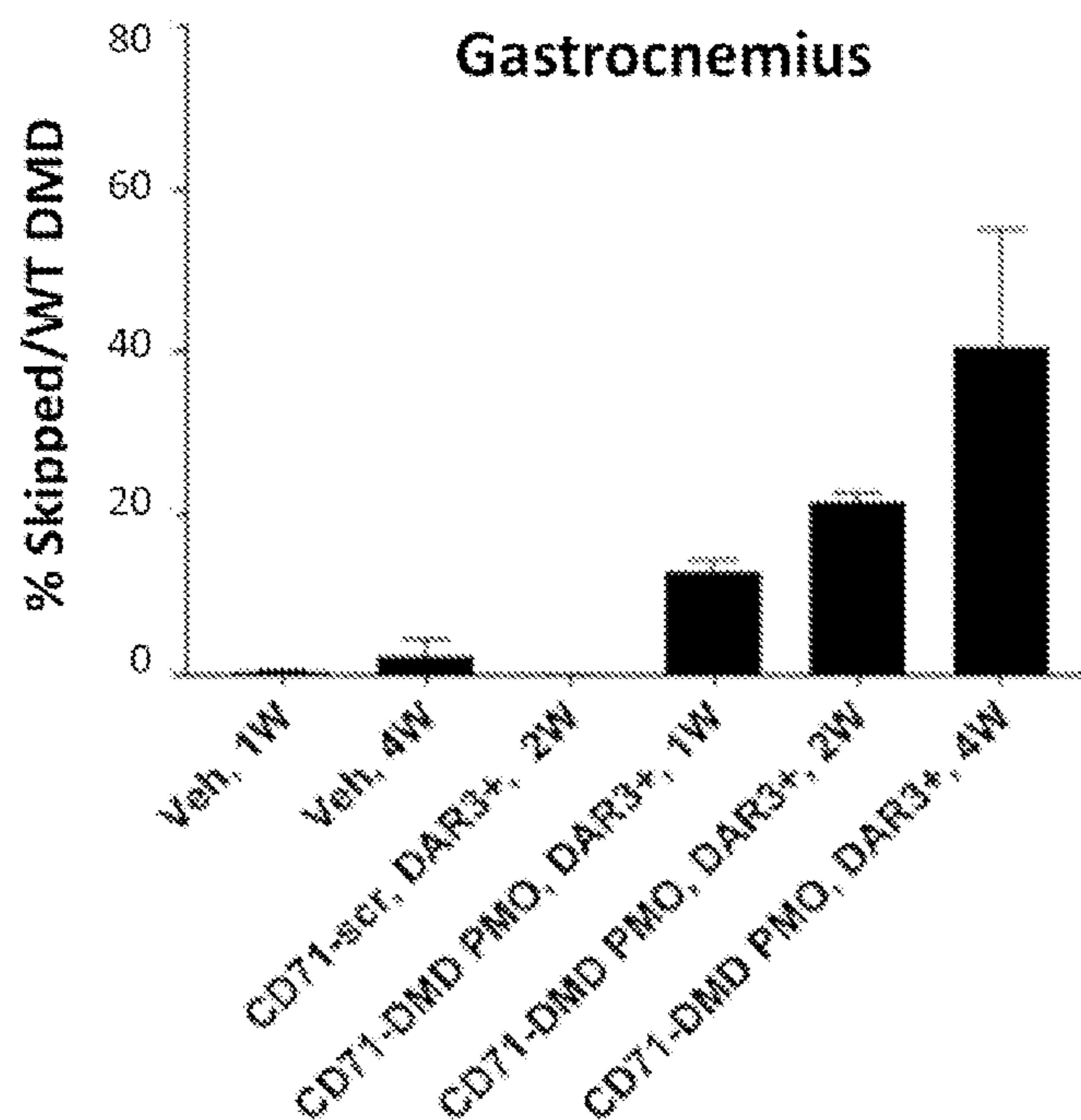


Fig. 15A

(57) **Abrégé/Abstract:**

Disclosed herein are molecules and pharmaceutical compositions that induce an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion. Also described herein include methods for treating a disease or disorder that comprises a molecule or a pharmaceutical composition that induces an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
12 July 2018 (12.07.2018)



(10) International Publication Number
WO 2018/129384 A1

(51) International Patent Classification:

A61K 38/16 (2006.01) C07K 7/08 (2006.01)
A61K 47/64 (2017.01) C07K 16/00 (2006.01)
C07K 7/06 (2006.01)

(21) International Application Number:

PCT/US2018/012672

(22) International Filing Date:

05 January 2018 (05.01.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/443,514 06 January 2017 (06.01.2017) US
62/561,939 22 September 2017 (22.09.2017) US

(71) Applicant: AVIDITY BIOSCIENCES LLC [US/US];
10975 North Torrey Pines Road, Suite 150, La Jolla, California 92037 (US).

(72) Inventors: LEVIN, Arthur A.; 10975 North Torrey Pines
Road, Suite 150, La Jolla, California 92037 (US). GEALL,

Andrew John; 10975 North Torrey Pines Road, Suite 150,
La Jolla, California 92037 (US). DOPPALAPUDI, Venka-
ta Ramana; 10975 North Torrey Pines Road, Suite 150,
La Jolla, California 92037 (US). COCHRAN, Michael
Caramian; 10975 North Torrey Pines Road, Suite 150, La
Jolla, California 92037 (US). HUANG, Hanhua; 10975
North Torrey Pines Road, Suite 150, La Jolla, California
92037 (US). BURKE, Rob; 10975 North Torrey Pines
Road, Suite 150, La Jolla, California 92037 (US).

(74) Agent: YANG, Xiaofan; Wilson Sonsini Goodrich &
Rosati, 650 Page Mill Road, Palo Alto, California 94304
(US).

(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, 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,

(54) Title: NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING

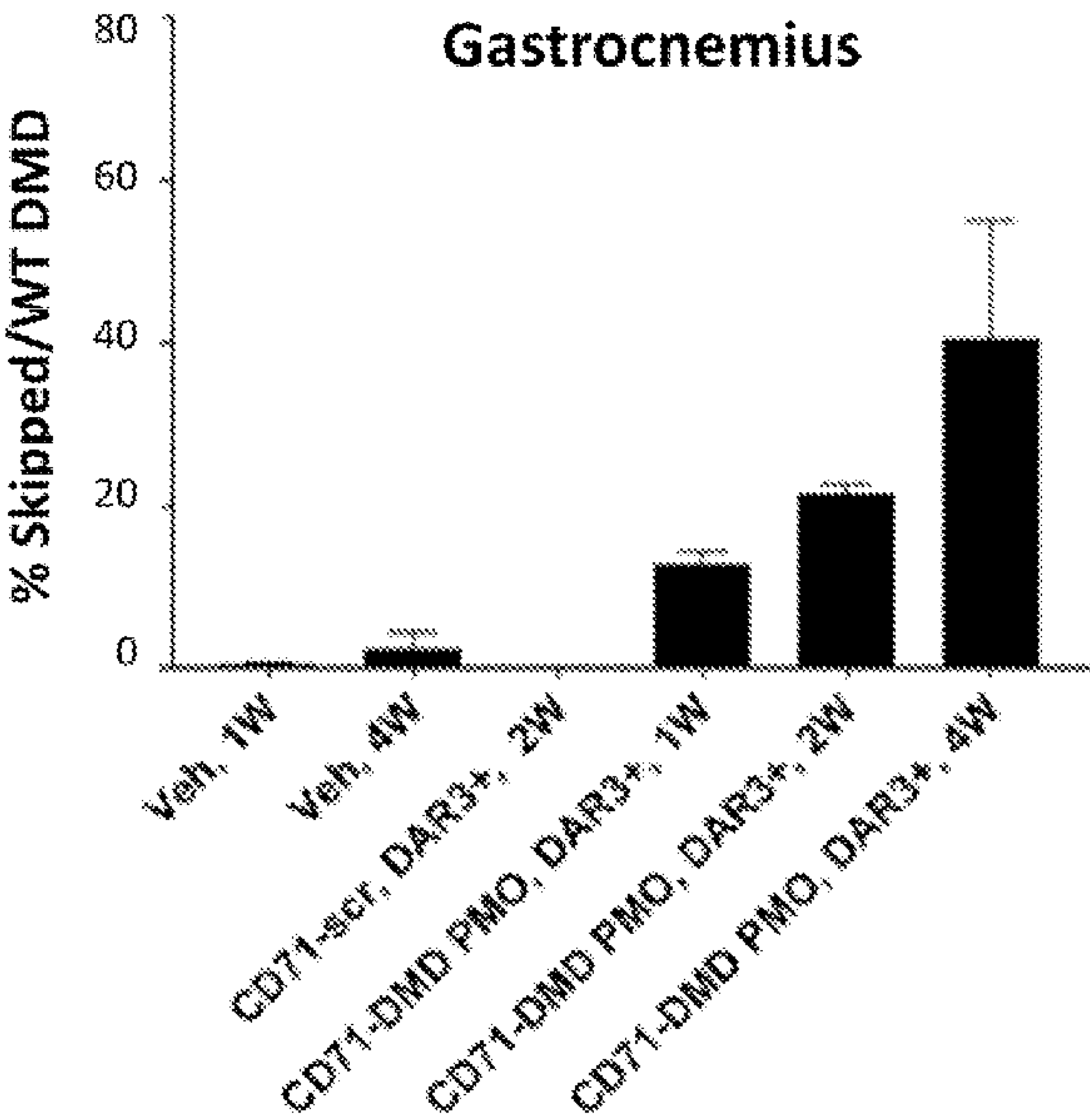


Fig. 15A

(57) Abstract: Disclosed herein are molecules and pharmaceutical compositions that induce an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion. Also described herein include methods for treating a disease or disorder that comprises a molecule or a pharmaceutical composition that induces an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

WO 2018/129384 A1

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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))*

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))*

NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/561,939 filed on September 22, 2017 and 62/443,514 filed on January 6, 2017, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 22, 2017, is named 45532-715_601_SL.txt and is 210,534 bytes in size.

BACKGROUND OF THE DISCLOSURE

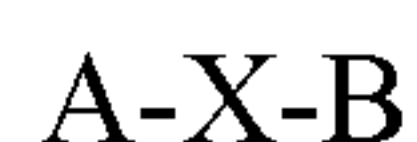
[0003] Modulation of RNA function is a developing area of therapeutic interest. Drugs that affect mRNA stability like antisense oligonucleotides and short interfering RNAs are one way to modulate RNA function. Another group of oligonucleotides can modulate RNA function by altering the processing of pre-mRNA to include or exclude specific regions of pre-mRNAs from the ultimate gene product: the encoded protein. As such, oligonucleotide therapeutics represent a means of modulating protein expression in disease states and as such have utility as therapeutics.

SUMMARY OF THE DISCLOSURE

[0004] Disclosed herein, in certain embodiments, are molecules and pharmaceutical compositions for modulating RNA processing.

[0005] Disclosed herein, in certain embodiments, are methods of treating a disease or disorder caused by an incorrectly spliced mRNA transcript in a subject in need thereof, the method comprising: administering to the subject a polynucleic acid molecule conjugate; wherein the polynucleic acid molecule conjugate is conjugated to a cell targeting binding moiety; wherein the polynucleotide optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; wherein the polynucleic acid molecule conjugate induces insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion in the incorrectly spliced mRNA transcript to generate a fully processed mRNA transcript; and wherein the fully processed mRNA transcript encodes a functional protein, thereby treating the disease or disorder in the subject. In some embodiments, the disease or disorder is further characterized by one or more mutations in the

mRNA. In some embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some embodiments, the disease or disorder is muscular dystrophy. In some embodiments, the disease or disorder is Duchenne muscular dystrophy. In some embodiments, the exon skipping is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some embodiments, the exon skipping is of exon 23 of the *DMD* gene. In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of a polynucleotide; and

X consists of a bond or first linker.

In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

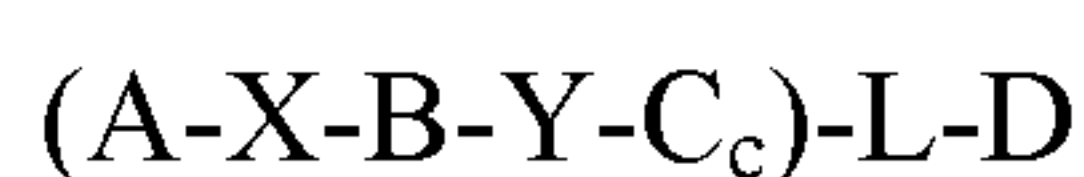
X consists of a bond or first linker; and

Y consists of a bond or second linker.

In some embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), or a peptide nucleic acid (PNA). In some embodiments, the at least one 2' modified nucleotide comprises a morpholino. In some embodiments, the at least one inverted basic moiety is at least one terminus. In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleic acid molecule is at least from about 10 to about 30 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least one of: from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification,

from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification. In some embodiments, the polynucleic acid molecule comprises from about 10% to about 20% modification. In some embodiments, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications. In some embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In some embodiments, the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications. In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides. In some embodiments, the polynucleic acid molecule comprises a single strand. In some embodiments, the polynucleic acid molecule comprises two or more strands. In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In some embodiments, X is a bond. In some embodiments, X is a C₁-C₆ alkyl group. In some embodiments, Y is a C₁-C₆ alkyl group. In some embodiments, X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C₁-C₆ alkyl group. In some embodiments, Y is a homobifunctional linker or a heterobifunctional linker. In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, the antibody or binding fragment thereof comprises a humanized antibody or binding

fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, C has a molecular weight of about 5000 Da. In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group. In some embodiments, methods further comprise D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is conjugated to the molecule conjugate of Formula (II) according to Formula (IV):



Formula IV

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists of a bond or first linker;

Y consists of a bond or second linker;

L consists of a bond or third linker;

D consists of an endosomolytic moiety; and

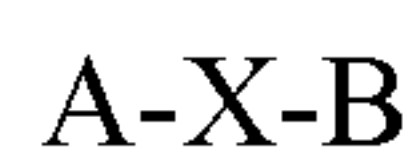
c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or an inverted abasic moiety; and D is conjugated anywhere on A, B, or C.

In some embodiments, D is INF7 or melittin. In some embodiments, L is a C₁-C₆ alkyl group. In some embodiments, L is a homobifunctional linker or a heterobifunctional linker. In some embodiments, methods further comprise at least a second binding moiety A. In some embodiments, the at least second binding moiety A is conjugated to A, to B, or to C.

[0006] Disclosed herein, in some embodiments, are methods of inducing an insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion in the incorrectly spliced mRNA transcript, the method comprising: contacting a target cell with a polynucleic acid molecule conjugate, wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; hybridizing the polynucleic acid molecule conjugate to the incorrectly

spliced mRNA transcript within the target cell to induce an insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion, wherein the incorrectly spliced mRNA transcript is capable of encoding a functional form of a protein; and translating the functional form of a protein from a fully processed mRNA transcript of the previous step. In some embodiments, the target cell is a target cell of a subject. In some embodiments, the incorrectly spliced mRNA transcript further induces a disease or disorder. In some embodiments, the disease or disorder is further characterized by one or more mutations in the mRNA. In some embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some embodiments, the disease or disorder is muscular dystrophy. In some embodiments, the disease or disorder is Duchenne muscular dystrophy. In some embodiments, the exon skipping is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some embodiments, the exon skipping is of exon 23 of the *DMD* gene. In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of a polynucleotide; and

X consists of a bond or first linker.

In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

In some embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

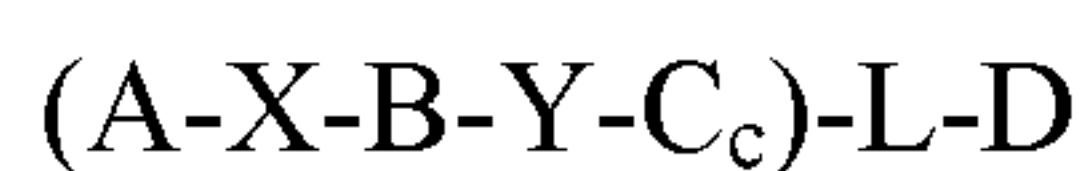
X consists of a bond or first linker; and

Y consists of a bond or second linker.

In some embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), peptide nucleic acid (PNA). In some embodiments, the at least one 2' modified nucleotide comprises a morpholino. In some embodiments, the at least one inverted basic moiety is at least one terminus. In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleic acid molecule is at least from about 10 to about 30 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least one of: from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about

60% to about 80% modification, and from about 70% to about 80% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification. In some embodiments, the polynucleic acid molecule comprises from about 10% to about 20% modification. In some embodiments, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications. In some embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In some embodiments, the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications. In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides. In some embodiments, the polynucleic acid molecule comprises a single strand. In some embodiments, the polynucleic acid molecule comprises two or more strands. In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a

non-polymeric linker group. In some embodiments, X is a bond. In some embodiments, X is a C₁-C₆ alkyl group. In some embodiments, Y is a C₁-C₆ alkyl group. In some embodiments, X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C₁-C₆ alkyl group. In some embodiments, Y is a homobifunctional linker or a heterobifunctional linker. In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, C has a molecular weight of about 5000 Da. In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group. In some embodiments, methods further comprise D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is conjugated to the molecule conjugate of Formula (II) according to Formula (IV):



Formula IV

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists of a bond or first linker;

Y is a bond or second linker;

L consists of a bond or third linker;

D consists of an endosomolytic moiety; and

c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or an inverted abasic moiety; and D is conjugated anywhere on A, B, or C.

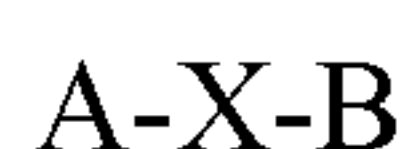
In some embodiments, D is INF7 or melittin. In some embodiments, L is a C₁-C₆ alkyl group. In some embodiments, L is a homobifunctional linker or a heterobifunctional linker. In some embodiments, methods further comprise at least a second binding moiety A. In some embodiments, the at least second binding moiety A is conjugated to A, to B, or to C. In some

embodiments, the method is an *in vivo* method. In some embodiments, the method is an *in vitro* method. In some embodiments, the subject is a human.

[0007] Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising: a molecule obtained by any one of the methods disclosed herein and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated as a nanoparticle formulation. In some embodiments, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0008] Disclosed herein, in certain embodiments, are kits comprising a molecule obtained by any one of the methods disclosed herein.

[0009] Disclosed herein, in certain embodiments, are compositions comprising a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a polynucleotide comprising a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 54-972. Disclosed herein, in certain embodiments, are compositions comprising a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a polynucleotide comprising a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 54-972. In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of the polynucleotide; and

X consists of a bond or first linker.

In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino.

[0010] Disclosed herein, in certain embodiments, are methods of treating a disease or disorder comprising: administering to a subject a polynucleic acid molecule conjugate; wherein the polynucleic acid molecule conjugate comprises a target cell binding moiety and a targeted pre-mRNA specific splice modulating polynucleic acid moiety; wherein the target cell binding moiety specifically binds to a targeted cell, and the targeted pre-mRNA specific splice modulating polynucleic acid moiety induces insertion, deletion, duplication, or alteration of a targeted pre-mRNA transcript in the targeted cell to induce a splicing event in the targeted pre-mRNA transcript to generate a mRNA transcript; and wherein the mRNA transcript encodes a protein that is modified when compared to the same protein in untreated target cells, thereby treating the disease or disorder in the subject. In certain embodiments, the splicing event is exon skipping. In certain embodiments, the splicing event is exon inclusion. In certain embodiments, the disease or disorder is further characterized by one or more mutations in the pre-mRNA. In certain embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In certain embodiments, the disease or disorder is muscular dystrophy. In certain embodiments, the disease or disorder is Duchenne muscular dystrophy. In certain embodiments, the splicing event is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of *DMD* gene. In certain embodiments, the splicing event is of exon 23 of *DMD* gene. In certain

embodiments, the splicing event is of an exon of *PAH*, *MSTN*, or *K-Ras* gene. In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of a polynucleotide; and

X consists of a bond or first linker.

In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists a bond or first linker; and

Y consists of a bond or second linker.

In certain embodiments, the polynucleic acid molecule conjugate comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

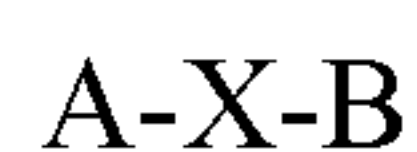
In certain embodiments, the polynucleic acid molecule conjugate optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido

(2'-O-NMA) modified nucleotide. In certain embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), or a peptide nucleic acid (PNA). In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino. In certain embodiments, the at least one inverted basic moiety is at least one terminus. In certain embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In certain embodiments, the polynucleic acid molecule comprises at least from about 10 to about 30 nucleotides in length. In certain embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In certain embodiments, the polynucleic acid molecule comprises a single strand. In certain embodiments, the polynucleic acid molecule comprises two or more strands. In certain embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In certain embodiments, the second polynucleotide comprises at least one modification. In certain embodiments, the first polynucleotide and the second polynucleotide comprise RNA molecules. In certain embodiments, the first polynucleotide and the second polynucleotide comprise siRNA molecules. In certain embodiments, X is a bond. In certain embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In certain embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In certain embodiments, X is a C₁-C₆ alkyl group. In certain embodiments, X or Y is a C₁-C₆ alkyl group. In certain embodiments, X or Y is a C₁-C₆ alkyl group. In certain embodiments, the binding moiety is an antibody or binding fragment thereof. In certain embodiments, the binding moiety is an antibody or binding fragment thereof. In certain embodiments, the binding moiety is an antibody or binding fragment thereof. In certain embodiments, C is polyethylene glycol. In certain embodiments, C is polyethylene glycol. In certain embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In certain embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In certain embodiments, methods further comprise D. In certain embodiments, D is conjugated to C or to A. In certain embodiments, methods further comprise at least a second binding moiety A. In certain embodiments, methods further comprise at least a second binding moiety A. In certain embodiments, methods further comprise at least a second binding moiety A.

[0011] Disclosed herein, in certain embodiments, are methods of inducing a splicing event in a targeted pre-mRNA transcript, comprising: (a) contacting a target cell with a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a target cell

binding moiety and a targeted pre-mRNA splice modulating polynucleic acid moiety; (b) hybridizing the targeted pre-mRNA splice modulating polynucleic acid moiety to the targeted pre-mRNA transcript within the target cell to induce the splicing event in the targeted pre-mRNA transcript to produce a mRNA transcript; and (c) optionally, translating the mRNA transcript of step (b) in the target cell to produce a protein. In certain embodiments, the splicing event is exon skipping. In certain embodiments, the splicing event is exon inclusion. In certain embodiments, the targeted pre-mRNA transcript induces a disease or disorder. In certain embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In certain embodiments, the polynucleic acid molecule conjugate:

a) comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of the polynucleotide; and

X consists of a bond or first linker;

b) comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker; or

c) comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

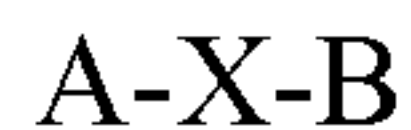
Y consists of a bond or second linker.

In certain embodiments, the polynucleic acid molecule conjugate optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In certain embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), peptide nucleic acid (PNA). In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino. In certain embodiments, the at least one inverted basic moiety is at least one terminus. In certain embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In certain embodiments, the polynucleic acid molecule comprises at least from about 10 to about 30 nucleotides in length. In certain embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In certain embodiments, the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications. In certain embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In certain embodiments, X is a bond. In certain embodiments, X is a C₁-C₆ alkyl group. In certain embodiments, Y is a C₁-C₆ alkyl group. In certain embodiments, X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C₁-C₆ alkyl group. In certain embodiments, Y is a homobifunctional linker or a heterobifunctional linker. In certain embodiments, the binding moiety is an antibody or binding fragment thereof. In certain embodiments, C is polyethylene glycol. In certain embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In certain embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In certain embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group. In certain embodiments, methods further comprise D. In certain embodiments, D is conjugated to C or to A. In certain embodiments, methods further comprise at least a second binding moiety A.

[0012] Disclosed herein, in certain embodiments, are polynucleic acid molecule conjugate compositions comprising a target cell binding moiety and a targeted pre-mRNA specific splice modulating polynucleic acid moiety wherein the targeted pre-mRNA specific splice modulating

polynucleic acid moiety comprises a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 54-972. In certain embodiments, the polynucleic acid molecule conjugate:

- a) comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of the polynucleotide; and

X consists of a bond or first linker;

- b) comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker; or

- c) comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

In certain embodiments, the pharmaceutical composition is formulated as a nanoparticle formulation.

DESCRIPTION OF THE DRAWINGS

[0013] Fig. 1 depicts a phosphorodiamidate morpholino oligomer (PMO) sequence with end nucleotides expanded (SEQ ID NO: 28).

[0014] **Fig. 2A** depicts a phosphorothioate antisense oligonucleotide (PS ASO) sequence with end nucleotides expanded (SEQ ID NO: 29).

[0015] **Fig. 2B** depicts a fully expanded phosphorothioate antisense oligonucleotide (PS ASO) sequence (SEQ ID NO: 29).

[0016] **Fig. 3** depicts methods used to quantify skipped DMD mRNA in total RNA using Taqman qPCR.

[0017] **Fig. 4** depicts a chromatogram of anti-CD71 mAb-PMO reaction mixture produced with hydrophobic interaction chromatography (HIC) method 2.

[0018] **Fig. 5A** depicts a chromatogram of anti-CD71 mAb produced using size exclusion chromatography (SEC) method 1.

[0019] **Fig. 5B** depicts a chromatogram of anti-CD71 mAb-PMO DAR 1,2 produced using size exclusion chromatography (SEC) method 1.

[0020] **Fig. 5C** depicts a chromatogram of anti-CD71 mAb-PMO DAR >2 produced using size exclusion chromatography (SEC) method 1.

[0021] **Fig. 6A** depicts a chromatogram of anti-CD71 mAb produced using hydrophobic interaction chromatography (HIC) method 2.

[0022] **Fig. 6B** depicts a chromatogram of purified anti-CD71 mAb-PMO DAR 1,2 conjugate produced using hydrophobic interaction chromatography (HIC) method 2.

[0023] **Fig. 6C** depicts a chromatogram of purified anti-CD71 mAb-PMO DAR >2 conjugate produced using hydrophobic interaction chromatography (HIC) method 2.

[0024] **Fig. 7A** depicts a chromatogram of fast protein liquid chromatography (FPLC) purification of anti-CD71 Fab-PMO using hydrophobic interaction chromatography (HIC) method 3.

[0025] **Fig. 7B** depicts a chromatogram of anti-CD71 Fab produced using SEC method 1.

[0026] **Fig. 7C** depicts a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using SEC method 1.

[0027] **Fig. 7D** depicts a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using SEC method 1.

[0028] **Fig. 7E** depicts a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using SEC method 1.

[0029] **Fig. 7F** depicts a chromatogram of anti-CD71 Fab produced using HIC method 4.

[0030] **Fig. 7G** depicts a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using HIC method 4.

[0031] **Fig. 7H** depicts a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using HIC method 4.

[0032] **Fig. 7I** depicts a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using HIC method 4.

[0033] **Fig. 8A** depicts a chromatogram of anti-CD71 mAb-PS ASO reaction mixture produced with SAX method 2.

[0034] **Fig. 8B** depicts a chromatogram of anti-CD71 mAb produced using SEC method 1.

[0035] **Fig. 8C** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SEC method 1.

[0036] **Fig. 8D** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SEC method 1.

[0037] **Fig. 8E** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SEC method 1.

[0038] **Fig. 8F** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SAX method 2.

[0039] **Fig. 8G** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SAX method 2.

[0040] **Fig. 8H** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SAX method 2.

[0041] **Fig. 9** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells using PMO and anti-CD71 mAb-PMO conjugate.

[0042] **Fig. 10** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells using PMO, anti-CD71 mAb-PMO, and anti-CD71 Fab-PMO conjugates.

[0043] **Fig. 11** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells PMO, ASO, conjugated anti-CD71 mAb-ASO of DAR1 (“ASC-DAR1”), conjugated anti-CD71 mAb-ASO of DAR2 (“ASC-DAR2”), and conjugated anti-CD71 mAb-ASO of DAR3 (“ASC-DAR3”).

[0044] **Fig. 12A** depicts an agarose gel from nested PCR detecting exon 23 skipping in gastrocnemius muscle of wild-type mice administered a single intravenous injection of anti-CD71 mAb-PMO conjugate.

[0045] **Fig. 12B** is a graph of quantification of PCR products from gastrocnemius muscle.

[0046] **Fig. 12C** is a graph of quantification of *in vivo* exon skipping using Taqman qPCR from gastrocnemius muscle from wild-type mice.

[0047] **Fig. 13A** depicts an agarose gel from nested PCR detecting exon 23 skipping in heart muscle from wild-type mice after a single intravenous injection.

[0048] **Fig. 13B** is a graph of quantification of PCR products from heart muscle.

[0049] **Fig. 14** depicts sequencing data of DNA fragments from skipped and wild-type PCR products (SEQ ID NOS 976-977, respectively).

[0050] **Fig. 15A** is a graph of quantification of *in vivo* exon skipping in wild type mice in gastrocnemius muscle using Taqman qPCR.

[0051] **Fig. 15B** is a graph of quantification of *in vivo* exon skipping in wild type mice in gastrocnemius muscle using nested PCR.

[0052] **Fig. 15C** is a graph of quantification of *in vivo* exon skipping in wild type mice in diaphragm muscle using Taqman qPCR.

[0053] **Fig. 15D** is a graph of quantification of *in vivo* exon skipping in wild type mice in diaphragm muscle using nested PCR.

[0054] **Fig. 15E** is a graph of quantification of *in vivo* exon skipping in wild type mice in heart muscle using Taqman qPCR.

[0055] **Fig. 15F** is a graph of quantification of *in vivo* exon skipping in wild type mice in heart muscle using nested PCR.

[0056] **Fig. 16A** depicts an agarose gel from PCR detecting CD71 mAb-PMO conjugate induction of *MSTN* exon 2 skipping in diaphragm muscle tissues in wild type mice after a single intravenous (i.v.) injection.

[0057] **Fig. 16B** depicts an agarose gel from PCR detecting CD71 mAb-PMO conjugate induction of *MSTN* exon 2 skipping in heart muscle tissues in wild type mice after a single intravenous (i.v.) injection.

[0058] **Fig. 16C** depicts an agarose gel from PCR detecting CD71 mAb-PMO conjugate induction of *MSTN* exon 2 skipping in gastrocnemius muscle tissues in wild type mice after a single intravenous (i.v.) injection.

[0059] **Fig. 17** depicts an agarose gel from PCR detecting ASGPR mAb-PMO conjugate induction of *PAH* exon 11 skipping in primary mouse hepatocytes.

[0060] **Fig. 18** depicts an agarose gel from PCR detecting ASGPR mAb-PMO conjugate induction of *PAH* exon 11 skipping in livers from wild type mice after a single intravenous (i.v.) injection.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0061] Nucleic acid (e.g., RNAi) therapy is a targeted therapy with high selectivity and specificity. However, in some instances, nucleic acid therapy is also hindered by poor intracellular uptake, insufficient intracellular concentrations in target cells, and low efficacy. To address these issues, various modifications of the nucleic acid composition are explored, such as for example, novel linkers for better stabilizing and/or lower toxicity, optimization of binding moiety for increased target specificity and/or target delivery, and nucleic acid polymer modifications for increased stability and/or reduced off-target effect.

[0062] In some instances, one such area where oligonucleotide is used is for treating muscular dystrophy. Muscular dystrophy encompasses several diseases that affect the muscle. Duchenne muscular dystrophy is a severe form of muscular dystrophy and caused by mutations in the *DMD* gene. In some instances, mutations in the *DMD* gene disrupt the translational reading frame and results in non-functional dystrophin protein.

[0063] Described herein, in certain embodiments, are methods and compositions relating nucleic acid therapy to induce an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion, which is used to restore the translational reading frame. In some embodiments, also described herein include methods and compositions for treating a disease or disorder characterized by an incorrectly processed mRNA transcript, in which after removal of an exon, the mRNA is capable of encoding a functional protein, thereby treating the disease or disorder. In additional embodiments, described herein include pharmaceutical compositions and kits for treating the same.

RNA Processing

[0064] RNA has a central role in regulation of gene expression and cell physiology. Proper processing of RNA is important for translational of functional protein. Alterations in RNA processing such as a result of incorrect splicing of RNA can result in disease. For example, mutations in a splice site causes exposure of a premature stop codon, a loss of an exon, or inclusion of an intron. In some instances, alterations in RNA processing results in an insertion, deletion, or duplication. In some instances, alterations in RNA processing results in an insertion, deletion, or duplication of an exon. Alterations in RNA processing, in some cases, results in an insertion, deletion, or duplication of an intron.

[0065] Alternative transcriptional or splicing events include, but are not limited to, exon skipping, alternative 3' splice site selection, alternative 5' splice site selection, intron retention, mutually exclusive exons, alternative promoter usage, and alternative polyadenylation. Splicing

events, in some embodiments, results in an insertion, deletion, or duplication of an exon, for example, by exon skipping or exon inclusion.

Exon Skipping

[0066] Exon skipping is a form of RNA splicing. In some cases, exon skipping occurs when an exon is skipped over or is spliced out of the processed mRNA. As a result of exon skipping, the processed mRNA does not contain the skipped exon. In some instances, exon skipping results in expression of an altered product.

[0067] In some instances, antisense oligonucleotides (AONs) are used to induce exon skipping. In some instances, AONs are short nucleic acid sequences that bind to specific mRNA or pre-mRNA sequences. For example, AONs bind splice sites or exonic enhancers. In some instances, binding of AONs to specific mRNA or pre-mRNA sequences generates double-stranded regions. In some instances, formation of double-stranded regions occurs at sites where the spliceosome or proteins associated with the spliceosome would normally bind and causes exons to be skipped. In some instances, skipping of exons results in restoration of the transcript reading frame and allows for production of a partially functional protein.

Exon Inclusion

[0068] In some instances, a mutation in RNA results in exon skipping. In some cases, a mutation is at least one of at the splice site, near the splice site, and at a distance from the splice site. In some instances, the mutations result in at least one of inactivating or weakening the splice site, disrupting exon splice enhancer or intron splice enhancer, and creating an exon splice silencer or intron splice enhancer. Mutations in some instances alter RNA secondary structure. In some cases, a mutation alters a RNA secondary structure result in disrupting the accessibility of signals important for exon recognition.

[0069] In some instances, use of AONs results in inclusion of the skipped exon. In some instances, the AONs bind to at least one of a splice site, a site near a splice site, and a site distant to a splice site. In some cases, AONs bind at site in the RNA to prevent disruption of an exon splice enhancer or intron splice enhancer. In some instances, AONs bind at site in the RNA to prevent creation of an exon splice silencer or intron splice silencer.

Intron Retention

[0070] In some instances, a mutation in RNA results in intron retention. Intron retention results in an intron remaining in the mature mRNA transcript. In some instances, presence of a retained

intron prevents or reduces translation of a functional protein. In some instances, intron retention occurs in a coding region, a non-coding region, at the 5' UTR, or at the 3' UTR. Where intron retention occurs in a coding region, in some instances, the retained intron encodes amino acids in frame, or is in misalignment which generates truncated proteins or non-functional proteins due to stop codon or frame shifts. In some instances, the intron is retained between two exons, located at the 5' UTR, or located at the 3' UTR.

[0071] In some instances, AONs are used to hybridize to a partially processed mRNA to initiate removal of a retained intron. In some instances, the AONs hybridize to an intronic splicing enhancer or an intronic splicing silencer. In some instances, the AONs hybridize at or a distance from a 5' splice site, 3' splice site, branchpoint, polypyrimidine tract, an intron silencer site, a cryptic intron splice site, a pseudo splice site, or an intron enhancer of the intron. In some instances, the AONs hybridize to an internal region of the intron.

Indications

[0072] In some embodiments, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of a disease or disorder characterized with a defective mRNA. In some embodiments, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of disease or disorder by inducing an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce a splicing event. In some embodiments, the splicing event is exon skipping or exon inclusion. In some embodiments, the splicing event is intron retention.

[0073] In some embodiments, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of disease or disorder by inducing an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

[0074] A large percentage of human protein-coding genes are alternatively spliced. In some instances, a mutation results in improperly spliced or partially spliced mRNA. For example, a mutation is in at least one of a splice site in a protein coding gene, a silencer or enhancer sequence, exonic sequences, or intronic sequences. In some instances, a mutation results in gene dysfunction. In some instances, a mutation results in a disease or disorder.

[0075] In some instances, a disease or disorder resulting from improperly spliced or partially spliced mRNA includes, but not limited to, a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.

[0076] In some instances, genetic diseases or disorders include an autosomal dominant disorder, an autosomal recessive disorder, X-linked dominant disorder, X-linked recessive disorder, Y-linked disorder, mitochondrial disease, or multifactorial or polygenic disorder.

[0077] In some instances, cardiovascular disease such as hypercholesterolemia results from improperly spliced or partially spliced mRNA. In hypercholesterolemia, it has been shown that a single nucleotide polymorphism in exon 12 of the low density lipoprotein receptor (LDLR) promotes exon skipping.

[0078] In some instances, improperly spliced or partially spliced mRNA results in cancer. For example, improperly spliced or partially spliced mRNA affects cellular processes involved in cancer including, but not limited to, proliferation, motility, and drug response. In some instances is a solid cancer or a hematologic cancer. In some instances, the cancer is bladder cancer, lung cancer, brain cancer, melanoma, breast cancer, Non-Hodgkin lymphoma, cervical cancer, ovarian cancer, colorectal cancer, pancreatic cancer, esophageal cancer, prostate cancer, kidney cancer, skin cancer, leukemia, thyroid cancer, liver cancer, or uterine cancer.

[0079] Improperly spliced or partially spliced mRNA in some instances causes a neuromuscular disease or disorder. Exemplary neuromuscular diseases include muscular dystrophy such as Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some instances, muscular dystrophy is genetic. In some instances, muscular dystrophy is caused by a spontaneous mutation. Becker muscular dystrophy and Duchenne muscular dystrophy have been shown to involve mutations in the *DMD* gene, which encodes the protein dystrophin. Facioscapulohumeral muscular dystrophy has been shown to involve mutations in double homeobox, 4 (*DUX4*) gene.

[0080] In some instances, improperly spliced or partially spliced mRNA causes Duchenne muscular dystrophy. Duchenne muscular dystrophy results in severe muscle weakness and is caused by mutations in the *DMD* gene that abolishes the production of functional dystrophin. In some instances, Duchenne muscular dystrophy is a result of a mutation in an exon in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 1, 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78 and 79 in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 8, 23,

35, 43, 44, 45, 50, 51, 52, 53, and 55 in the *DMD* gene. In some instances, multiple exons are mutated. For example, mutation of exons 48-50 is common in Duchenne muscular dystrophy patients. In some instances, Duchenne muscular dystrophy is a result of mutation of exon 51. In some instances, Duchenne muscular dystrophy is a result of mutation of exon 23. In some instances, a mutation involves a deletion of an exon. In some instances, a mutation involves a duplication of an exon. In some instances, a mutation involves a point mutation in an exon. For example, it has been shown that some patients have a nonsense point mutation in exon 51 of the *DMD* gene.

[0081] In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of muscular dystrophy. In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of Duchenne muscular dystrophy.

Polynucleic Acid Molecule

[0082] In some embodiments, a polynucleic acid molecule described herein that induces an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion. In some instances, the polynucleic acid molecule restores the translational reading frame. In some instances, the polynucleic acid molecule results in a functional and truncated protein.

[0083] In some instances, a polynucleic acid molecule targets a mRNA sequence. In some instances, the polynucleic acid molecule targets a splice site. In some instances, the polynucleic acid molecule targets a *cis*-regulatory element. In some instances, the polynucleic acid molecule targets a *trans*-regulatory element. In some instances, the polynucleic acid molecule targets exonic splice enhancers or intronic splice enhancers. In some instances, the polynucleic acid molecule targets exonic splice silencers or intronic splice silencers.

[0084] In some instances, a polynucleic acid molecule targets a sequence found in introns or exons. For example, the polynucleic acid molecule targets a sequence found in an exon that mediates splicing of said exon. In some instances, the polynucleic acid molecule targets an exon recognition sequence. In some instances, the polynucleic acid molecule targets a sequence upstream of an exon. In some instances, the polynucleic acid molecule targets a sequence downstream of an exon.

[0085] As described above, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in a disease or disorder not limited to a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.

[0086] In some instances, a polynucleic acid molecule targets an exon that is mutated in a gene that causes a disease or disorder. Exemplary diseases or disorders include, but are not limited to, familial dysautonomia (FD), spinal muscular atrophy (SMA), medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, Hutchinson-Gilford progeria syndrome (HGPS), myotonic dystrophy type I (DM1), myotonic dystrophy type II (DM2), autosomal dominant retinitis pigmentosa (RP), Duchenne muscular dystrophy (DMD), microcephalic steodysplastic primordial dwarfism type 1 (MOPD1) (Taybi-Linder syndrome (TALS)), frontotemporal dementia with parkinsonism-17 (FTDP-17), Fukuyama congenital muscular dystrophy (FCMD), amyotrophic lateral sclerosis (ALS), hypercholesterolemia, and cystic fibrosis (CF). Exemplary genes that are involved in the disease or disorder include, but are not limited to, *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, and *K-Ras*. In some embodiments, the gene is *DMD*, *PAH*, *MSTN*, or *K-Ras*.

[0087] In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 1, 2, or 3 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 2 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 11 of *PAH*.

[0088] In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of at least one of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some embodiments, a polynucleic acid molecule described herein targets either the 5' intron-exon junction or the 3' exon-intron junction of exon 1, 2, or 3 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is either the 5' intron-exon junction or the 3' exon-intron junction of exon 2 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is either the 5' intron-exon junction or the 3' exon-

intron junction of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH*. In some embodiments, a polynucleic acid molecule described herein targets a region that is either the 5' intron-exon junction or the 3' exon-intron junction of exon 11 of *PAH*.

[0089] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of at least one of exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, *TDP-43*, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 5' intron-exon junction of exon 1, 2, or 3 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 5' intron-exon junction of exon 2 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 5' intron-exon junction of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 5' intron-exon junction of exon 11 of *PAH*.

[0090] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of at least one of exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, *TDP-43*, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 3' exon-intron junction of exon 1, 2, or 3 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 3' exon-intron junction of exon 2 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 3' exon-intron junction of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH*. In some embodiments, a polynucleic acid molecule described herein targets a region that is at the 3' exon-intron junction of exon 11 of *PAH*.

[0091] In some cases, the polynucleic acid molecule described herein targets a splice site of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, *TDP-43*, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some embodiments, a polynucleic acid molecule described herein targets a splice site of exon 1, 2, or 3 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a splice site of exon 2 of *MSTN*. In some embodiments, a polynucleic acid molecule described herein targets a splice site of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH*. In some embodiments, a polynucleic acid molecule described herein targets a splice site of exon 11 of *PAH*. As used herein, a splice site includes a canonical splice site, a cryptic splice site or an alternative splice site that is capable of inducing an insertion,

deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

[0092] In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, *TDP-43*, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 1, 2, or 3 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 2 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of *PAH* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 11 of the *PAH* gene.

[0093] In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, *TDP-43*, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 1, 2, or 3 of the *MSTN* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to at least one of exon 2 of the *MSTN* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of the *PAH* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to at least one of exon 11 of the *PAH* gene.

[0094] In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*,

TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 1, 2, or 3 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 2 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of the *PAH* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 11 of the *PAH* gene.

[0095] In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to at least one of an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 1, 2, or 3 of the *MSTN* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 2 of the *MSTN* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of the *PAH* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 11 of the *PAH* gene.

[0096] In some instances, a polynucleic acid molecule described herein targets an internal region within an exon of a gene that causes a disease or disorder. In some embodiments, the gene is *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 1, 2, or 3 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 2 of the *MSTN* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 of the *PAH* gene. In some instances, a

polynucleic acid molecule described herein targets an internal region within exon 11 of the *PAH* gene.

[0097] In some cases, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in a neuromuscular disease or disorder. In some cases, a neuromuscular disease or disorder is Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some cases, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some cases, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in Duchenne muscular dystrophy.

[0098] In some instances, a polynucleic acid molecule targets an exon that is mutated in the *DMD* gene that causes Duchenne muscular dystrophy. Exemplary exons that are mutated in the *DMD* gene that causes Duchenne muscular dystrophy include, but not limited to, exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63. In some instances, the polynucleic acid molecule targets a sequence adjacent to a mutated exon. For example, if there is a deletion of exon 50, the polynucleic acid molecule targets a sequence in exon 51 so that exon 51 is skipped. In another instance, if there is a mutation in exon 23, the polynucleic acid molecule targets a sequence in exon 22 so that exon 23 is skipped.

[0099] In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 8 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 23 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 35 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 43 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 44 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 45 of the *DMD* gene. In

some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 48 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 49 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 50 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 51 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 52 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 53 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 55 of the *DMD* gene.

[0100] In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0101] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 8 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 23 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 35 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 43 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 44 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 45 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 50 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 51 of the *DMD* gene. In some cases, the

polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 52 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 53 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 55 of the *DMD* gene.

[0102] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 8 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 23 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 35 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 43 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 44 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 45 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 50 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 51 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 52 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 53 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 55 of the *DMD* gene.

[0103] In some instances, a polynucleic acid molecule described herein targets a splice site of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 23 of the *DMD* gene. In

some cases, a polynucleic acid molecule described herein targets a splice site of exon 35 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 43 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 44 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 45 of the *DMD* gene.

In some instances, a polynucleic acid molecule described herein targets a splice site of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 51 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 52 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 53 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 55 of the *DMD* gene. As used herein, a splice site includes a canonical splice site, a cryptic splice site or an alternative splice site that is capable of inducing an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

[0104] In some embodiments, a polynucleic acid molecule described herein target a partially spliced mRNA sequence comprising additional exons involved in Duchenne muscular dystrophy such as exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63.

[0105] In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt,

50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 55 of the *DMD* gene.

[0106] In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to at

least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene.

[0107] In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt

downstream (or from the 3') of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 55 of the *DMD* gene.

[0108] In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0109] In some instances, a polynucleic acid molecule described herein targets an internal region within exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon

44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 55 of the *DMD* gene.

[0110] In some instances, the polynucleic acid molecule hybridizes to a target region that is within at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is within at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0111] In some embodiments, a polynucleic acid molecule described herein targets a partially spliced mRNA sequence comprising exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to exon 51.

[0112] In some instances, the polynucleic acid molecule hybridizes to a target region that is within exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon 51 junction or the 3' exon 51-intron junction.

[0113] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid

molecule comprises a sequence having at least 70% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule consists of a target sequence of interest.

[0114] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest.

[0115] In some embodiments, the polynucleic acid molecule described herein comprises RNA or DNA. In some cases, the polynucleic acid molecule comprises RNA. In some instances, RNA comprises short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), double-stranded RNA (dsRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), or heterogeneous nuclear RNA (hnRNA). In some instances, RNA comprises shRNA. In some instances, RNA comprises miRNA. In some instances, RNA comprises dsRNA. In some instances, RNA comprises tRNA. In some instances, RNA comprises rRNA. In some instances, RNA comprises

hnRNA. In some instances, the RNA comprises siRNA. In some instances, the polynucleic acid molecule comprises siRNA.

[0116] In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0117] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances, the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 50 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 45 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 40 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 35 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 20 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 12 and about 30 nucleotides in length.

[0118] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide. In some instances, the polynucleic acid molecule comprises a second polynucleotide. In some

instances, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide is a sense strand or passenger strand. In some instances, the second polynucleotide is an antisense strand or guide strand.

[0119] In some embodiments, the polynucleic acid molecule is a first polynucleotide. In some embodiments, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0120] In some instances, the first polynucleotide is about 50 nucleotides in length. In some instances, the first polynucleotide is about 45 nucleotides in length. In some instances, the first polynucleotide is about 40 nucleotides in length. In some instances, the first polynucleotide is about 35 nucleotides in length. In some instances, the first polynucleotide is about 30 nucleotides in length. In some instances, the first polynucleotide is about 25 nucleotides in length. In some instances, the first polynucleotide is about 20 nucleotides in length. In some instances, the first polynucleotide is about 19 nucleotides in length. In some instances, the first polynucleotide is about 18 nucleotides in length. In some instances, the first polynucleotide is about 17 nucleotides in length. In some instances, the first polynucleotide is about 16 nucleotides in length. In some instances, the first polynucleotide is about 15 nucleotides in length. In some instances, the first polynucleotide is about 14 nucleotides in length. In some instances, the first polynucleotide is about 13 nucleotides in length. In some instances, the first polynucleotide is about 12 nucleotides in length. In some instances, the first polynucleotide is about 11 nucleotides in length. In some instances, the first polynucleotide is about 10 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 12 and about 30 nucleotides in length.

[0121] In some embodiments, the polynucleic acid molecule is a second polynucleotide. In some embodiments, the second polynucleotide is from about 10 to about 50 nucleotides in length. In

some instances, the second polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0122] In some instances, the second polynucleotide is about 50 nucleotides in length. In some instances, the second polynucleotide is about 45 nucleotides in length. In some instances, the second polynucleotide is about 40 nucleotides in length. In some instances, the second polynucleotide is about 35 nucleotides in length. In some instances, the second polynucleotide is about 30 nucleotides in length. In some instances, the second polynucleotide is about 25 nucleotides in length. In some instances, the second polynucleotide is about 20 nucleotides in length. In some instances, the second polynucleotide is about 19 nucleotides in length. In some instances, the second polynucleotide is about 18 nucleotides in length. In some instances, the second polynucleotide is about 17 nucleotides in length. In some instances, the second polynucleotide is about 16 nucleotides in length. In some instances, the second polynucleotide is about 15 nucleotides in length. In some instances, the second polynucleotide is about 14 nucleotides in length. In some instances, the second polynucleotide is about 13 nucleotides in length. In some instances, the second polynucleotide is about 12 nucleotides in length. In some instances, the second polynucleotide is about 11 nucleotides in length. In some instances, the second polynucleotide is about 10 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 12 and about 30 nucleotides in length.

[0123] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the polynucleic acid molecule further comprises a blunt terminus, an overhang, or a combination thereof. In some instances, the blunt terminus is a 5' blunt terminus, a 3' blunt terminus, or both. In some cases, the overhang is a 5' overhang, 3' overhang, or both. In some cases, the overhang comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-base

pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, 4, 5, or 6 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, or 4 non-base pairing nucleotides. In some cases, the overhang comprises 1 non-base pairing nucleotide. In some cases, the overhang comprises 2 non-base pairing nucleotides. In some cases, the overhang comprises 3 non-base pairing nucleotides. In some cases, the overhang comprises 4 non-base pairing nucleotides.

[0124] In some embodiments, the sequence of the polynucleic acid molecule is at least 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 50% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 60% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 70% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 80% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 90% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 95% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 99% complementary to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule is 100% complementary to a target sequence described herein.

[0125] In some embodiments, the sequence of the polynucleic acid molecule has 5 or less mismatches to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule has 4 or less mismatches to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 1 or less mismatches to a target sequence described herein.

[0126] In some embodiments, the specificity of the polynucleic acid molecule that hybridizes to a target sequence described herein is a 95%, 98%, 99%, 99.5% or 100% sequence complementarity of the polynucleic acid molecule to a target sequence. In some instances, the hybridization is a high stringent hybridization condition.

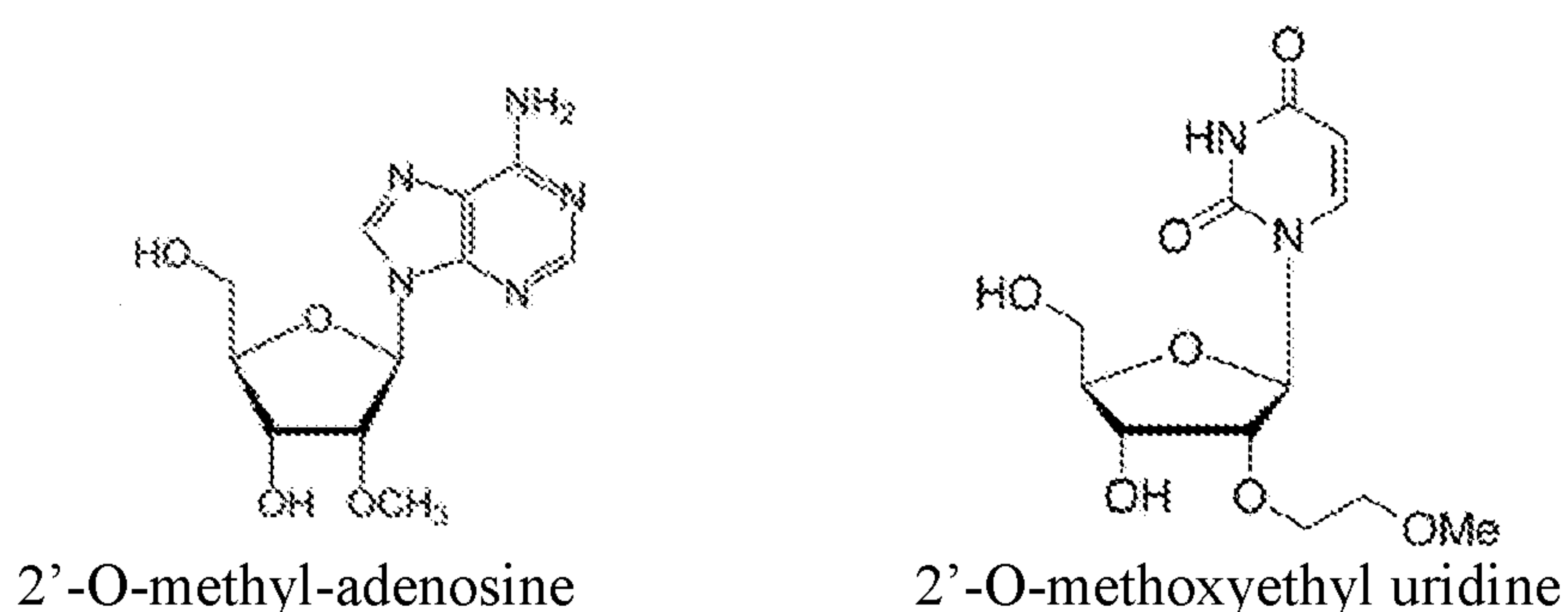
[0127] In some embodiments, the polynucleic acid molecule has reduced off-target effect. In some instances, “off-target” or “off-target effects” refer to any instance in which a polynucleic acid polymer directed against a given target causes an unintended effect by interacting either directly or

indirectly with another mRNA sequence, a DNA sequence or a cellular protein or other moiety. In some instances, an “off-target effect” occurs when there is a simultaneous degradation of other transcripts due to partial homology or complementarity between that other transcript and the sense and/or antisense strand of the polynucleic acid molecule.

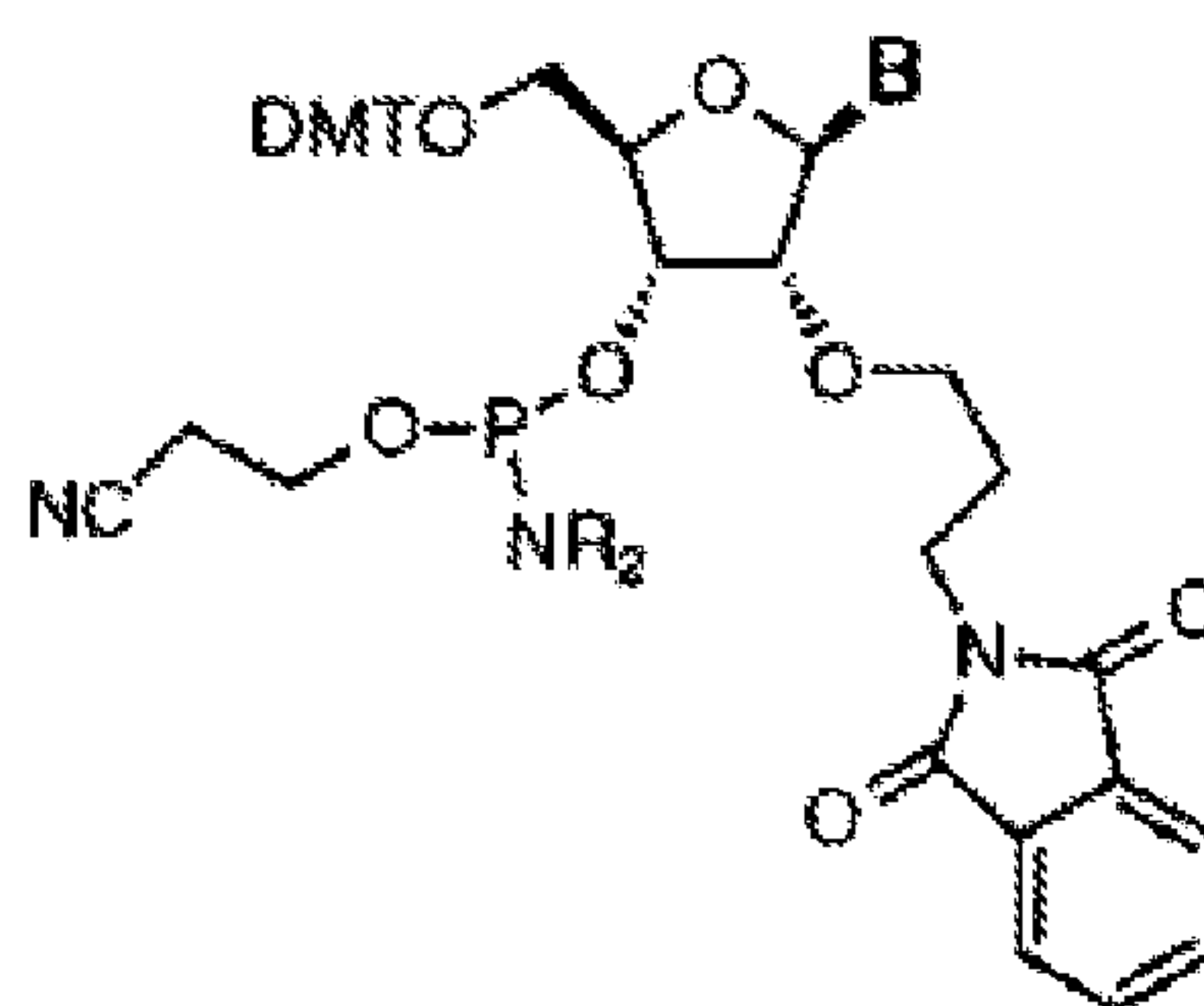
[0128] In some embodiments, the polynucleic acid molecule comprises natural or synthetic or artificial nucleotide analogues or bases. In some cases, the polynucleic acid molecule comprises combinations of DNA, RNA and/or nucleotide analogues. In some instances, the synthetic or artificial nucleotide analogues or bases comprise modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

[0129] In some embodiments, nucleotide analogues or artificial nucleotide base comprise a nucleic acid with a modification at a 2' hydroxyl group of the ribose moiety. In some instances, the modification includes an H, OR, R, halo, SH, SR, NH₂, NHR, NR₂, or CN, wherein R is an alkyl moiety. Exemplary alkyl moiety includes, but is not limited to, halogens, sulfurs, thiols, thioethers, thioesters, amines (primary, secondary, or tertiary), amides, ethers, esters, alcohols and oxygen. In some instances, the alkyl moiety further comprises a modification. In some instances, the modification comprises an azo group, a keto group, an aldehyde group, a carboxyl group, a nitro group, a nitroso group, a nitrile group, a heterocycle (e.g., imidazole, hydrazino or hydroxylamino) group, an isocyanate or cyanate group, or a sulfur containing group (e.g., sulfoxide, sulfone, sulfide, and disulfide). In some instances, the alkyl moiety further comprises a hetero substitution. In some instances, the carbon of the heterocyclic group is substituted by a nitrogen, oxygen or sulfur. In some instances, the heterocyclic substitution includes but is not limited to, morpholino, imidazole, and pyrrolidino.

[0130] In some instances, the modification at the 2' hydroxyl group is a 2'-O-methyl modification or a 2'-O-methoxyethyl (2'-O-MOE) modification. In some cases, the 2'-O-methyl modification adds a methyl group to the 2' hydroxyl group of the ribose moiety whereas the 2'-O-methoxyethyl modification adds a methoxyethyl group to the 2' hydroxyl group of the ribose moiety. Exemplary chemical structures of a 2'-O-methyl modification of an adenosine molecule and 2'-O-methoxyethyl modification of an uridine are illustrated below.

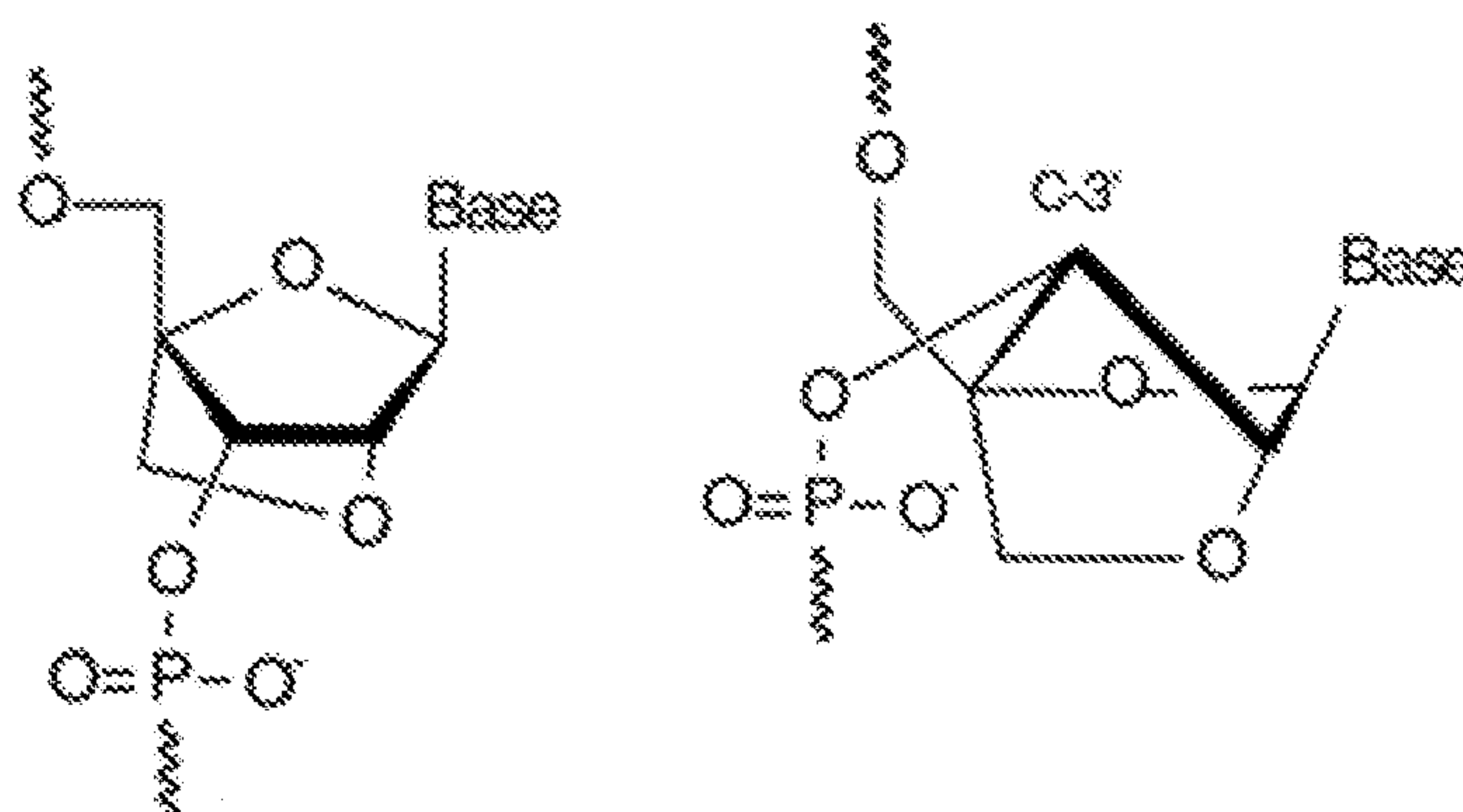


[0131] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties. An exemplary chemical structure of a 2'-O-aminopropyl nucleoside phosphoramidite is illustrated below.



2'-O-aminopropyl nucleoside phosphoramidite

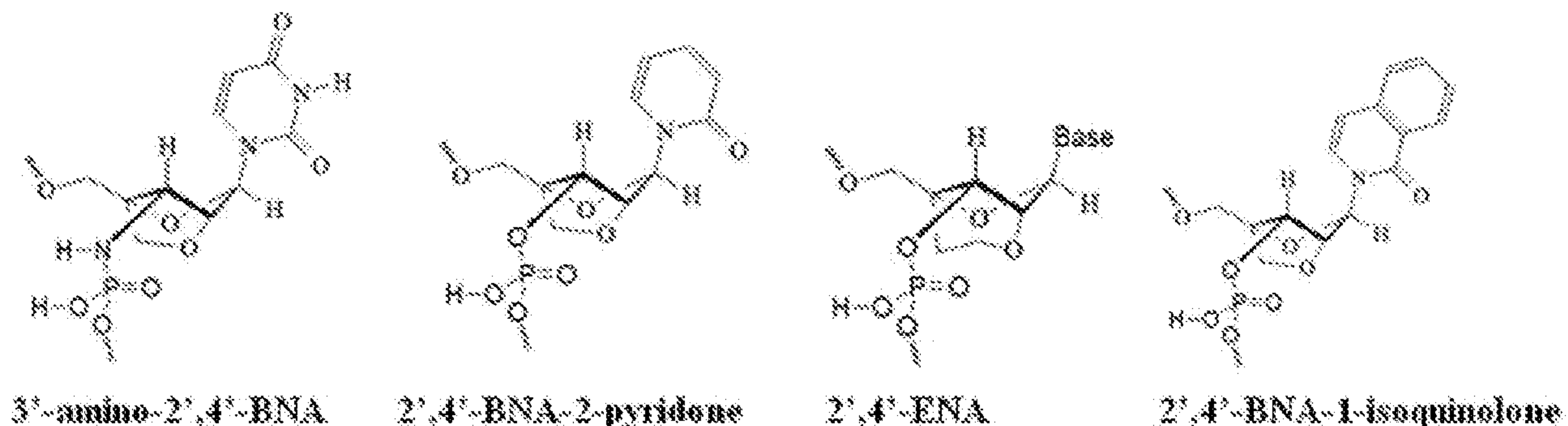
[0132] In some instances, the modification at the 2' hydroxyl group is a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2'-C,4'-C-oxy-methylene-linked bicyclic ribonucleotide monomer. Exemplary representations of the chemical structure of LNA are illustrated below. The representation shown to the left highlights the chemical connectivities of an LNA monomer. The representation shown to the right highlights the locked 3'-endo (³E) conformation of the furanose ring of an LNA monomer.



LNA (Locked Nucleic Acids)

[0133] In some instances, the modification at the 2' hydroxyl group comprises ethylene nucleic acids (ENA) such as for example 2'-4'-ethylene-bridged nucleic acid, which locks the sugar conformation into a C_{3'}'-endo sugar puckering conformation. ENA are part of the bridged nucleic

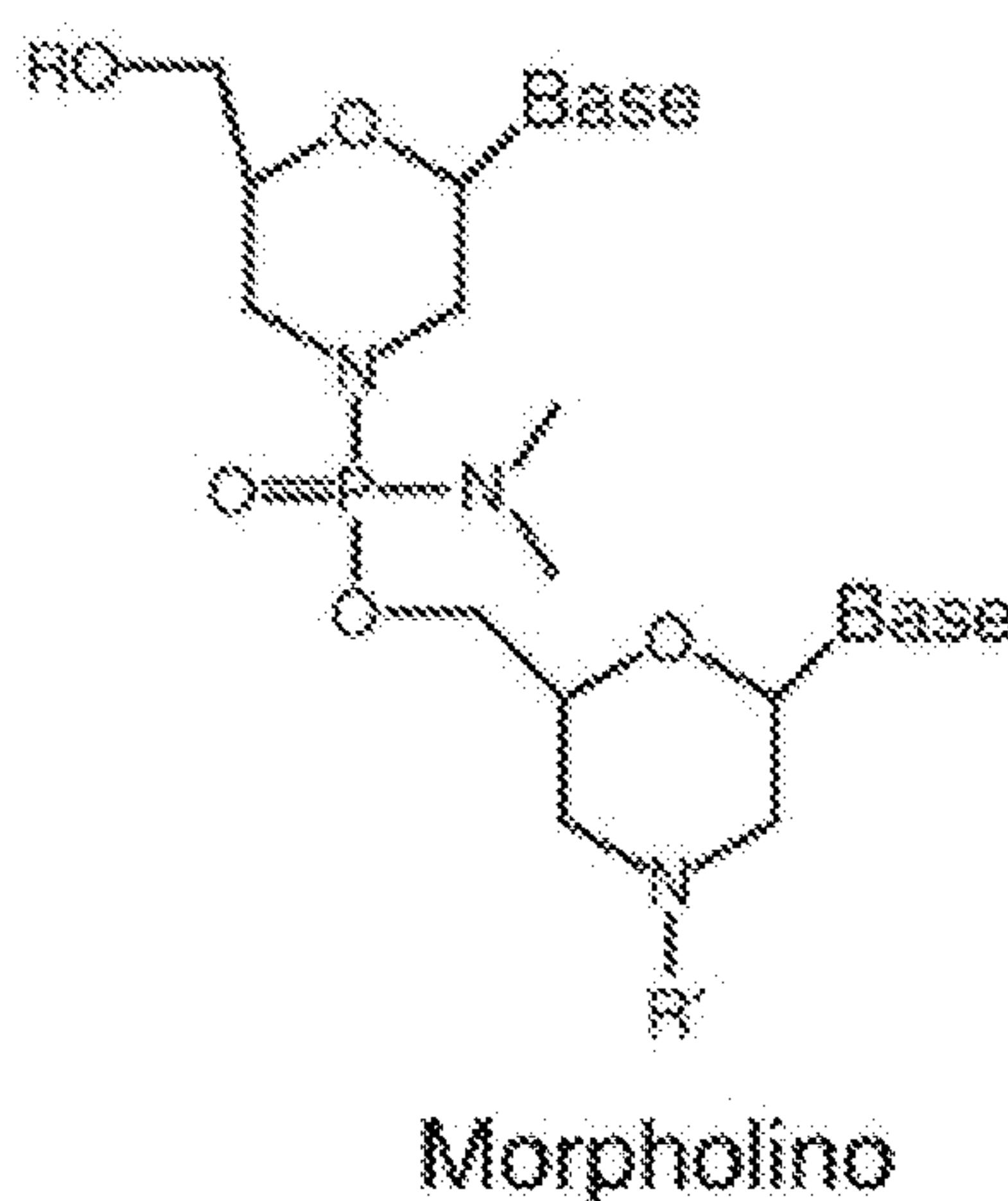
acids class of modified nucleic acids that also comprises LNA. Exemplary chemical structures of the ENA and bridged nucleic acids are illustrated below.



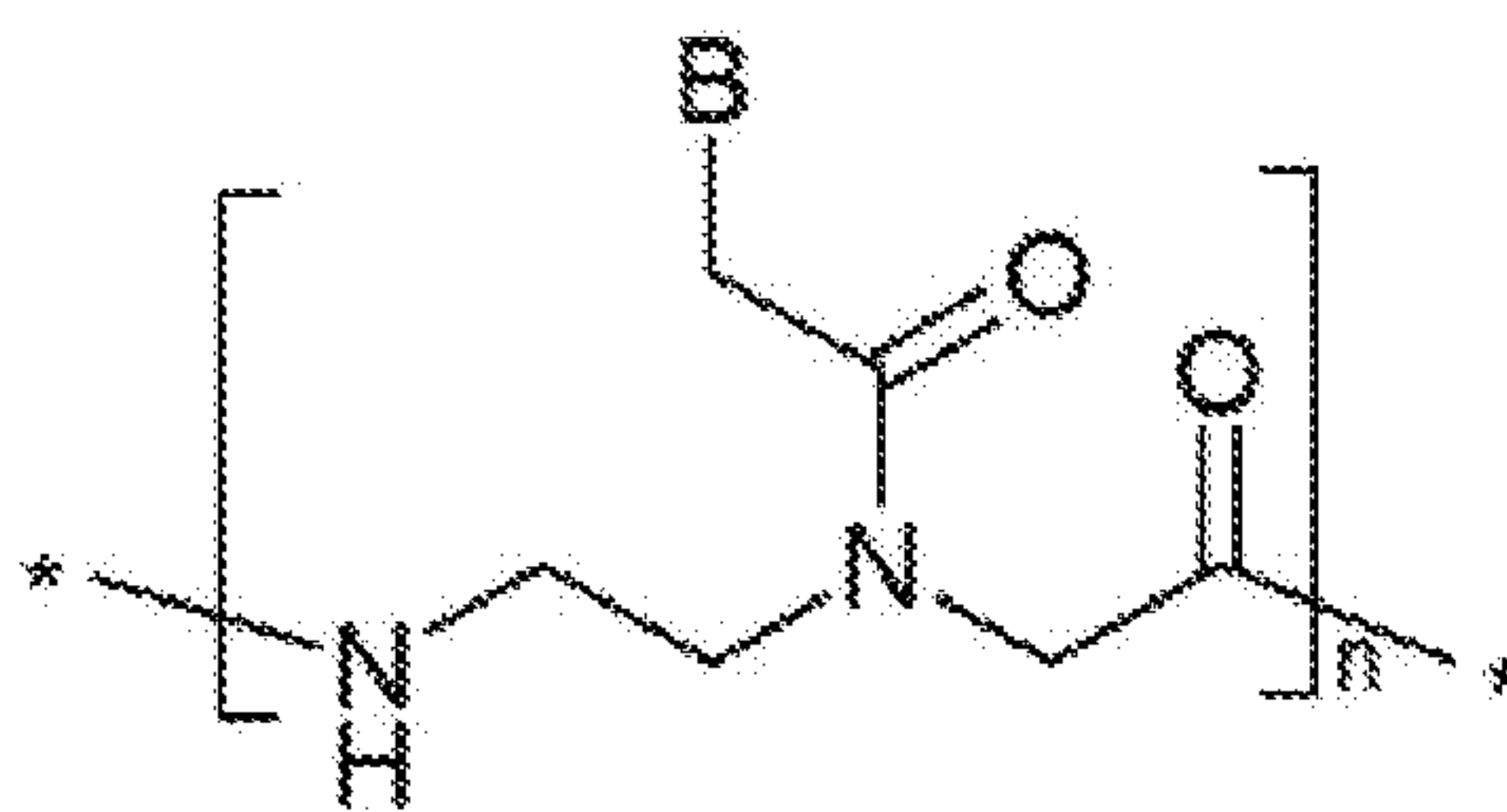
[0134] In some embodiments, additional modifications at the 2' hydroxyl group include 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

[0135] In some embodiments, nucleotide analogues comprise modified bases such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N, N, -dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5- (2- amino) propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2- methylguanosine, 7-methylguanosine, 2, 2-dimethylguanosine, 5- methylaminoethyluridine, 5-methoxyuridine, deazanucleotides such as 7-deaza- adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O-and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0136] In some embodiments, nucleotide analogues further comprise morpholinos, peptide nucleic acids (PNAs), methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, 1', 5'- anhydrohexitol nucleic acids (HNAs), or a combination thereof. Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure by deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides.



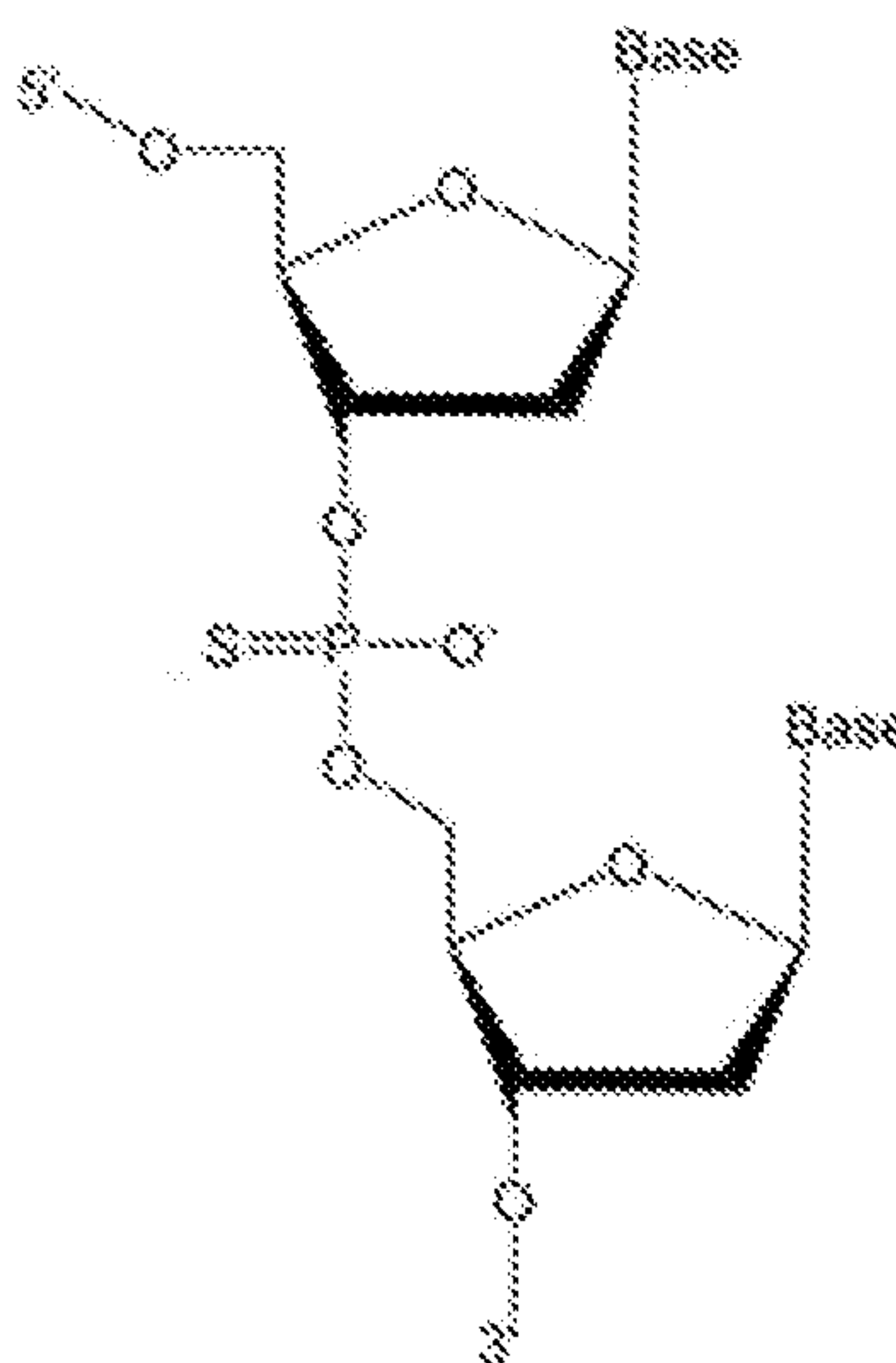
[0137] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.



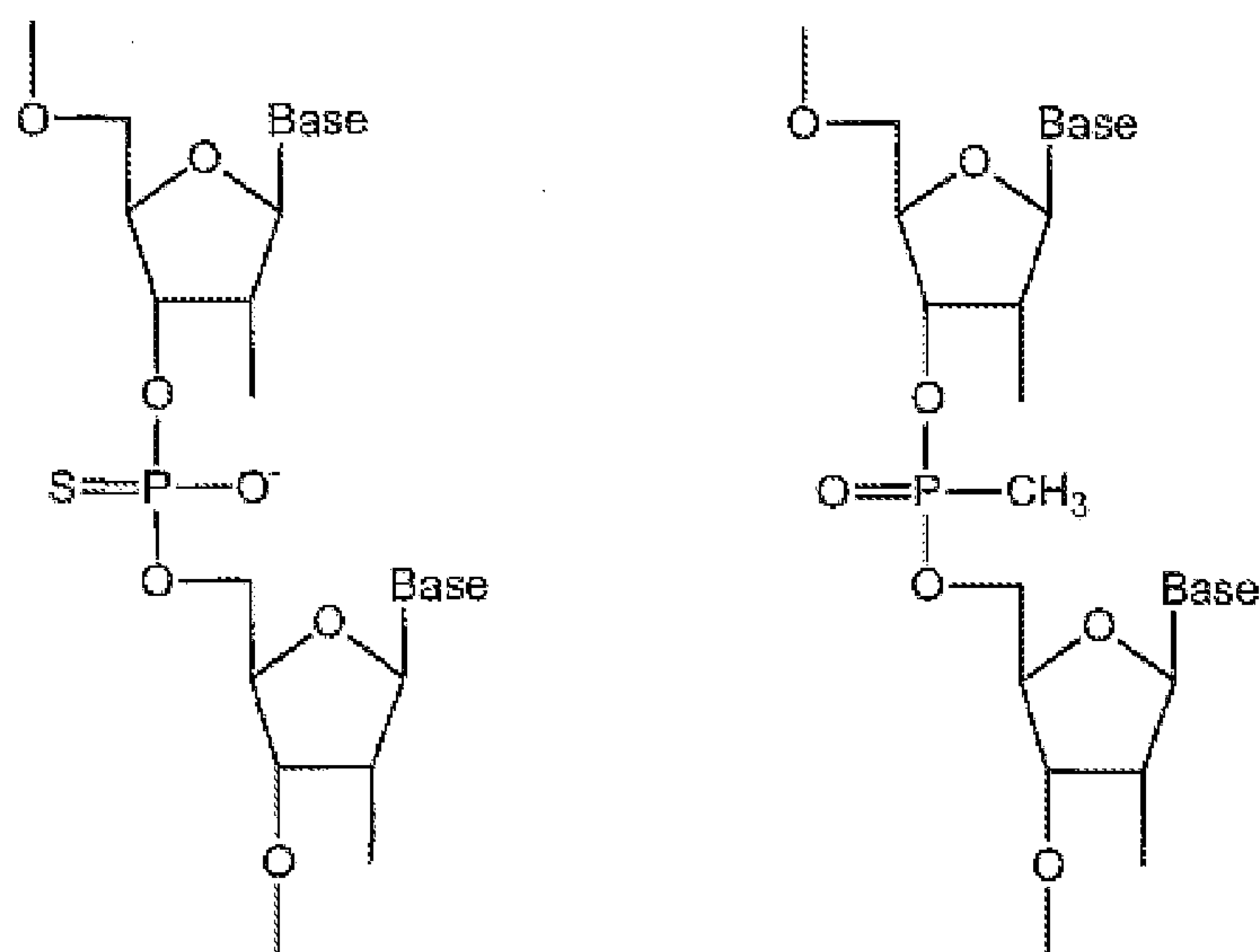
PNA

[0138] In some embodiments, one or more modifications optionally occur at the internucleotide linkage. In some instances, modified internucleotide linkage include, but is not limited to, phosphorothioates, phosphorodithioates, methylphosphonates, 5'-alkylenephosphonates, 5'-methylphosphonate, 3'-alkylene phosphonates, borontrifluoridates, borano phosphate esters and selenophosphates of 3'-5'linkage or 2'-5'linkage, phosphotriesters, thionoalkylphosphotriesters,

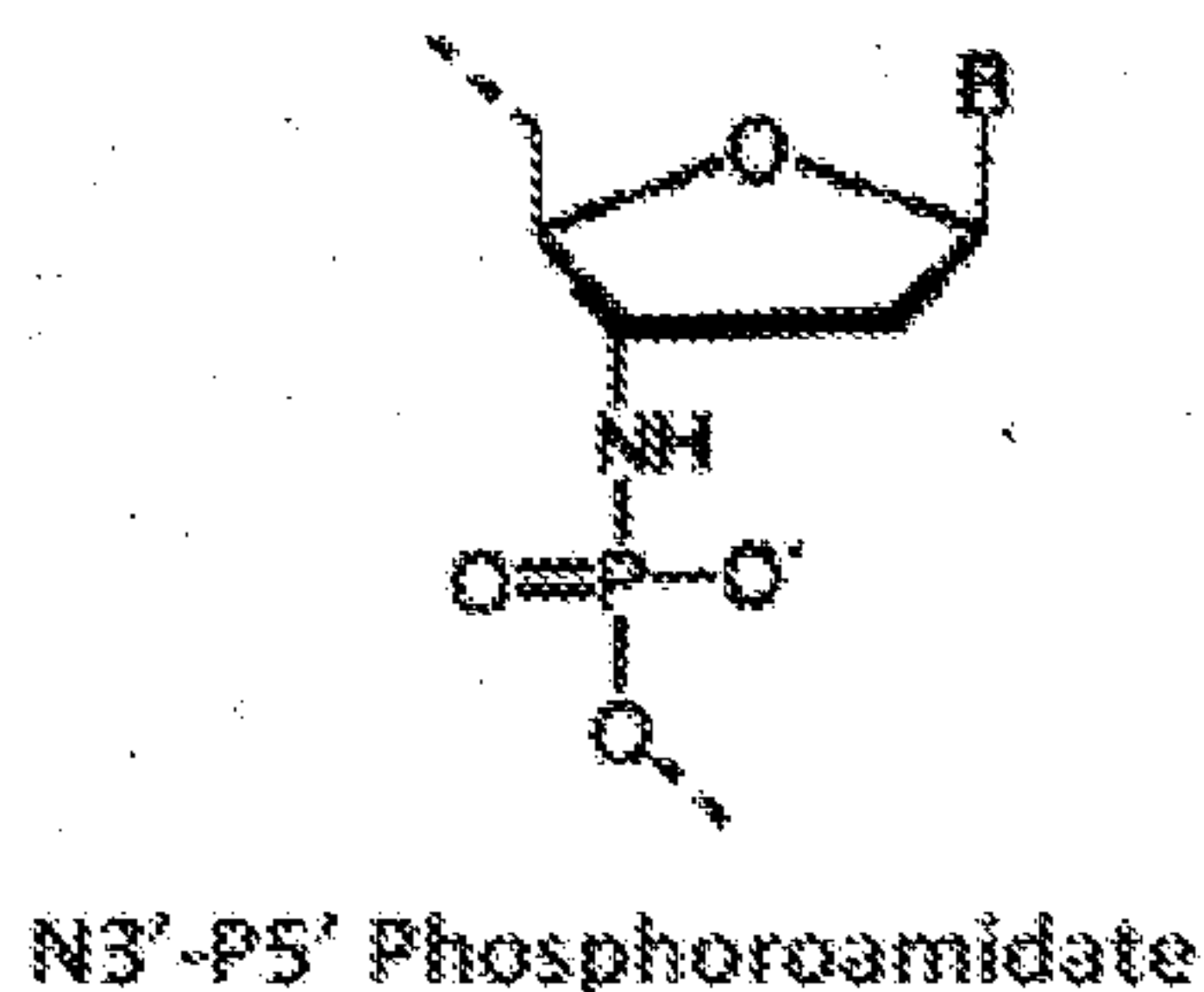
hydrogen phosphonate linkages, alkyl phosphonates, alkylphosphonothioates, arylphosphonothioates, phosphoroselenoates, phosphorodiselenoates, phosphinates, phosphoramidates, 3'-alkylphosphoramidates, aminoalkylphosphoramidates, thionophosphoramidates, phosphoropiperazidates, phosphoroanilothioates, phosphoroanilidates, ketones, sulfones, sulfonamides, carbonates, carbamates, methylenehydrazos, methylenedimethylhydrazos, formacetals, thioformacetals, oximes, methyleneiminos, methylenemethyliminos, thioamidates, linkages with riboacetyl groups, aminoethyl glycine, silyl or siloxane linkages, alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms, linkages with morpholino structures, amides, polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly, and combinations thereof. Phosphorothioate antisense oligonucleotides (PS ASO) are antisense oligonucleotides comprising a phosphorothioate linkage. An exemplary PS ASO is illustrated below.



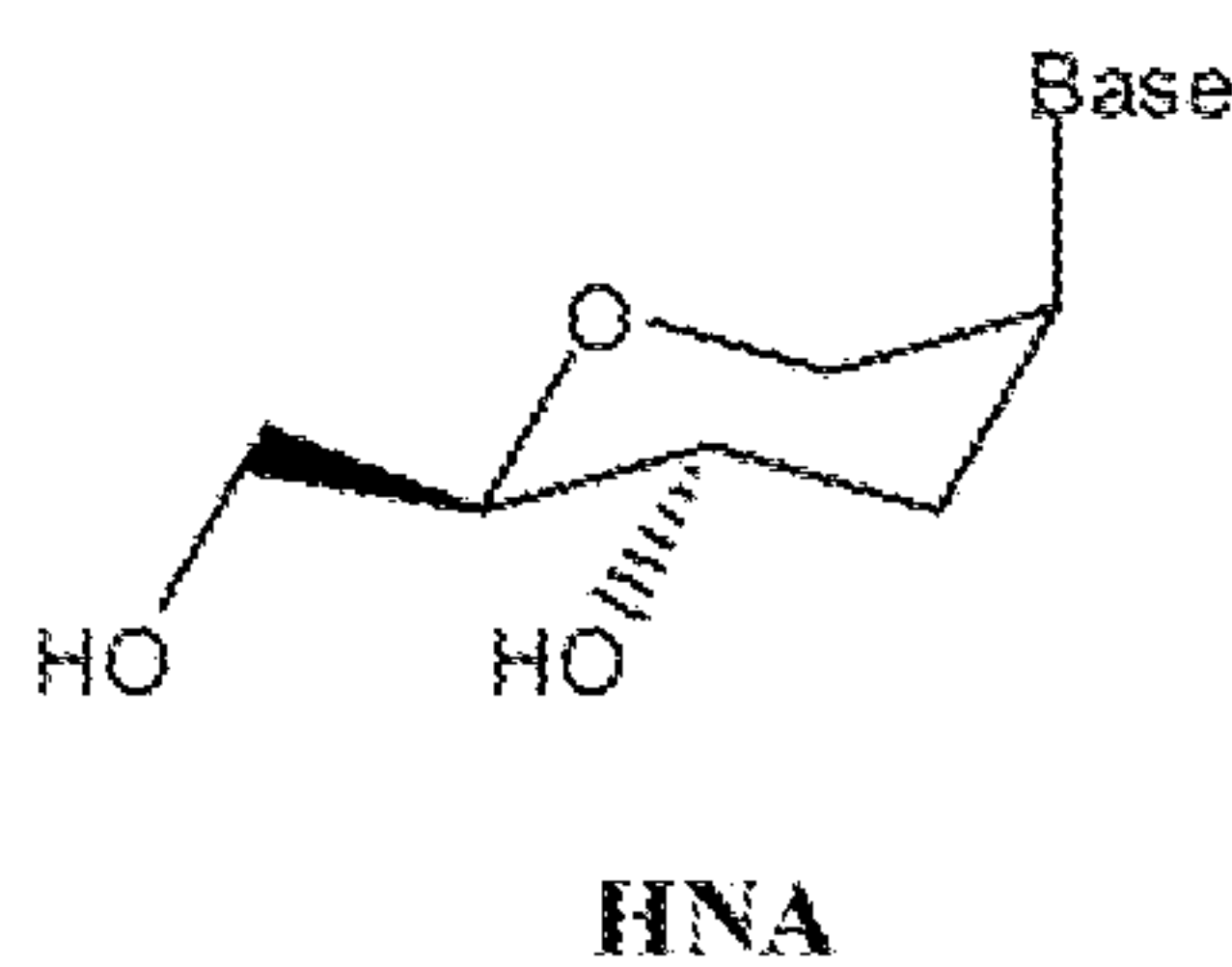
[0139] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left) and methylphosphonate nucleotide (right) are illustrated below.



[0140] In some instances, a modified nucleotide includes, but is not limited to, 2'-fluoro N3-P5'-phosphoramidites illustrated as:



[0141] In some instances, a modified nucleotide includes, but is not limited to, hexitol nucleic acid (or 1', 5'- anhydrohexitol nucleic acids (HNA)) illustrated as:



[0142] In some embodiments, one or more modifications further optionally include modifications of the ribose moiety, phosphate backbone and the nucleoside, or modifications of the nucleotide analogues at the 3' or the 5' terminus. For example, the 3' terminus optionally include a 3' cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus is optionally conjugated with an aminoalkyl group, e.g., a 3' C5-aminoalkyl dT. In an additional alternative, the 3'-terminus is optionally conjugated with an abasic site, e.g., with an apurinic or apyrimidinic site. In some instances, the 5'-terminus is conjugated with an aminoalkyl group, e.g., a 5'-O-alkylamino substituent. In some cases, the 5'-terminus is conjugated with an abasic site, e.g., with an apurinic or apyrimidinic site.

[0143] In some embodiments, the polynucleic acid molecule comprises one or more of the artificial nucleotide analogues described herein. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methyl modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methoxyethyl (2'-O-MOE) modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of thiolphosphonate nucleotides.

[0144] In some instances, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification.

[0145] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification.

[0146] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification.

[0147] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification.

[0148] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification.

[0149] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification.

[0150] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification.

[0151] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification.

[0152] In some cases, the polynucleic acid molecule comprises from about 10% to about 20% modification.

[0153] In some cases, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications.

[0154] In additional cases, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification.

[0155] In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications.

[0156] In some instances, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13,

about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides.

[0157] In some instances, from about 5% to about 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 10% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 15% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 20% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 25% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 30% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 35% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 40% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 45% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 50% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 55% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 60% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 65% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 70% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 75% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 80% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 85% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 90% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 95% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 96% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 97% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 98% of a polynucleic acid molecule comprise the artificial

nucleotide analogues described herein. In some instances, about 99% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 100% of a polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof.

[0158] In some embodiments, the polynucleic acid molecule comprises from about 1 to about 25 modifications in which the modification comprises an artificial nucleotide analogues described herein. In some embodiments, a polynucleic acid molecule comprises about 1 modification in which the modification comprises an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 2 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 3 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 4 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 5 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 6 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 7 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 8 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 9 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 10 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 11 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 12 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 13 modifications in which the modifications comprise

an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 14 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 15 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 16 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 17 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 18 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 19 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 20 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 21 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 22 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 23 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 24 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 25 modifications in which the modifications comprise an artificial nucleotide analogue described herein.

[0159] In some embodiments, a polynucleic acid molecule is assembled from two separate polynucleotides wherein one polynucleotide comprises the sense strand and the second polynucleotide comprises the antisense strand of the polynucleic acid molecule. In other embodiments, the sense strand is connected to the antisense strand via a linker molecule, which in some instances is a polynucleotide linker or a non-nucleotide linker.

[0160] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides in the sense strand comprises 2'-O-methylpyrimidine nucleotides and purine nucleotides in the sense strand comprise 2'-deoxy purine nucleotides. In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides present in the sense strand comprise 2'-deoxy-2'-fluoro pyrimidine

nucleotides and wherein purine nucleotides present in the sense strand comprise 2'-deoxy purine nucleotides.

[0161] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides when present in said antisense strand are 2'-O-methyl purine nucleotides.

[0162] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides when present in said antisense strand comprise 2'-deoxy-purine nucleotides.

[0163] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the sense strand includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In other embodiments, the terminal cap moiety is an inverted deoxy abasic moiety.

[0164] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a phosphate backbone modification at the 3' end of the antisense strand. In some instances, the phosphate backbone modification is a phosphorothioate.

[0165] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a glyceryl modification at the 3' end of the antisense strand.

[0166] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and in which the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more,

pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0167] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and in which the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0168] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the

5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

[0169] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises about 1 to about 25 or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0170] In some embodiments, a polynucleic acid molecule described herein is a chemically-modified short interfering nucleic acid molecule having about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more phosphorothioate internucleotide linkages in each strand of the polynucleic acid molecule.

[0171] In another embodiment, a polynucleic acid molecule described herein comprises 2'-5' internucleotide linkages. In some instances, the 2'-5' internucleotide linkage(s) is at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both sequence strands. In addition instances, the 2'-5' internucleotide linkage(s) is present at various other positions within one or both sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide

linkage of a pyrimidine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2'-5' internucleotide linkage.

[0172] In some embodiments, a polynucleic acid molecule is a single stranded polynucleic acid molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the polynucleic acid molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the polynucleic acid are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the polynucleic acid are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and a terminal cap modification, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the polynucleic acid molecule optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the polynucleic acid molecule, wherein the terminal nucleotides further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the polynucleic acid molecule optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

[0173] In some cases, one or more of the artificial nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease when compared to natural polynucleic acid molecules. In some instances, artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or combinations thereof are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease. In some instances, 2'-O-methyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-

3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-aminopropyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-deoxy modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, LNA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, ENA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, HNA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, morpholinos is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, PNA modified polynucleic acid molecule is resistant to nucleases (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, methylphosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, thiophosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, the 5' conjugates described herein inhibit 5'-3' exonucleolytic cleavage. In some instances, the 3' conjugates described herein inhibit 3'-5' exonucleolytic cleavage.

[0174] In some embodiments, one or more of the artificial nucleotide analogues described herein have increased binding affinity toward their mRNA target relative to an equivalent natural

polynucleic acid molecule. The one or more of the artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiophosphonate nucleotides, or 2'-fluoro N3-P5'-phosphoramidites have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, LNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, ENA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, PNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances,

HNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, morpholino modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, methylphosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, thiolphosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some cases, the increased affinity is illustrated with a lower K_d , a higher melt temperature (T_m), or a combination thereof.

[0175] In some embodiments, a polynucleic acid molecule described herein is a chirally pure (or stereo pure) polynucleic acid molecule, or a polynucleic acid molecule comprising a single enantiomer. In some instances, the polynucleic acid molecule comprises L-nucleotide. In some instances, the polynucleic acid molecule comprises D-nucleotides. In some instance, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of its mirror enantiomer. In some cases, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of a racemic mixture. In some instances, the polynucleic acid molecule is a polynucleic acid molecule described in: U.S. Patent Publication Nos: 2014/194610 and 2015/211006; and PCT Publication No.: WO2015107425.

[0176] In some embodiments, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is a DNA aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is Alphamer (Centauri Therapeutics), which comprises an aptamer portion that recognizes a specific cell-surface target and a portion that presents a specific epitopes for attaching to circulating antibodies. In some instance, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety as described in: U.S. Patent Nos: 8,604,184, 8,591,910, and 7,850,975.

[0177] In additional embodiments, a polynucleic acid molecule described herein is modified to increase its stability. In some embodiment, the polynucleic acid molecule is RNA (e.g., siRNA). In some instances, the polynucleic acid molecule is modified by one or more of the modifications described above to increase its stability. In some cases, the polynucleic acid molecule is modified at the 2' hydroxyl position, such as by 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl

(2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modification or by a locked or bridged ribose conformation (e.g., LNA or ENA). In some cases, the polynucleic acid molecule is modified by 2'-O-methyl and/or 2'-O-methoxyethyl ribose. In some cases, the polynucleic acid molecule also includes morpholinos, PNAs, HNA, methylphosphonate nucleotides, thiolphosphonate nucleotides, and/or 2'-fluoro N3-P5'-phosphoramidites to increase its stability. In some instances, the polynucleic acid molecule is a chirally pure (or stereo pure) polynucleic acid molecule. In some instances, the chirally pure (or stereo pure) polynucleic acid molecule is modified to increase its stability. Suitable modifications to the RNA to increase stability for delivery will be apparent to the skilled person.

[0178] In some embodiments, a polynucleic acid molecule describe herein has RNAi activity that modulates expression of RNA encoded by a gene involved in a disease or disorder such as, but not limited to, *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*. In some instances, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*, wherein one of the strands of the double-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras* or RNA encoded by at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras* or a portion thereof, and wherein the second strand of the double-stranded siRNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras* or RNA encoded by at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras* or a portion thereof. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*, wherein each strand of the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides, and wherein each strand comprises at least about 14, 17, or 19 nucleotides that are complementary to the nucleotides of the other strand. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *IKBKAP*, *SMN2*, *MCAD*, *LMNA*, *DMPK*, *ZNF9*, *MAPT*, *FKTN*, TDP-43, *LDLR*, *CFTR*, *DMD*, *PAH*, *MSTN*, or *K-Ras*, wherein each strand of the siRNA

molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In some instances, the RNAi activity occurs within a cell. In other instances, the RNAi activity occurs in a reconstituted in vitro system.

[0179] In some embodiments, a polynucleic acid molecule describe herein has RNAi activity that modulates expression of RNA encoded by a gene involved in muscular dystrophy such as, but not limited to, *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*. In some instances, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein one of the strands of the double-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or RNA encoded by at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or a portion thereof, and wherein the second strand of the double-stranded siRNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or RNA encoded by at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or a portion thereof. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein each strand of the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides, and wherein each strand comprises at least about 14, 17, or 19 nucleotides that are complementary to the nucleotides of the other strand. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein each strand of the siRNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In some instances, the RNAi activity occurs within a cell. In other instances, the RNAi activity occurs in a reconstituted in vitro system.

[0180] In some embodiments, a polynucleic acid molecule describe herein has RNAi activity that modulates expression of RNA encoded by the *DMD* gene. In some instances, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the single-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of *DMD* or RNA encoded by *DMD* or a portion thereof. In some cases, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides. In some cases, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the siRNA

molecule comprises about 19 to about 23 nucleotides. In some instances, the RNAi activity occurs within a cell. In other instances, the RNAi activity occurs in a reconstituted in vitro system.

[0181] In some instances, the polynucleic acid molecule is a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In some instances, the polynucleic acid molecule is assembled from two separate polynucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (e.g., each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19, 20, 21, 22, 23, or more base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the polynucleic acid molecule is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the polynucleic acid molecule are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

[0182] In some cases, the polynucleic acid molecule is a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In other cases, the polynucleic acid molecule is a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide is processed either in vivo or in vitro to generate an active polynucleic acid molecule capable of mediating RNAi. In additional cases, the polynucleic acid molecule also comprises a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such polynucleic acid molecule does not require the presence within the polynucleic acid molecule of nucleotide sequence corresponding to the

target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide further comprises a terminal phosphate group, such as a 5'-phosphate (see for example Martinez et al., 2002, *Cell*, 110, 563-574 and Schwarz et al., 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate.

[0183] In some instances, an asymmetric is a linear polynucleic acid molecule comprising an antisense region, a loop portion that comprises nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a loop region comprising about 4 to about 8 nucleotides, and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region. In some cases, the asymmetric hairpin polynucleic acid molecule also comprises a 5'-terminal phosphate group that is chemically modified. In additional cases, the loop portion of the asymmetric hairpin polynucleic acid molecule comprises nucleotides, non-nucleotides, linker molecules, or conjugate molecules.

[0184] In some embodiments, an asymmetric duplex is a polynucleic acid molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region.

[0185] In some cases, an universal base refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

Polynucleic Acid Molecule Synthesis

[0186] In some embodiments, a polynucleic acid molecule described herein is constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a polynucleic acid molecule is chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the

molecules or to increase the physical stability of the duplex formed between the polynucleic acid molecule and target nucleic acids. Exemplary methods include those described in: U.S. Patent Nos. 5,142,047; 5,185,444; 5,889,136; 6,008,400; and 6,111,086; PCT Publication No. WO2009099942; or European Publication No. 1579015. Additional exemplary methods include those described in: Griffey et al., "2'-O-aminopropyl ribonucleotides: a zwitterionic modification that enhances the exonuclease resistance and biological activity of antisense oligonucleotides," *J. Med. Chem.* **39**(26):5100-5109 (1997)); Obika, et al. "Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar pucker". *Tetrahedron Letters* **38** (50): 8735 (1997); Koizumi, M. "ENA oligonucleotides as therapeutics". *Current opinion in molecular therapeutics* **8** (2): 144-149 (2006); and Abramova et al., "Novel oligonucleotide analogues based on morpholino nucleoside subunits-antisense technologies: new chemical possibilities," *Indian Journal of Chemistry* **48B**:1721-1726 (2009). Alternatively, the polynucleic acid molecule is produced biologically using an expression vector into which a polynucleic acid molecule has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleic acid molecule will be of an antisense orientation to a target polynucleic acid molecule of interest).

[0187] In some embodiments, a polynucleic acid molecule is synthesized via a tandem synthesis methodology, wherein both strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate fragments or strands that hybridize and permit purification of the duplex.

[0188] In some instances, a polynucleic acid molecule is also assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the molecule.

[0189] Additional modification methods for incorporating, for example, sugar, base and phosphate modifications include: Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. *Nature*, 1990, 344, 565-568; Pieken et al. *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman et al. International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, *J. Biol. Chem.*, 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al., U.S. Pat. No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., U.S. Ser. No. 60/082,404 which was filed on Apr. 20, 1998; Karpeisky et al., 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina et al., 1997, *Bioorg. Med. Chem.*, 5, 1999-2010. Such publications describe

general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis.

[0190] In some instances, while chemical modification of the polynucleic acid molecule internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications sometimes cause toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages in some cases is minimized. In such cases, the reduction in the concentration of these linkages lowers toxicity, increases efficacy and higher specificity of these molecules.

Nucleic Acid-Polypeptide Conjugate

[0191] In some embodiments, a polynucleic acid molecule is further conjugated to a polypeptide A for delivery to a site of interest. In some cases, a polynucleic acid molecule is conjugated to a polypeptide A and optionally a polymeric moiety.

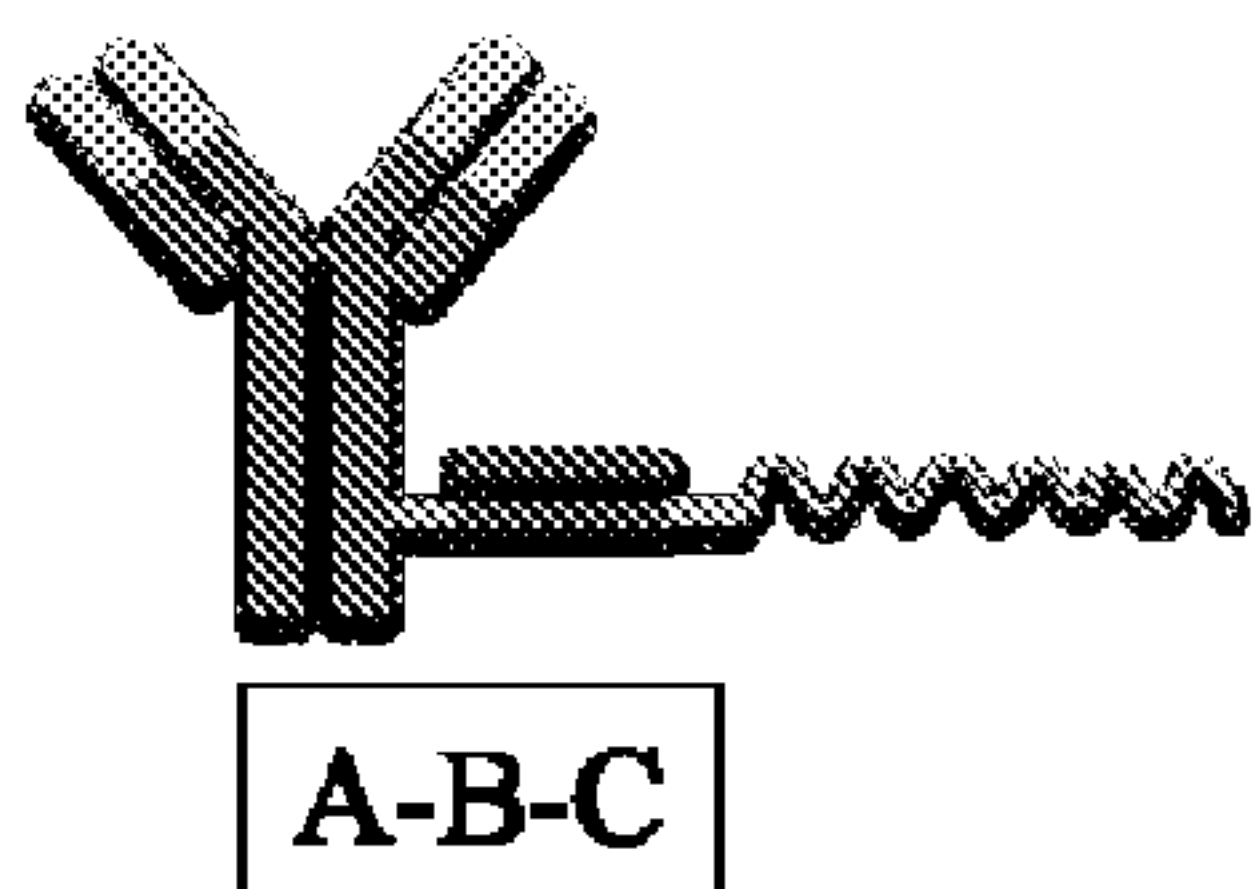
[0192] In some instances, at least one polypeptide A is conjugated to at least one B. In some instances, the at least one polypeptide A is conjugated to the at least one B to form an A-B conjugate. In some embodiments, at least one A is conjugated to the 5' terminus of B, the 3' terminus of B, an internal site on B, or in any combinations thereof. In some instances, the at least one polypeptide A is conjugated to at least two B. In some instances, the at least one polypeptide A is conjugated to at least 2, 3, 4, 5, 6, 7, 8, or more B.

[0193] In some embodiments, at least one polypeptide A is conjugated at one terminus of at least one B while at least one C is conjugated at the opposite terminus of the at least one B to form an A-B-C conjugate. In some instances, at least one polypeptide A is conjugated at one terminus of the at least one B while at least one of C is conjugated at an internal site on the at least one B. In some instances, at least one polypeptide A is conjugated directly to the at least one C. In some instances, the at least one B is conjugated indirectly to the at least one polypeptide A via the at least one C to form an A-C-B conjugate.

[0194] In some instances, at least one B and/or at least one C, and optionally at least one D are conjugated to at least one polypeptide A. In some instances, the at least one B is conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the at least one polypeptide A or are conjugated via an internal site to the at least one polypeptide A. In some cases, the at least one C is conjugated either directly to the at least one polypeptide A or indirectly via the at least one B. If indirectly via the at least one B, the at least one C is conjugated either at the same terminus as the at least one polypeptide A on B, at opposing terminus from the at least one polypeptide A, or independently at an internal site. In some instances, at least one additional polypeptide A is further conjugated to the

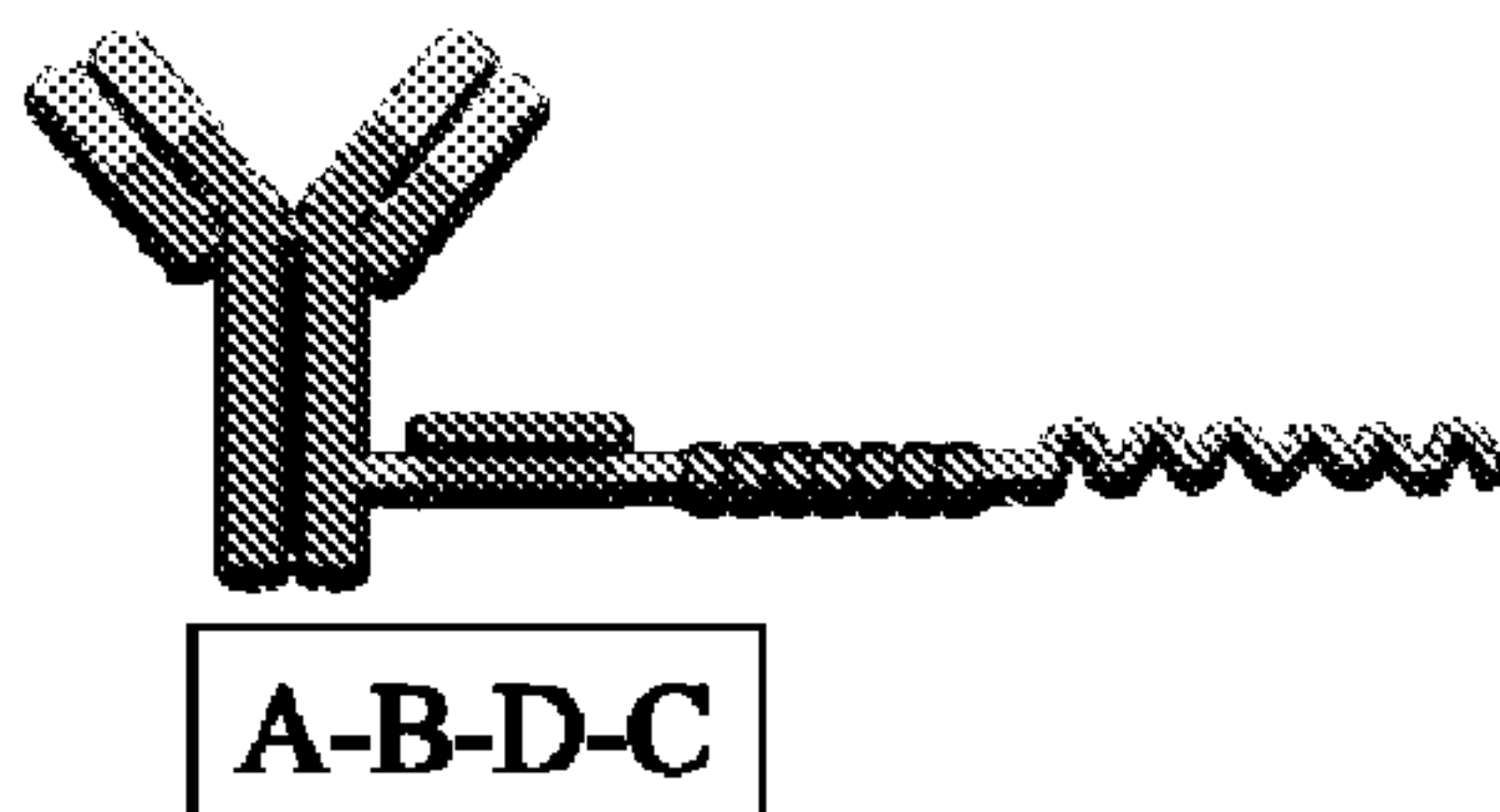
at least one polypeptide A, to B, or to C. In additional instances, the at least one D is optionally conjugated either directly or indirectly to the at least one polypeptide A, to the at least one B, or to the at least one C. If directly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-D-B conjugate or is optionally conjugated to the at least one B and the at least one C to form an A-D-B-C conjugate. In some instances, the at least one D is directly conjugated to the at least one polypeptide A and indirectly to the at least one B and the at least one C to form a D-A-B-C conjugate. If indirectly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-B-D conjugate or is optionally conjugated to the at least one B and the at least one C to form an A-B-D-C conjugate. In some instances, at least one additional D is further conjugated to the at least one polypeptide A, to B, or to C.

[0195] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



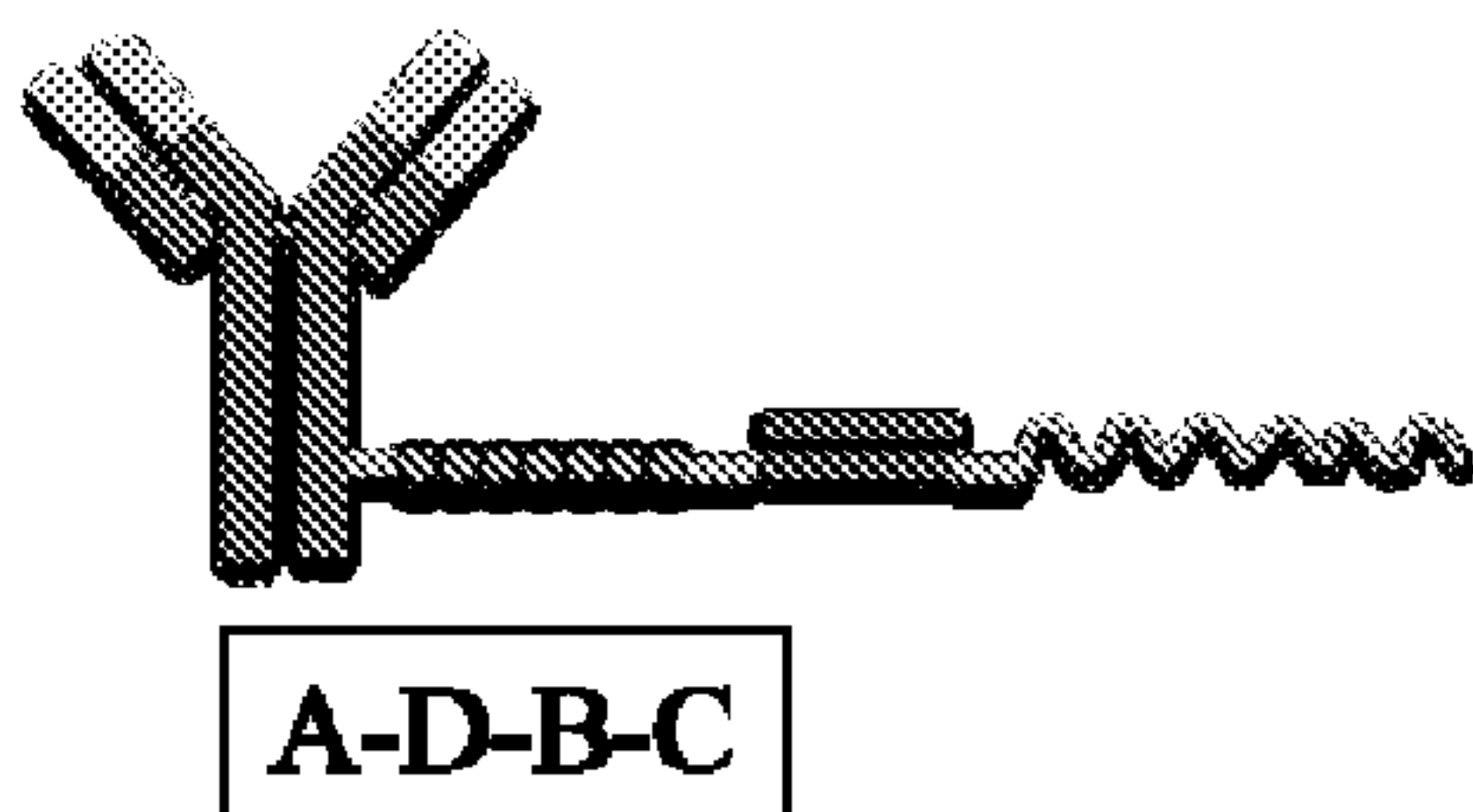
illustrated:

[0196] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



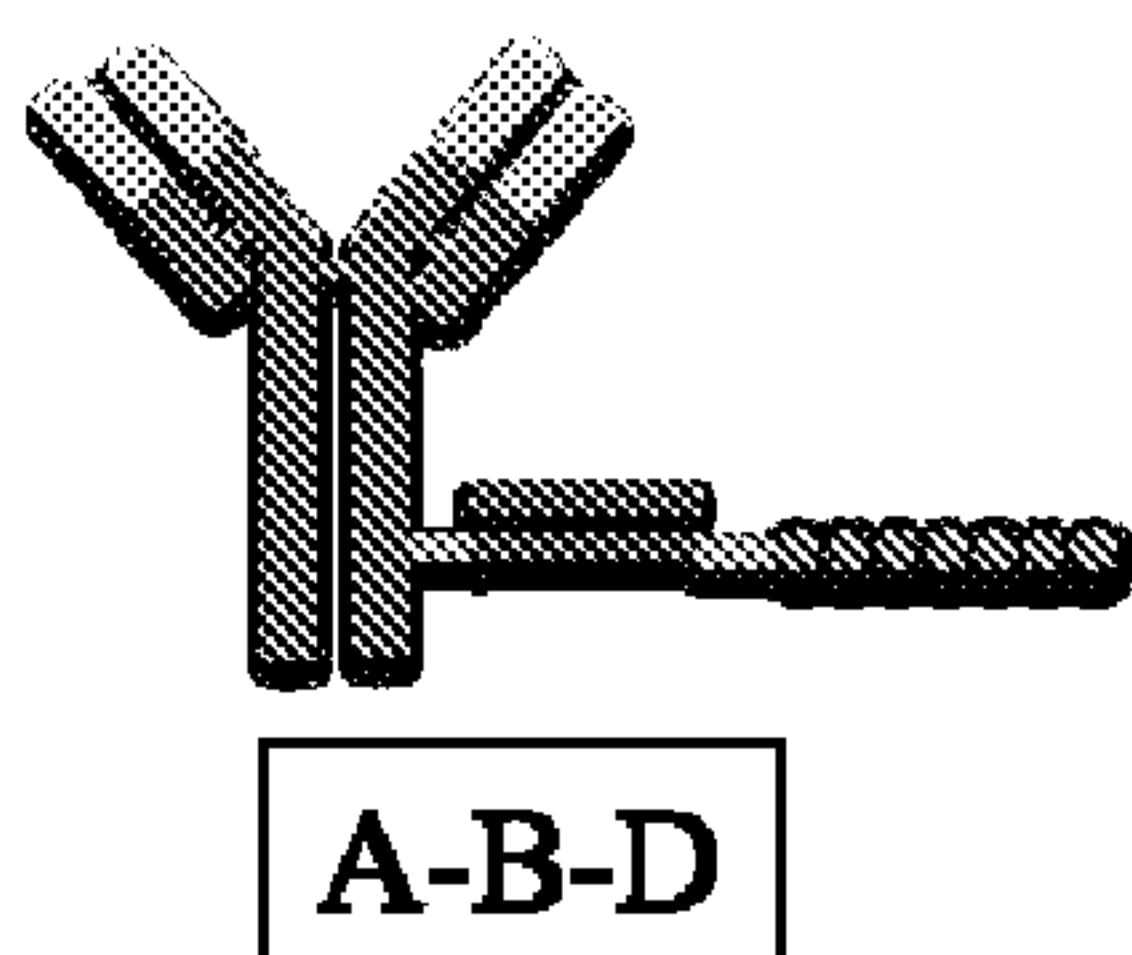
illustrated:

[0197] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



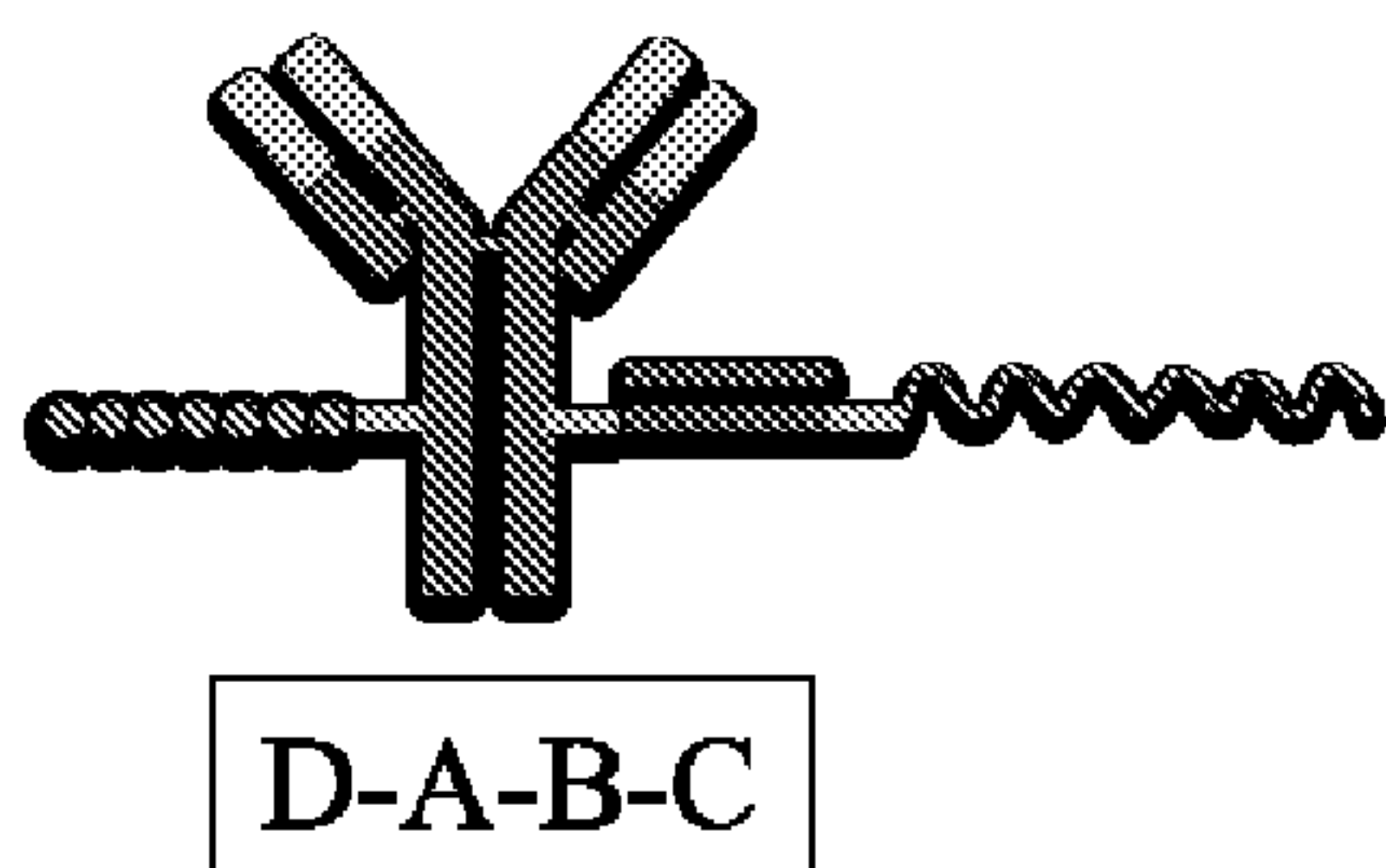
illustrated:

[0198] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



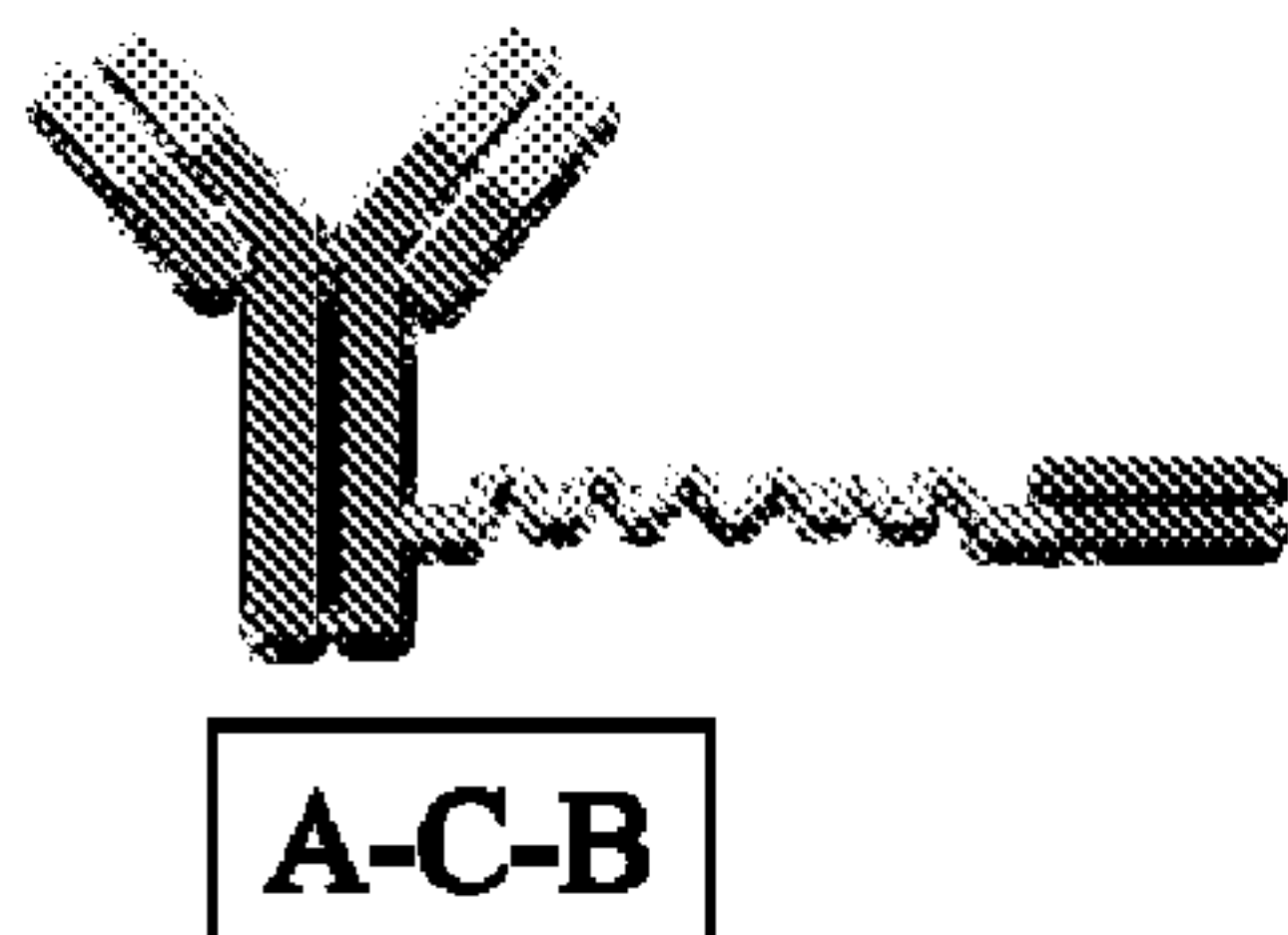
illustrated:

[0199] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



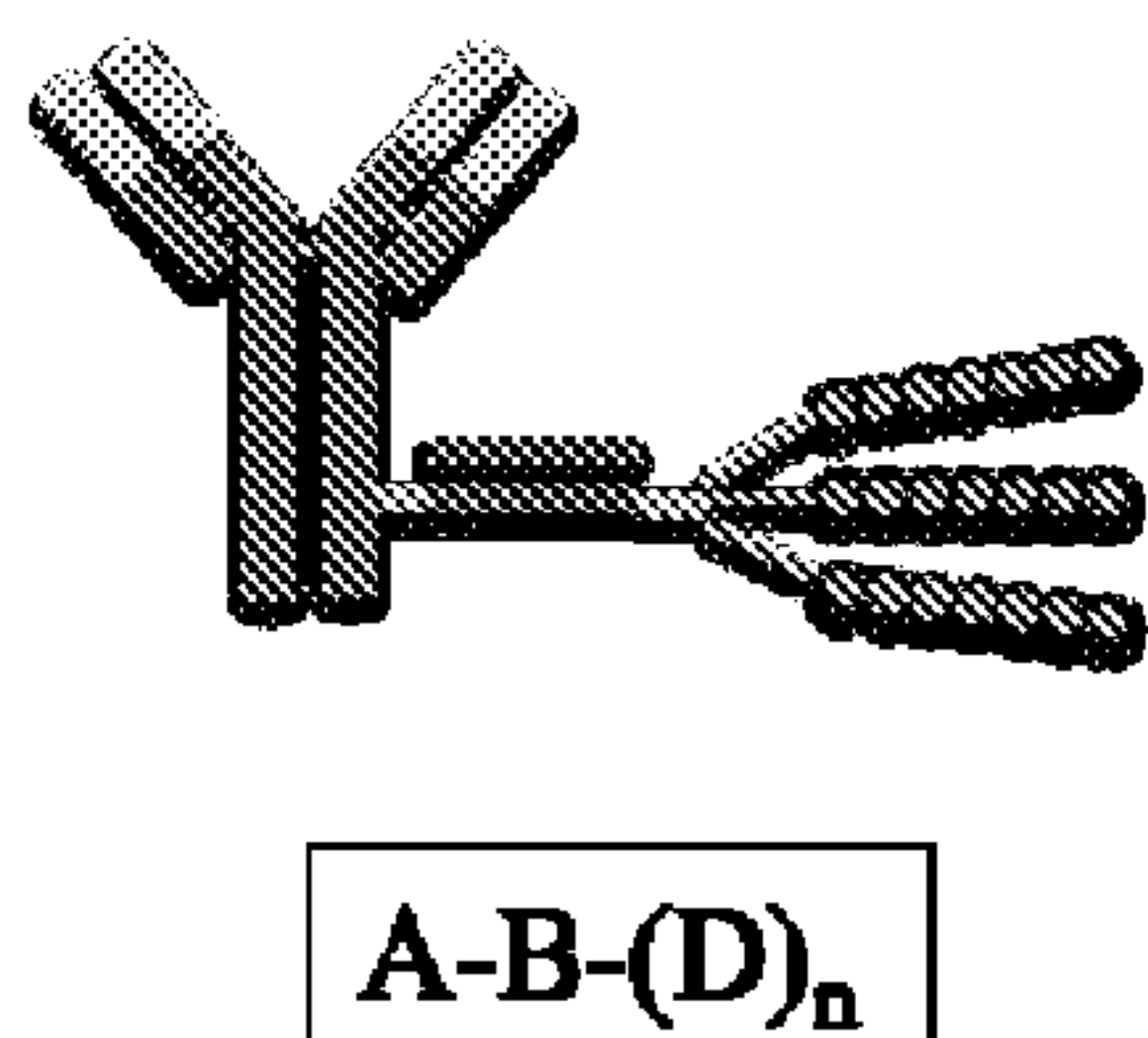
illustrated:

[0200] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



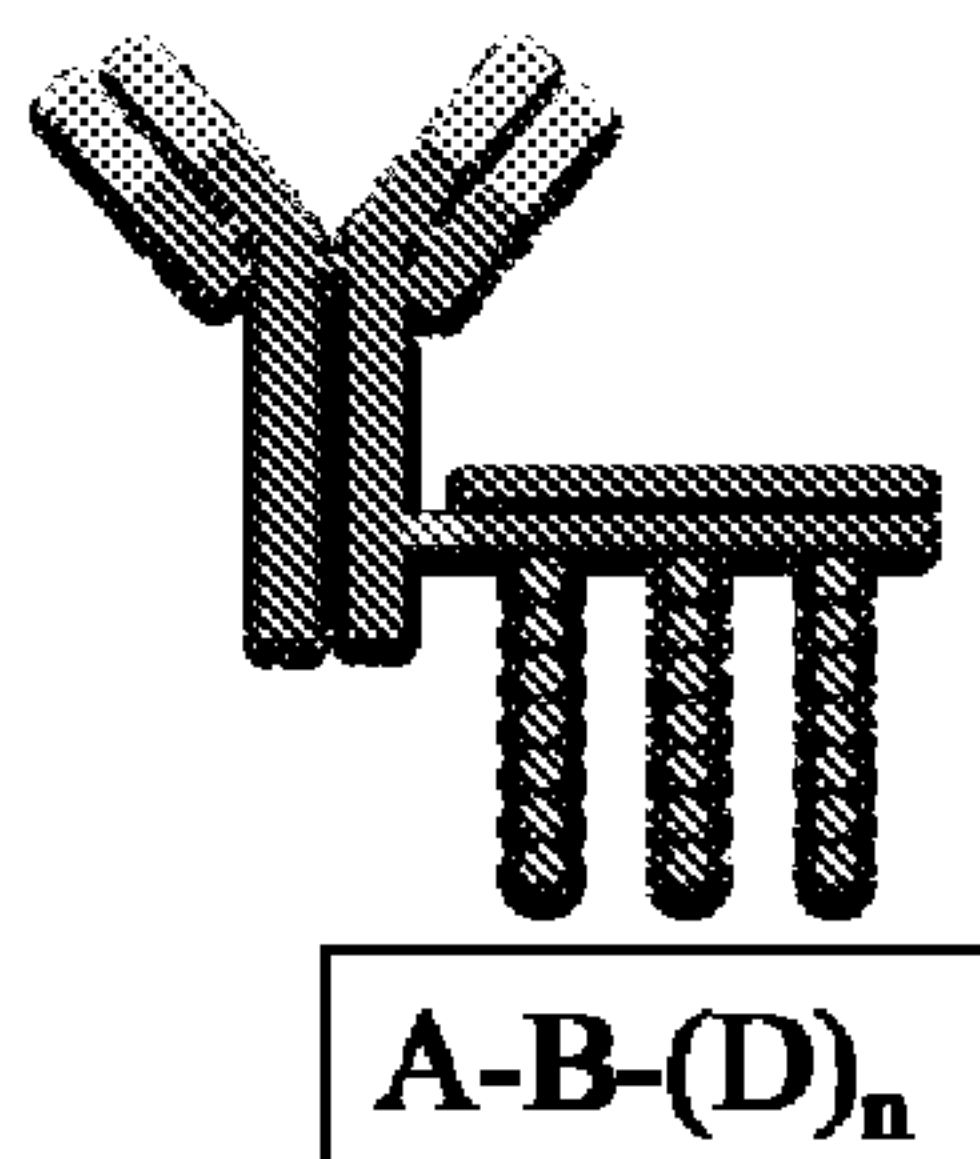
illustrated:

[0201] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



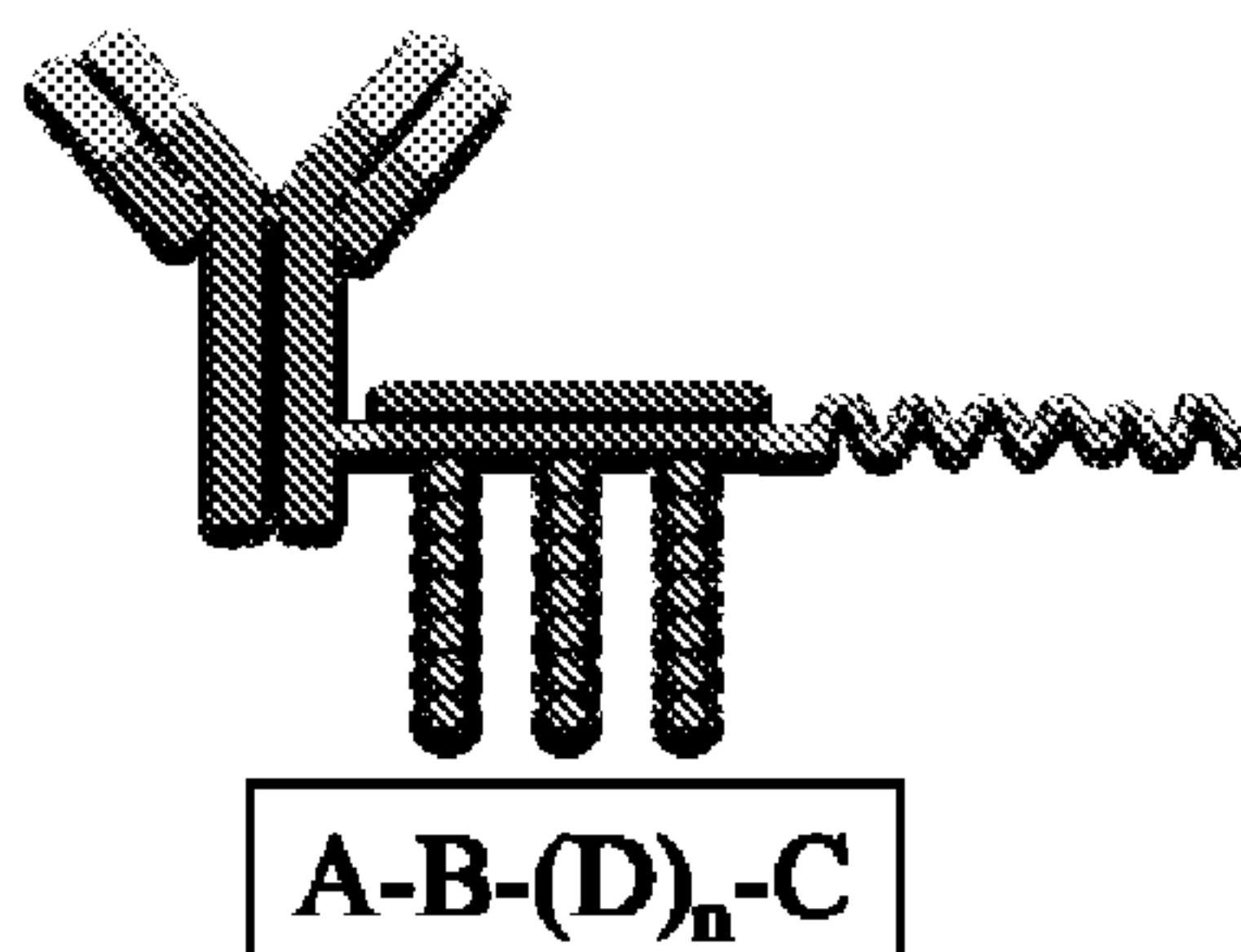
illustrated:

[0202] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



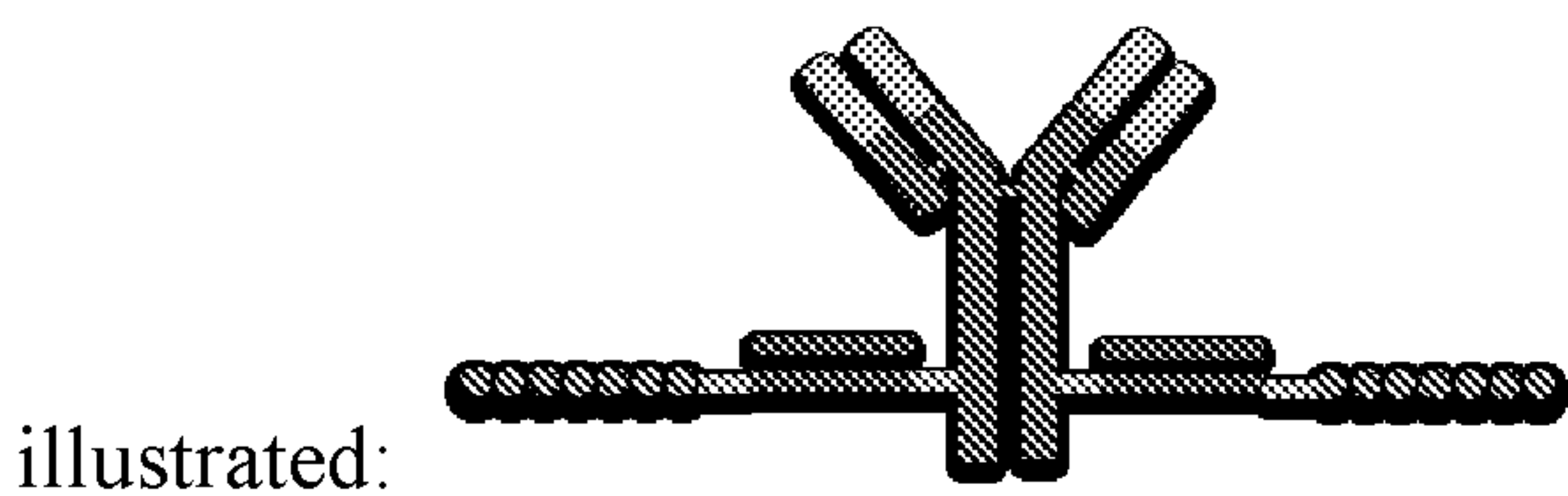
illustrated:

[0203] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as

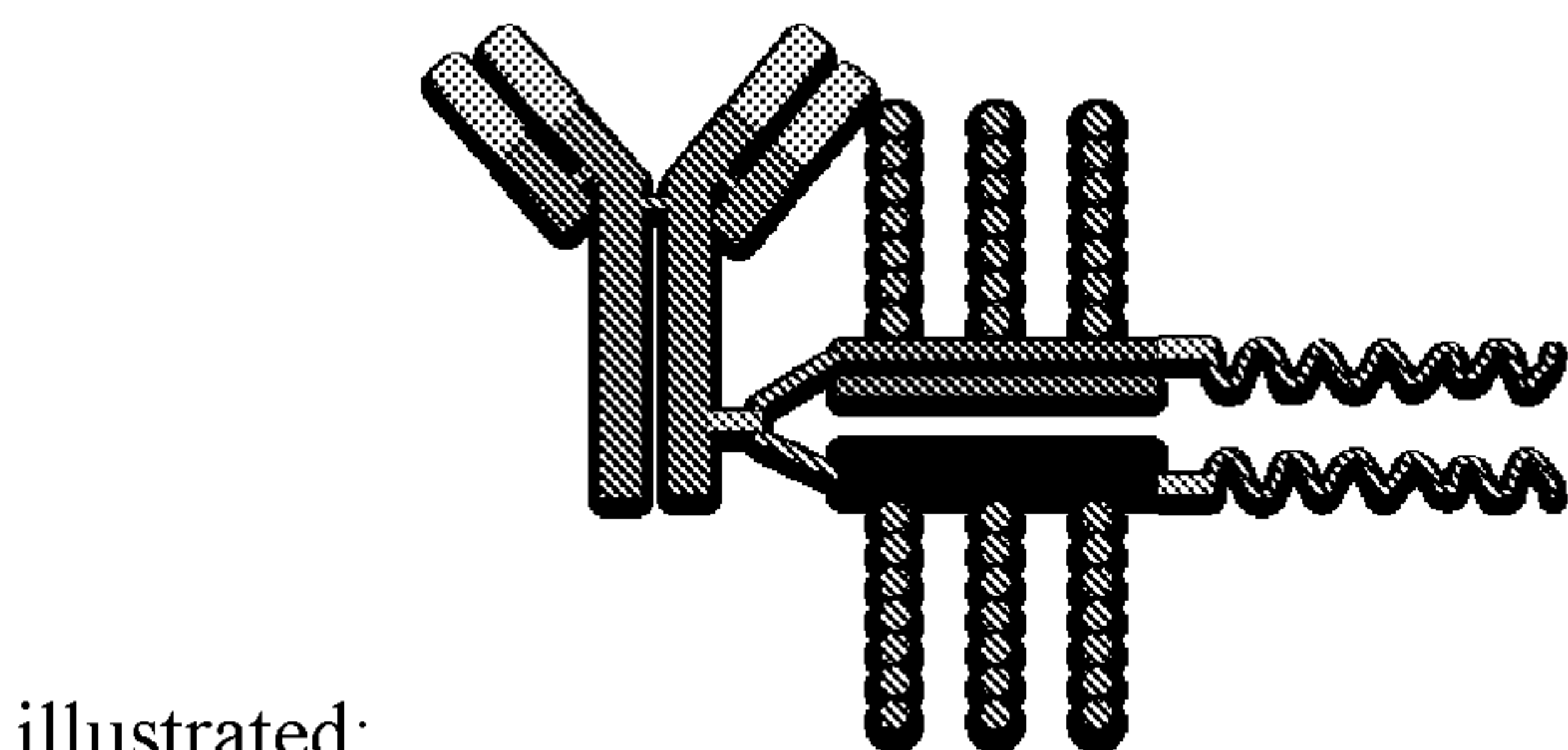


illustrated:

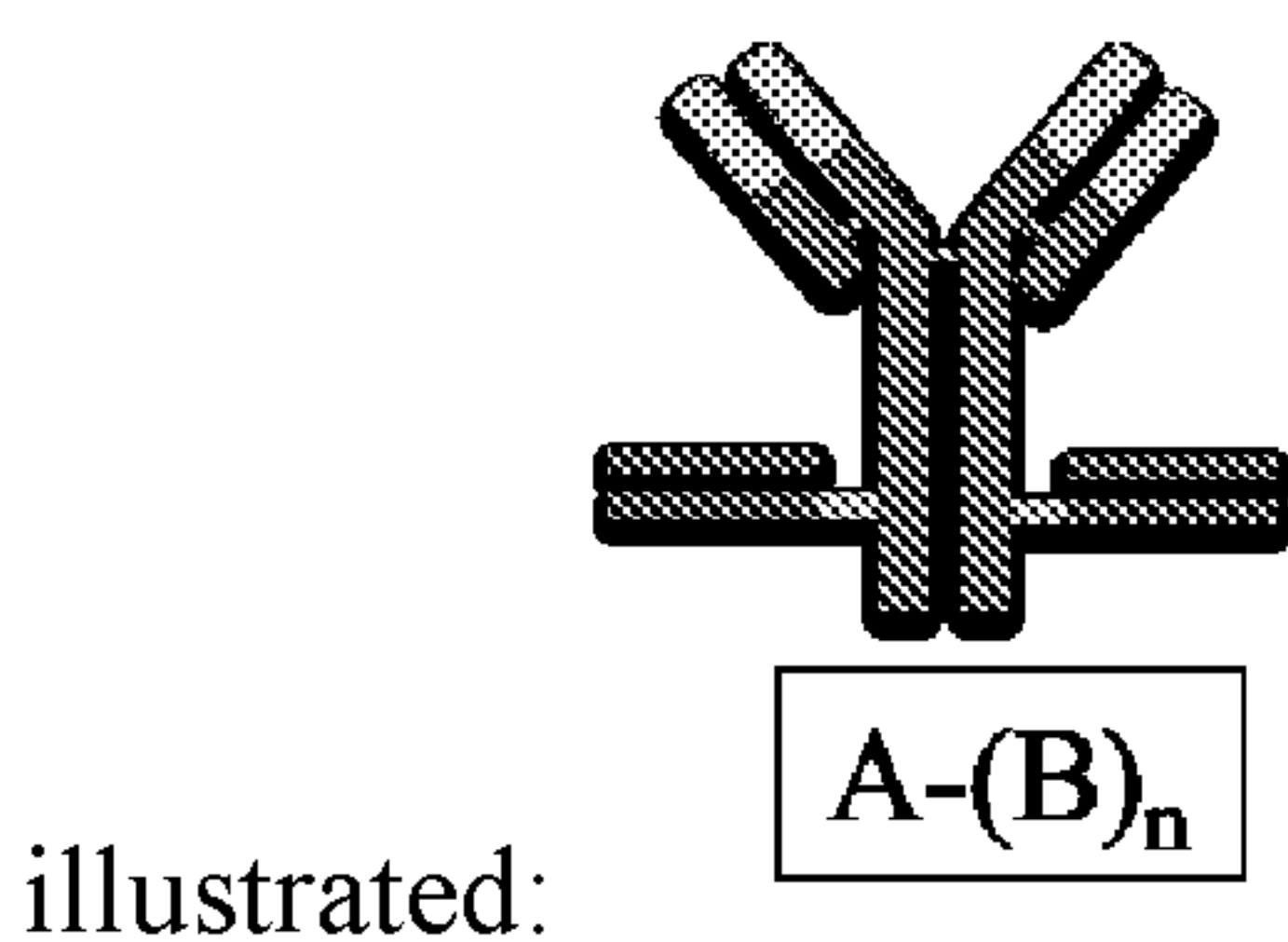
[0204] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as

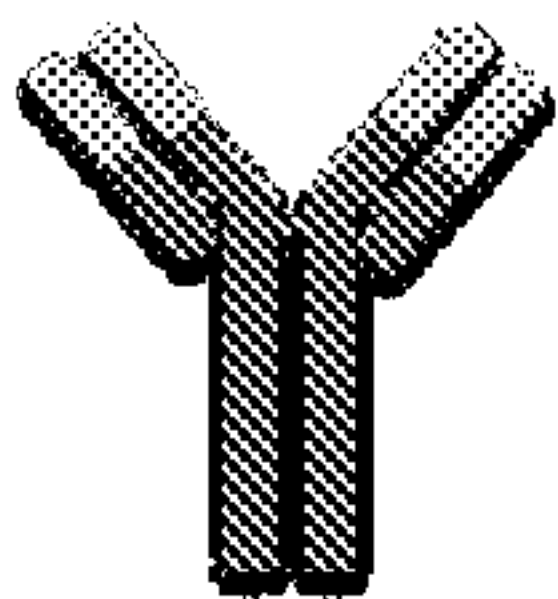


[0205] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



[0206] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



[0207] The  as illustrated above is for representation purposes only and encompasses a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof.

Binding Moiety

[0208] In some embodiments, the binding moiety A is a polypeptide. In some instances, the polypeptide is an antibody or its fragment thereof. In some cases, the fragment is a binding fragment. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab')₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv),

single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof.

[0209] In some instances, A is an antibody or binding fragment thereof. In some instances, A is a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab)'₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof. In some instances, A is a humanized antibody or binding fragment thereof. In some instances, A is a murine antibody or binding fragment thereof. In some instances, A is a chimeric antibody or binding fragment thereof. In some instances, A is a monoclonal antibody or binding fragment thereof. In some instances, A is a monovalent Fab'. In some instances, A is a divalent Fab₂. In some instances, A is a single-chain variable fragment (scFv).

[0210] In some embodiments, the binding moiety A is a bispecific antibody or binding fragment thereof. In some instances, the bispecific antibody is a trifunctional antibody or a bispecific mini-antibody. In some cases, the bispecific antibody is a trifunctional antibody. In some instances, the trifunctional antibody is a full length monoclonal antibody comprising binding sites for two different antigens.

[0211] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab₂, F(ab)'₃ fragments, bis-scFv, (scFv)₂, diabody, minibody, triabody, tetrabody or a bi-specific T-cell engager (BiTE). In some embodiments, the bi-specific T-cell engager is a fusion protein that contains two single-chain variable fragments (scFvs) in which the two scFvs target epitopes of two different antigens.

[0212] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab₂. In some instances, A is a bispecific F(ab)'₃ fragment. In some cases, A is a bispecific bis-scFv. In some cases, A is a bispecific (scFv)₂. In some embodiments, A is a bispecific diabody. In some embodiments, A is a bispecific minibody. In some embodiments, A is a bispecific triabody. In other embodiments, A is a bispecific tetrabody. In other embodiments, A is a bi-specific T-cell engager (BiTE).

[0213] In some embodiments, the binding moiety A is a trispecific antibody. In some instances, the trispecific antibody comprises F(ab)'₃ fragments or a triabody. In some instances, A is a trispecific F(ab)'₃ fragment. In some cases, A is a triabody. In some embodiments, A is a trispecific antibody as described in Dimas, *et al.*, "Development of a trispecific antibody designed

to simultaneously and efficiently target three different antigens on tumor cells,” *Mol. Pharmaceutics*, **12**(9): 3490-3501 (2015).

[0214] In some embodiments, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein on a muscle cell. Exemplary cell surface proteins recognized by an antibody or binding fragment thereof include, but are not limited to, Sca-1, CD34, Myo-D, myogenin, MRF4, NCAM, CD43, and CD95 (Fas).

[0215] In some instances, the cell surface protein comprises clusters of differentiation (CD) cell surface markers. Exemplary CD cell surface markers include, but are not limited to, CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), and the like.

[0216] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a CD cell surface marker. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), or a combination thereof.

[0217] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) non-specifically. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue or a cysteine residue, in a non-site specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine

residue in a non-site specific manner. In some cases, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a cysteine residue in a non-site specific manner.

[0218] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) in a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue, a cysteine residue, at the 5'-terminus, at the 3'-terminus, an unnatural amino acid, or an enzyme-modified or enzyme-catalyzed residue, via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a cysteine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 5'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 3'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an unnatural amino acid via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an enzyme-modified or enzyme-catalyzed residue via a site-specific manner.

[0219] In some embodiments, one or more polynucleic acid molecule (B) is conjugated to a binding moiety A. In some instances, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 1 polynucleic acid molecule is conjugated to one binding moiety A. In some instances, about 2 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 3 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 4 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 5 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 6 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 7 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 8 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 9 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 10 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 11 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 12 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 13 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 14 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 15 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 16

polynucleic acid molecules are conjugated to one binding moiety A. In some cases, the one or more polynucleic acid molecules are the same. In other cases, the one or more polynucleic acid molecules are different.

[0220] In some embodiments, the number of polynucleic acid molecule (B) conjugated to a binding moiety A forms a ratio. In some instances, the ratio is referred to as a DAR (drug-to-antibody) ratio, in which the drug as referred to herein is the polynucleic acid molecule (B). In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12 or greater.

[0221] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding

moiety A is about 10. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 13. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 14. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 15. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 16.

[0222] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 12.

[0223] In some instances, a conjugate comprising polynucleic acid molecule (B) and binding moiety A has improved activity as compared to a conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, improved activity results in enhanced biologically relevant functions, e.g., improved stability, affinity, binding, functional activity, and efficacy in treatment or prevention of a disease state. In some instances, the disease state is a result of one or more mutated exons of a gene. In some instances, the conjugate comprising polynucleic acid molecule (B) and binding moiety A results in increased exon skipping of the one or more mutated exons as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, exon skipping is increased by at least or about 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% in the conjugate comprising polynucleic acid molecule (B) and binding moiety A as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A.

[0224] In some embodiments, an antibody or its binding fragment is further modified using conventional techniques known in the art, for example, by using amino acid deletion, insertion, substitution, addition, and/or by recombination and/or any other modification (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. In some instances, the modification further comprises a modification for modulating interaction with Fc receptors. In some instances, the one or more modifications include those described in, for example, International Publication No. WO97/34631, which discloses amino

acid residues involved in the interaction between the Fc domain and the FcRn receptor. Methods for introducing such modifications in the nucleic acid sequence underlying the amino acid sequence of an antibody or its binding fragment is well known to the person skilled in the art.

[0225] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0226] In some instances, the term “single-chain” as used herein means that the first and second domains of a bi-specific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

[0227] In some instances, a bispecific single chain antibody construct relates to a construct comprising two antibody derived binding domains. In such embodiments, bi-specific single chain antibody construct is tandem bi-scFv or diabody. In some instances, a scFv contains a VH and VL domain connected by a linker peptide. In some instances, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities.

[0228] In some embodiments, binding to or interacting with as used herein defines a binding/interaction of at least two antigen-interaction-sites with each other. In some instances, antigen-interaction-site defines a motif of a polypeptide that shows the capacity of specific interaction with a specific antigen or a specific group of antigens. In some cases, the binding/interaction is also understood to define a specific recognition. In such cases, specific recognition refers to that the antibody or its binding fragment is capable of specifically interacting with and/or binding to at least two amino acids of each of a target molecule. For example, specific recognition relates to the specificity of the antibody molecule, or to its ability to discriminate between the specific regions of a target molecule. In additional instances, the specific interaction of the antigen-interaction-site with its specific antigen results in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. In further embodiments, the binding is exemplified by the specificity of a "key-lock-principle". Thus in some instances, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. In such cases, the specific interaction of the antigen-interaction-site with its specific antigen results as well in a simple binding of the site to the antigen.

[0229] In some instances, specific interaction further refers to a reduced cross-reactivity of the antibody or its binding fragment or a reduced off-target effect. For example, the antibody or its binding fragment that bind to the polypeptide/protein of interest but do not or do not essentially

bind to any of the other polypeptides are considered as specific for the polypeptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor, for example, the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

Conjugation Chemistry

[0230] In some embodiments, a polynucleic acid molecule B is conjugated to a binding moiety. In some instances, the binding moiety comprises amino acids, peptides, polypeptides, proteins, antibodies, antigens, toxins, hormones, lipids, nucleotides, nucleosides, sugars, carbohydrates, polymers such as polyethylene glycol and polypropylene glycol, as well as analogs or derivatives of all of these classes of substances. Additional examples of binding moiety also include steroids, such as cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons (e.g., saturated, unsaturated, or contains substitutions), enzyme substrates, biotin, digoxigenin, and polysaccharides. In some instances, the binding moiety is an antibody or binding fragment thereof. In some instances, the polynucleic acid molecule is further conjugated to a polymer, and optionally an endosomolytic moiety.

[0231] In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety by a chemical ligation process. In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a native ligation. In some instances, the conjugation is as described in: Dawson, et al. "Synthesis of proteins by native chemical ligation," *Science* **1994**, 266, 776–779; Dawson, et al. "Modulation of Reactivity in Native Chemical Ligation through the Use of Thiol Additives," *J. Am. Chem. Soc.* **1997**, 119, 4325–4329; Hackeng, et al. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology," *Proc. Natl. Acad. Sci. USA* **1999**, 96, 10068–10073; or Wu, et al. "Building complex glycopeptides: Development of a cysteine-free native chemical ligation protocol," *Angew. Chem. Int. Ed.* **2006**, 45, 4116–4125. In some instances, the conjugation is as described in U.S. Patent No. 8,936,910. In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety either site-specifically or non-specifically via native ligation chemistry.

[0232] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing a "traceless" coupling technology (Philochem). In some instances, the "traceless" coupling technology utilizes an N-terminal 1,2-aminothiol group on the binding moiety which is then conjugate with a polynucleic acid molecule containing an aldehyde group. (see Casi *et al.*, "Site-specific traceless coupling of potent cytotoxic drugs to recombinant antibodies for pharmacodelivery," *JACS* **134**(13): 5887-5892 (2012))

[0233] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an unnatural amino acid incorporated into the binding moiety. In some instances, the unnatural amino acid comprises *p*-acetylphenylalanine (pAcPhe). In some instances, the keto group of pAcPhe is selectively coupled to an alkoxy-amine derivatived conjugating moiety to form an oxime bond. (see Axup *et al.*, “Synthesis of site-specific antibody-drug conjugates using unnatural amino acids,” *PNAS* **109**(40): 16101-16106 (2012)).

[0234] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an enzyme-catalyzed process. In some instances, the site-directed method utilizes SMARTag™ technology (Redwood). In some instances, the SMARTag™ technology comprises generation of a formylglycine (FGly) residue from cysteine by formylglycine-generating enzyme (FGE) through an oxidation process under the presence of an aldehyde tag and the subsequent conjugation of FGly to an alkylhydrazine-functionalized polynucleic acid molecule via hydrazino-Pictet-Spengler (HIPS) ligation. (see Wu *et al.*, “Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag,” *PNAS* **106**(9): 3000-3005 (2009); Agarwal, *et al.*, “A Pictet-Spengler ligation for protein chemical modification,” *PNAS* **110**(1): 46-51 (2013))

[0235] In some instances, the enzyme-catalyzed process comprises microbial transglutaminase (mTG). In some cases, the polynucleic acid molecule is conjugated to the binding moiety utilizing a microbial transglutaminase catalyzed process. In some instances, mTG catalyzes the formation of a covalent bond between the amide side chain of a glutamine within the recognition sequence and a primary amine of a functionalized polynucleic acid molecule. In some instances, mTG is produced from *Streptomyces mobarensis*. (see Strop *et al.*, “Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates,” *Chemistry and Biology* **20**(2) 161-167 (2013))

[0236] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in PCT Publication No. WO2014/140317, which utilizes a sequence-specific transpeptidase.

[0237] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in U.S. Patent Publication Nos. 2015/0105539 and 2015/0105540.

Production of Antibodies or Binding Fragments Thereof

[0238] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of

polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0239] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0240] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0241] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0242] In some embodiments, techniques developed for the production of “chimeric antibodies” (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0243] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments

of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0244] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0245] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0246] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the

recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0247] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk⁻, hgp⁻ or ap⁻ cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hyg^r, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0248] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0249] In some instances, any method known in the art for purification or analysis of an antibody or antibody conjugates is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of

proteins. Exemplary chromatography methods included, but are not limited to, strong anion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, and fast protein liquid chromatography.

Polymer Conjugating Moiety

[0250] In some embodiments, a polymer moiety C is further conjugated to a polynucleic acid molecule described herein, a binding moiety described herein, or in combinations thereof. In some instances, a polymer moiety C is conjugated a polynucleic acid molecule. In some cases, a polymer moiety C is conjugated to a binding moiety. In other cases, a polymer moiety C is conjugated to a polynucleic acid molecule-binding moiety molecule. In additional cases, a polymer moiety C is conjugated, as illustrated *supra*.

[0251] In some instances, the polymer moiety C is a natural or synthetic polymer, consisting of long chains of branched or unbranched monomers, and/or cross-linked network of monomers in two or three dimensions. In some instances, the polymer moiety C includes a polysaccharide, lignin, rubber, or polyalkylene oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxypolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethylenterephthalat (PET, PETG), polyethylene terephthalate (PETE), polytetramethylene glycol (PTG), or polyurethane as well as mixtures thereof. As used herein, a mixture refers to the use of different polymers within the same compound as well as in reference to block copolymers. In some cases, block copolymers are polymers wherein at least one section of a polymer is build up from monomers of another polymer. In some instances, the polymer moiety C comprises polyalkylene oxide. In some instances, the polymer moiety C comprises PEG. In some instances, the polymer moiety C comprises polyethylene imide (PEI) or hydroxy ethyl starch (HES).

[0252] In some instances, C is a PEG moiety. In some instances, the PEG moiety is conjugated at the 5' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 3' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated at the 3' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 5' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated to an internal site of the polynucleic acid molecule. In some instances, the PEG moiety, the binding moiety, or a combination thereof, are conjugated to an internal site of the polynucleic acid molecule. In some instances, the conjugation is a direct conjugation. In some instances, the conjugation is via native ligation.

[0253] In some embodiments, the polyalkylene oxide (e.g., PEG) is a polydispers or monodispers compound. In some instances, polydispers material comprises disperse distribution of different molecular weight of the material, characterized by mean weight (weight average) size and dispersity. In some instances, the monodisperse PEG comprises one size of molecules. In some embodiments, C is poly- or monodispersed polyalkylene oxide (e.g., PEG) and the indicated molecular weight represents an average of the molecular weight of the polyalkylene oxide, e.g., PEG, molecules.

[0254] In some embodiments, the molecular weight of the polyalkylene oxide (e.g., PEG) is about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da.

[0255] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some embodiments, C is PEG and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some instances, the molecular weight of C is about 200 Da. In some instances, the molecular weight of C is about 300 Da. In some instances, the molecular weight of C is about 400 Da. In some instances, the molecular weight of C is about 500 Da. In some instances, the molecular weight of C is about 600 Da. In some instances, the molecular weight of C is about 700 Da. In some instances, the molecular weight of C is about 800 Da. In some instances, the molecular weight of C is about 900 Da. In some instances, the molecular weight of C is about 1000 Da. In some instances, the molecular weight of C is about 1100 Da. In some instances, the molecular weight of C is about 1200 Da. In some instances, the molecular weight of C is about 1300 Da. In some instances, the molecular weight of C is about 1400 Da. In some instances, the molecular weight of C is about 1450 Da. In some instances, the molecular weight of C is about 1500 Da. In some instances, the molecular weight of C is about 1600 Da. In some instances, the molecular weight of C is about 1700 Da. In some instances, the molecular weight of C is about 1800 Da. In some instances, the molecular weight of C is about 1900 Da. In some instances, the molecular weight of C is about

2000 Da. In some instances, the molecular weight of C is about 2100 Da. In some instances, the molecular weight of C is about 2200 Da. In some instances, the molecular weight of C is about 2300 Da. In some instances, the molecular weight of C is about 2400 Da. In some instances, the molecular weight of C is about 2500 Da. In some instances, the molecular weight of C is about 2600 Da. In some instances, the molecular weight of C is about 2700 Da. In some instances, the molecular weight of C is about 2800 Da. In some instances, the molecular weight of C is about 2900 Da. In some instances, the molecular weight of C is about 3000 Da. In some instances, the molecular weight of C is about 3250 Da. In some instances, the molecular weight of C is about 3350 Da. In some instances, the molecular weight of C is about 3500 Da. In some instances, the molecular weight of C is about 3750 Da. In some instances, the molecular weight of C is about 4000 Da. In some instances, the molecular weight of C is about 4250 Da. In some instances, the molecular weight of C is about 4500 Da. In some instances, the molecular weight of C is about 4600 Da. In some instances, the molecular weight of C is about 4750 Da. In some instances, the molecular weight of C is about 5000 Da. In some instances, the molecular weight of C is about 5500 Da. In some instances, the molecular weight of C is about 6000 Da. In some instances, the molecular weight of C is about 6500 Da. In some instances, the molecular weight of C is about 7000 Da. In some instances, the molecular weight of C is about 7500 Da. In some instances, the molecular weight of C is about 8000 Da. In some instances, the molecular weight of C is about 10,000 Da. In some instances, the molecular weight of C is about 12,000 Da. In some instances, the molecular weight of C is about 20,000 Da. In some instances, the molecular weight of C is about 35,000 Da. In some instances, the molecular weight of C is about 40,000 Da. In some instances, the molecular weight of C is about 50,000 Da. In some instances, the molecular weight of C is about 60,000 Da. In some instances, the molecular weight of C is about 100,000 Da.

[0256] In some embodiments, the polyalkylene oxide (e.g., PEG) comprises discrete ethylene oxide units (e.g., four to about 48 ethylene oxide units). In some instances, the polyalkylene oxide comprising the discrete ethylene oxide units is a linear chain. In other cases, the polyalkylene oxide comprising the discrete ethylene oxide units is a branched chain.

[0257] In some instances, the polymer moiety C is a polyalkylene oxide (e.g., PEG) comprising discrete ethylene oxide units. In some cases, the polymer moiety C comprises between about 4 and about 48 ethylene oxide units. In some cases, the polymer moiety C comprises about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about

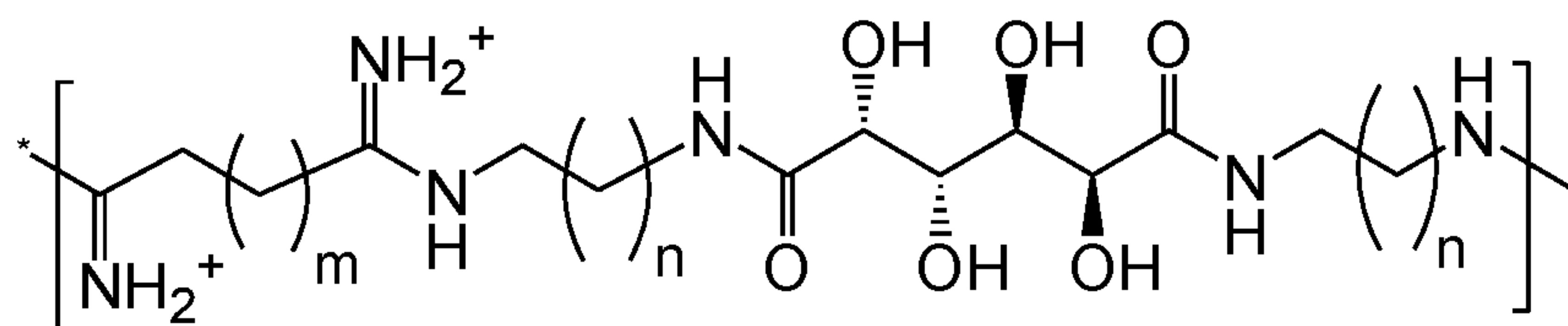
36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, or about 48 ethylene oxide units.

[0258] In some instances, the polymer moiety C is a discrete PEG comprising, e.g., between about 4 and about 48 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, or about 48 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 4 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 5 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 6 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 7 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 8 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 9 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 10 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 11 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 12 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 13 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 14 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 15 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 16 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 17 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 18 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 19 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 20 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 21 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 22 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 23 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 24 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 25 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 26 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG

comprising, e.g., about 27 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 28 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 29 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 30 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 31 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 32 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 33 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 34 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 35 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 36 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 37 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 38 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 39 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 40 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 41 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 42 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 43 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 44 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 45 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 46 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 47 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 48 ethylene oxide units.

[0259] In some cases, the polymer moiety C is dPEG® (Quanta Biodesign Ltd).

[0260] In some embodiments, the polymer moiety C comprises a cationic mucic acid-based polymer (cMAP). In some instances, cMAP comprises one or more subunit of at least one repeating subunit, and the subunit structure is represented as Formula (V):



Formula V

[0261] wherein m is independently at each occurrence 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 4-6 or 5; and n is independently at each occurrence 1, 2, 3, 4, or 5. In some embodiments, m and n are, for example, about 10.

[0262] In some instances, cMAP is further conjugated to a PEG moiety, generating a cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer.

In some instances, the PEG moiety is in a range of from about 500 Da to about 50,000 Da. In some instances, the PEG moiety is in a range of from about 500 Da to about 1000 Da, greater than 1000 Da to about 5000 Da, greater than 5000 Da to about 10,000 Da, greater than 10,000 to about 25,000 Da, greater than 25,000 Da to about 50,000 Da, or any combination of two or more of these ranges.

[0263] In some instances, the polymer moiety C is cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some cases, the polymer moiety C is cMAP-PEG copolymer. In other cases, the polymer moiety C is an mPEG-cMAP-PEGm triblock polymer. In additional cases, the polymer moiety C is a cMAP-PEG-cMAP triblock polymer.

[0264] In some embodiments, the polymer moiety C is conjugated to the polynucleic acid molecule, the binding moiety, and optionally to the endosomolytic moiety as illustrated *supra*.

Endosomolytic Moiety

[0265] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic moiety. In some cases, the endosomolytic moiety is a cellular compartmental release component, such as a compound capable of releasing from any of the cellular compartments known in the art, such as the endosome, lysosome, endoplasmic reticulum (ER), golgi apparatus, microtubule, peroxisome, or other vesicular bodies with the cell. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide, an endosomolytic polymer, an endosomolytic lipid, or an endosomolytic small molecule. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide. In other cases, the endosomolytic moiety comprises an endosomolytic polymer.

Endosomolytic Polypeptides

[0266] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic polypeptide. In some cases, the endosomolytic polypeptide is a pH-dependent membrane active peptide. In some cases, the endosomolytic polypeptide is an amphipathic polypeptide. In additional cases, the endosomolytic polypeptide is a peptidomimetic. In some instances, the endosomolytic polypeptide comprises INF, melittin, meucin, or their respective derivatives thereof. In some instances, the endosomolytic polypeptide comprises INF or its derivatives thereof. In other cases, the endosomolytic polypeptide comprises melittin or its

derivatives thereof. In additional cases, the endosomolytic polypeptide comprises meucin or its derivatives thereof.

[0267] In some instances, INF7 is a 24 residue polypeptide those sequence comprises CGIFGEIEELIEEGLENLIDWGNA (SEQ ID NO: 1), or GLFEAIEGFIENGWEGMIDGWYGC (SEQ ID NO: 2). In some instances, INF7 or its derivatives comprise a sequence of: GLFEAIEGFIENGWEGMIWDYGSGSCG (SEQ ID NO: 3), GLFEAIEGFIENGWEGMIDGWYG-(PEG)6-NH₂ (SEQ ID NO: 4), or GLFEAIEGFIENGWEGMIWDYG-SGSC-K(GalNAc)₂ (SEQ ID NO: 5).

[0268] In some cases, melittin is a 26 residue polypeptide those sequence comprises CLIGAILKVLATGLPTLISWIKNKRKQ (SEQ ID NO: 6), or GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 7). In some instances, melittin comprises a polypeptide sequence as described in U.S. Patent No. 8,501,930.

[0269] In some instances, meucin is an antimicrobial peptide (AMP) derived from the venom gland of the scorpion *Mesobuthus eupeus*. In some instances, meucin comprises of meucin-13 those sequence comprises IFGAIAGLLKNIF-NH₂ (SEQ ID NO: 8) and meucin-18 those sequence comprises FFGHLFKLATKIIPSLFQ (SEQ ID NO: 9).

[0270] In some instances, the endosomolytic polypeptide comprises a polypeptide in which its sequence is at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% sequence identity to INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof. In some instances, the endosomolytic moiety comprises INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof.

[0271] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1-5. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2-5. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2-5. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2-5.

[0272] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 6 or 7. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7. In some cases, the endosomolytic moiety comprises SEQ ID NO: 6. In some cases, the endosomolytic moiety comprises SEQ ID NO: 7. In some cases, the endosomolytic moiety consists of SEQ ID NO: 6. In some cases, the endosomolytic moiety consists of SEQ ID NO: 7.

[0273] In some instances, the endosomolytic moiety is meucin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 8 or 9. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9. In some cases, the endosomolytic moiety comprises SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises SEQ ID NO: 9. In some cases, the endosomolytic moiety consists of SEQ ID NO: 8. In some cases, the endosomolytic moiety consists of SEQ ID NO: 9.

[0274] In some instances, the endosomolytic moiety comprises a sequence as illustrated in **Table 1**.

Table 1.

Name	Origin	Amino Acid Sequence	SEQ ID NO:	Type
Pep-1	NLS from Simian Virus 40 large antigen and Reverse transcriptase of HIV	KETWWETWWTEWSQPKKKRKV	10	Primary amphipathic
pVEC	VE-cadherin	LLILRRRRIRKQAHASK	11	Primary amphipathic
VT5	Synthetic peptide	DPKGDPKGVTVTVTVTGTGKGDP KPD	12	β -sheet amphipathic
C105Y	1-antitrypsin	CSIPPEVKFNKPFVYLI	13	-
Transportan	Galanin and mastoparan	GWTLSAGYLLGKINLKALAALAKKIL	14	Primary amphipathic
TP10	Galanin and mastoparan	AGYLLGKINLKALAALAKKIL	15	Primary amphipathic
MPG	A hydrophobic domain from the fusion	GALFLGFLGAAGSTMGA	16	β -sheet amphipathic

	sequence of HIV gp41 and NLS of SV40 T antigen			
gH625	Glycoprotein gH of HSV type I	HGLASTLTRWAHYNALIRAF	17	Secondary amphipathic α -helical
CADY	PPTG1 peptide	GLWRALWRLLRSLWRLWRA	18	Secondary amphipathic α -helical
GALA	Synthetic peptide	WEAALAEALAEALAEHLAEALAE ALEALAA	19	Secondary amphipathic α -helical
INF	Influenza HA2 fusion peptide	GLFEAIEGFIENGWEGMIDGWYGC	20	Secondary amphipathic α -helical/ pH-dependent membrane active peptide
HA2E5-TAT	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDGWYG	21	Secondary amphipathic α -helical/ pH-dependent membrane active peptide
HA2-penetratin	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDGRQIKI WFQNRRMKW KK-amide	22	pH-dependent membrane active peptide
HA-K4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDG-SSK KKK	23	pH-dependent membrane active peptide
HA2E4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFEAIAAGFIENGWEGMIDGGGYC	24	pH-dependent membrane active peptide
H5WYG	HA2 analogue	GLFHAI AHFIHGGWH GLIHGWYG	25	pH-dependent membrane active peptide
GALA-INF3-(PEG)6-NH	INF3 fusion peptide	GLFEAIEGFIENGWEGLAELAEAL EALAA-(PEG)6-NH2	26	pH-dependent membrane active peptide
CM18-TAT11	Cecropin-A-Melittin ₂₋₁₂ (CM ₁₈) fusion peptide	KWKLFKKIGAVLKVLTTG-YGRKKRRQRRR	27	pH-dependent

				membrane active peptide
--	--	--	--	-------------------------------

[0275] In some cases, the endosomolytic moiety comprises a Bak BH3 polypeptide which induces apoptosis through antagonization of suppressor targets such as Bcl-2 and/or Bcl-x_L. In some instances, the endosomolytic moiety comprises a Bak BH3 polypeptide described in Albarran, *et al.*, “Efficient intracellular delivery of a pro-apoptotic peptide with a pH-responsive carrier,” *Reactive & Functional Polymers* **71**: 261-265 (2011).

[0276] In some instances, the endosomolytic moiety comprises a polypeptide (e.g., a cell-penetrating polypeptide) as described in PCT Publication Nos. WO2013/166155 or WO2015/069587.

Linkers

[0277] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In other instances, the linker is a non-cleavable linker.

[0278] In some cases, the linker is a non-polymeric linker. A non-polymeric linker refers to a linker that does not contain a repeating unit of monomers generated by a polymerization process. Exemplary non-polymeric linkers include, but are not limited to, C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), homobifunctional cross linkers, heterobifunctional cross linkers, peptide linkers, traceless linkers, self-immolative linkers, maleimide-based linkers, or combinations thereof. In some cases, the non-polymeric linker comprises a C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), a homobifunctional cross linker, a heterobifunctional cross linker, a peptide linker, a traceless linker, a self-immolative linker, a maleimide-based linker, or a combination thereof. In additional cases, the non-polymeric linker does not comprise more than two of the same type of linkers, e.g., more than two homobifunctional cross linkers, or more than two peptide linkers. In further cases, the non-polymeric linker optionally comprises one or more reactive functional groups.

[0279] In some instances, the non-polymeric linker does not encompass a polymer that is described above. In some instances, the non-polymeric linker does not encompass a polymer encompassed by the polymer moiety C. In some cases, the non-polymeric linker does not encompass a polyalkylene oxide (e.g., PEG). In some cases, the non-polymeric linker does not encompass a PEG.

[0280] In some instances, the linker comprises a homobifunctional linker. Exemplary homobifunctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3'3'-dithiobis(sulfosuccinimidyl propionate (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N'-disuccinimidyl carbonate (DSC), dimethyl adipimide (DMA), dimethyl pimelimide (DMP), dimethyl suberimide (DMS), dimethyl-3,3'-dithiobispropionimide (DTBP), 1,4-di-3'-(2'-pyridyldithio)propionamido)butane (DPDPB), bismaleimido-hexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4'-difluoro-3,3'-dinitrophenylsulfone (DFDNPS), bis-[β -(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbonyldiimidazole, o-toluidine, 3,3'-dimethylbenzidine, benzidine, α,α' -p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N'-ethylene-bis(iodoacetamide), or N,N'-hexamethylene-bis(iodoacetamide).

[0281] In some embodiments, the linker comprises a heterobifunctional linker. Exemplary heterobifunctional linker include, but are not limited to, amine-reactive and sulfhydryl cross-linkers such as N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (sulfo-LC-sPDP), succinimidyl- α -methyl- α -(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α -methyl- α -(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacetyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ -maleimidobutyryloxy)succinimide ester (GMBs), N-(γ -maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (sIAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-(((4-iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-

carboxyl-hydrazide-8 (M_2C_2H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), amine-reactive and photoreactive cross-linkers such as N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(p-azidosalicylamido)ethyl-1,3'-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3'-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3'-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(p-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3'-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumain-3-acetate (sulfo-sAMCA), p-nitrophenyl diazopyruvate (pNPDP), p-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as 1-(p-Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(p-azidosalicylamido)butyl]-3'-(2'-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as p-azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(p-azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as p-azidophenyl glyoxal (APG).

[0282] In some instances, the linker comprises a reactive functional group. In some cases, the reactive functional group comprises a nucleophilic group that is reactive to an electrophilic group present on a binding moiety. Exemplary electrophilic groups include carbonyl groups—such as aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide or acid anhydride. In some embodiments, the reactive functional group is aldehyde. Exemplary nucleophilic groups include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0283] In some embodiments, the linker comprises a maleimide group. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC) described above.

[0284] In some embodiments, the maleimide group is a self-stablizing maleimide. In some instances, the self-stablizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, *et al.*, “Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates,” *Nat. Biotechnol.* **32**(10):1059-1062 (2014). In some instances, the linker comprises a self-stablizing maleimide. In some instances, the linker is a self-stablizing maleimide.

[0285] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, 6, 7, 8, or more amino acid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety. In some instances, the peptide moiety comprises Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 973), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 974), or Gly-Phe-Leu-Gly (SEQ ID NO: 975). In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 973), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 974), or Gly-Phe-Leu-Gly (SEQ ID NO: 975). In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0286] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[0287] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group. In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[0288] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination

linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Patent No. 9,089,614 or PCT Publication No. WO2015038426.

[0289] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of polynucleotide B to the binding moiety A. In some instances, the dendritic type linker comprises PAMAM dendrimers.

[0290] In some embodiments, the linker is a traceless linker or a linker in which after cleavage does not leave behind a linker moiety (e.g., an atom or a linker group) to a binding moiety A, a polynucleotide B, a polymer C, or an endosomolytic moiety D. Exemplary traceless linkers include, but are not limited to, germanium linkers, silicium linkers, sulfur linkers, selenium linkers, nitrogen linkers, phosphorus linkers, boron linkers, chromium linkers, or phenylhydrazide linker. In some cases, the linker is a traceless aryl-triazene linker as described in Hejesen, *et al.*, “A traceless aryl-triazene linker for DNA-directed chemistry,” *Org Biomol Chem* **11**(15): 2493-2497 (2013). In some instances, the linker is a traceless linker described in Blaney, *et al.*, “Traceless solid-phase organic synthesis,” *Chem. Rev.* **102**: 2607-2024 (2002). In some instances, a linker is a traceless linker as described in U.S. Patent No. 6,821,783.

[0291] In some instances, the linker is a linker described in U.S. Patent Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

[0292] In some embodiments, X, Y, and L are independently a bond or a linker. In some instances, X, Y, and L are independently a bond. In some cases, X, Y, and L are independently a linker.

[0293] In some instances, X is a bond or a linker, e.g., a non-polymeric linker. In some instances, X is a bond. In some instances, X is a non-polymeric linker. In some instances, the non-polymeric linker is a C₁-C₆ alkyl group. In some cases, X is a C₁-C₆ alkyl group, such as for example, a C₅, C₄, C₃, C₂, or C₁ alkyl group. In some cases, the C₁-C₆ alkyl group is an unsubstituted C₁-C₆ alkyl group. As used in the context of a non-polymeric linker, and in particular in the context of X, alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X includes a homobifunctional linker or a heterobifunctional linker described *supra*. In some cases, X includes a heterobifunctional linker. In some cases, X includes sMCC. In other instances, X includes a heterobifunctional linker optionally conjugated to a C₁-C₆ alkyl group. In other instances, X includes sMCC optionally conjugated to a C₁-C₆ alkyl group. In additional

instances, X does not encompass a polymer encompassed by the polymer moiety C, e.g., X does not encompass a polyalkylene oxide (e.g., a PEG molecule).

[0294] In some instances, Y is a bond or a linker, e.g., a non-polymeric linker. In some instances, Y is a bond. In other cases, Y is a non-polymeric linker. In some embodiments, Y is a C₁-C₆ alkyl group. In some instances, Y is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, Y is a homobifunctional linker described *supra*. In some instances, Y is a heterobifunctional linker described *supra*. In some instances, Y comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, Y comprises a peptide moiety, such as Val-Cit. In some instances, Y comprises a benzoic acid group, such as PABA. In additional instances, Y comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, Y comprises a mc group. In additional instances, Y comprises a mc-val-cit group. In additional instances, Y comprises a val-cit-PABA group. In additional instances, Y comprises a mc-val-cit-PABA group. In some cases, Y does not encompass a polymer encompassed by the polymer moiety C, e.g., Y does not encompass a polyalkylene oxide (e.g., a PEG molecule).

[0295] In some instances, L is a bond or a linker, optionally a non-polymeric linker. In some cases, L is a bond. In other cases, L is a linker, optionally a non-polymeric linker. In some embodiments, L is a C₁-C₆ alkyl group. In some instances, L is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, L is a homobifunctional linker described *supra*. In some instances, L is a heterobifunctional linker described *supra*. In some instances, L comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, L comprises a peptide moiety, such as Val-Cit. In some instances, L comprises a benzoic acid group, such as PABA. In additional instances, L comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, L comprises a mc group. In additional instances, L comprises a mc-val-cit group. In additional instances, L comprises a val-cit-PABA group. In additional instances, L comprises a mc-val-cit-PABA group. In some cases, L, when optionally as a non-polymeric linker, does not encompass a polymer encompassed by the polymer moiety C, e.g., Y does not encompass a polyalkylene oxide (e.g., a PEG molecule).

Pharmaceutical Formulation

[0296] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular), oral, intranasal, buccal, rectal, or transdermal

administration routes. In some instances, the pharmaceutical composition describe herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intra-arterial, intraperitoneal, intrathecal, intracerebral, intracerebroventricular, or intracranial) administration. In other instances, the pharmaceutical composition describe herein is formulated for oral administration. In still other instances, the pharmaceutical composition describe herein is formulated for intranasal administration.

[0297] In some embodiments, the pharmaceutical formulations include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0298] In some instances, the pharmaceutical formulation includes multiparticulate formulations. In some instances, the pharmaceutical formulation includes nanoparticle formulations. In some instances, nanoparticles comprise cMAP, cyclodextrin, or lipids. In some cases, nanoparticles comprise solid lipid nanoparticles, polymeric nanoparticles, self-emulsifying nanoparticles, liposomes, microemulsions, or micellar solutions. Additional exemplary nanoparticles include, but are not limited to, paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes and quantum dots. In some instances, a nanoparticle is a metal nanoparticle, e.g., a nanoparticle of scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, yttrium, zirconium, niobium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, gadolinium, aluminum, gallium, indium, tin, thallium, lead, bismuth, magnesium, calcium, strontium, barium, lithium, sodium, potassium, boron, silicon, phosphorus, germanium, arsenic, antimony, and combinations, alloys or oxides thereof.

[0299] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0300] In some instances, a nanoparticle is further coated with molecules for attachment of functional elements (e.g., with one or more of a polynucleic acid molecule or binding moiety described herein). In some instances, a coating comprises chondroitin sulfate, dextran sulfate, carboxymethyl dextran, alginic acid, pectin, carrageenan, fucoidan, agarose, agarpectin, porphyrin, karaya gum, gellan gum, xanthan gum, hyaluronic acids, glucosamine, galactosamine, chitin (or chitosan), polyglutamic acid, polyaspartic acid, lysozyme, cytochrome C, ribonuclease, trypsinogen,

chymotrypsinogen, α -chymotrypsin, polylysine, polyarginine, histone, protamine, ovalbumin or dextrin or cyclodextrin. In some instances, a nanoparticle comprises a graphene-coated nanoparticle.

[0301] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0302] In some instances, the nanoparticle formulation comprises paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes or quantum dots. In some instances, a polynucleic acid molecule or a binding moiety described herein is conjugated either directly or indirectly to the nanoparticle. In some instances, at least 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more polynucleic acid molecules or binding moieties described herein are conjugated either directly or indirectly to a nanoparticle.

[0303] In some embodiments, the pharmaceutical formulation comprise a delivery vector, e.g., a recombinant vector, the delivery of the polynucleic acid molecule into cells. In some instances, the recombinant vector is DNA plasmid. In other instances, the recombinant vector is a viral vector. Exemplary viral vectors include vectors derived from adeno-associated virus, retrovirus, adenovirus, or alphavirus. In some instances, the recombinant vectors capable of expressing the polynucleic acid molecules provide stable expression in target cells. In additional instances, viral vectors are used that provide for transient expression of polynucleic acid molecules.

[0304] In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrolidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphatidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New

York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999).

[0305] In some instances, the pharmaceutical formulations further include pH adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0306] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0307] In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they provide a more stable environment. Salts dissolved in buffered solutions (which also provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel[®]; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac[®] (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0308] In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term "disintegrate" include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel[®], or sodium starch glycolate such as Promogel[®] or Explotab[®], a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel[®], Avicel[®] PH101, Avicel[®] PH102, Avicel[®] PH105, Elcema[®] P100, Emcocel[®], Vivacel[®], Ming Tia[®], and Solka-Floc[®], methylcellulose, croscarmellose, or a cross-

linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol[®]), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum[®] HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0309] In some instances, the pharmaceutical formulations include filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrans, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0310] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumarate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex[®]), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet[®], boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax[™], sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid[™], Cab-O-Sil[®], a starch such as corn starch, silicone oil, a surfactant, and the like.

[0311] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers also function as dispersing agents or wetting agents.

[0312] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium docusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutool, propylene glycol, and dimethyl isosorbide and the like.

[0313] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0314] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol has a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulose, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0315] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic[®] (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0316] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0317] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium docusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Therapeutic Regimens

[0318] In some embodiments, the pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is

administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

[0319] In some embodiments, one or more pharmaceutical compositions are administered simultaneously, sequentially, or at an interval period of time. In some embodiments, one or more pharmaceutical compositions are administered simultaneously. In some cases, one or more pharmaceutical compositions are administered sequentially. In additional cases, one or more pharmaceutical compositions are administered at an interval period of time (e.g., the first administration of a first pharmaceutical composition is on day one followed by an interval of at least 1, 2, 3, 4, 5, or more days prior to the administration of at least a second pharmaceutical composition).

[0320] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simultaneously. In some cases, the two or more different pharmaceutical compositions are coadministered sequentially without a gap of time between administrations. In other cases, the two or more different pharmaceutical compositions are coadministered sequentially with a gap of about 0.5 hour, 1 hour, 2 hour, 3 hour, 12 hours, 1 day, 2 days, or more between administrations.

[0321] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously; alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0322] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

[0323] In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0324] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages is altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[0325] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD50 and ED50. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Article of Manufacture

[0326] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more of the compositions and methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0327] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0328] For example, the container(s) include target nucleic acid molecule described herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0329] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[0330] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0331] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Certain Terminology

[0332] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter

claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

[0333] As used herein, ranges and amounts can be expressed as “about” a particular value or range. About also includes the exact amount. Hence “about 5 μ L” means “about 5 μ L” and also “5 μ L.” Generally, the term “about” includes an amount that would be expected to be within experimental error.

[0334] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0335] As used herein, the terms “individual(s),” “subject(s)” and “patient(s)” mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician’s assistant, an orderly or a hospice worker).

[0336] As used herein the terms “*DMD*,” “*DMD* gene,” and equivalents thereof refer to the *DMD* gene that encodes for the protein dystrophin. In addition, the terms “*DMD*” and “*DMD* gene” are used interchangeably, and both terms refer to the dystrophin gene.

EXAMPLES

[0337] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

[0338] Example 1. Antisense oligonucleotide sequences and synthesis

[0339] Phosphorodiamidate morpholino oligomers (PMO), phosphorothioate antisense oligonucleotides (PS ASO), and antisense oligonucleotides (ASOs) were synthesized.

[0340] The PMO sequence was 5’GGCCAAACCTCGGCTTACCTGAAAT3’ Primary amine (SEQ ID NO: 28) and can be seen in **Fig. 1** with end nucleotides expanded. The PMO contains a C3-NH₂ conjugation handle at the 3’ end of the molecule for conjugation. PMOs were fully assembled on solid phase using standard solid phase synthesis protocols and purified over HPLC.

[0341] The PS ASO sequence was Amine-C6-GGCCAAACCUCGGCUUACCU (SEQ ID NO: 29) and can be seen in **Figs. 2A-2B** with end nucleotides expanded. The structure of the PS ASO comprised a phosphate backbone that was 100% phosphorothioate linkages and all the ribose

sugars contained a 2' 2'OMe modification. The PS ASO also contained a C6-NH₂ conjugation handle at the 5' end of the molecule for conjugation. The PS ASOs were fully assembled on the solid phase using standard solid phase phosphoramidite chemistry and purified over HPLC.

[0342] ASOs were fully assembled on the solid phase using standard solid phase phosphoramidite chemistry and purified over HPLC. ASOs contained a C6-NH₂ conjugation handle at the 5' end of the molecule for conjugation.

[0343] **Example 2. Detection of *DMD* exon skipping**

[0344] *Methods for Determining DMD Exon 23 Skipping in Differentiated C1C12 Cells*

[0345] Mouse myoblast C2C12 cells were plated at 50,000-100,000/well in 24-well plates in 0.5 mL 10% FBS RPMI 1640 media and incubated at 37 °C with 5% CO₂ overnight. On the second day, cells were switched to differentiation media (2% horse serum RPMI 1640 and 1 µM insulin) and incubated for 3-5 days. Following incubation, samples were added and incubated for 24 hours. After the sample treatment, 1 mL of fresh media (with no compounds) was changed every day for 2 more days. At 72 hours after the start of treatments, cells were harvested. RNAs were isolated using InviTrap RNA Cell HTS 96 Kit (B-Bridge International #7061300400) and reverse transcribed using High Capacity cDNA Reverse transcription Kit (ThermoFisher #4368813). PCR reactions were performed using DreamTaq™ PCR Mastermix (ThermoFisher #K1072). The primary PCR used primers in exon 20 (Ex20F 5'-CAGAATTCTGCCAATTGCTGAG) (SEQ ID NO: 30) and exon 26 (Ex26R 5'-TTCTTCAGCTTGTGTCATCC) (SEQ ID NO: 31) to amplify both skipped and unskipped molecules using the protocol in **Table 2**.

Table 2. PCR Protocol

Hot Start	95 °C for 2 minutes
Denaturation	95 °C for 0.5 minute
Annealing of primers	50°C for 0.5 minute
Primer extension	72°C for 1 minute
Final extension	72°C for 5 minutes
Number of Cycles	10

[0346] For the nested PCR, primary PCR reactions were diluted with water 100X, and 5 µl was used for nested PCR reaction (50 µl total reaction volume). Nested PCR used primers in exon 20 (Ex20F2: 5'- ACCCAGTCTACCACCCTATC) (SEQ ID NO: 32) and exon 25 (Ex25R: 5'- CTCTTTATCTTCTGCCCACCTT) (SEQ ID NO: 33) to amplify both skipped and unskipped molecules using the protocol in **Table 3**.

Table 3. Nested PCR Protocol

Hot Start	95 °C for 2 minutes
Denaturation	95 °C for 0.5 minute
Annealing of primers	50°C for 0.5 minute
Primer extension	72°C for 1 minute
Final extension	72°C for 5 minutes
Number of Cycles	35

[0347] PCR reactions were analyzed using 4% TAE agarose gels. The wild-type (WT) *DMD* product had an expected size of 788 base pairs and the skipped *DMD* $\Delta 23$ of 575 base pairs.

[0348] *Animals*

[0349] All animal studies were conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the “Guide for the Care and Use of Laboratory Animals” (National Research Council publication, 8th Ed., revised in 2011). All mice were obtained from either Charles River Laboratories or Harlan Laboratories.

[0350] *In vivo mouse model*

[0351] WT CD-1 mice (4-6 weeks old) were dosed via intravenous (iv) injection with the indicated antisense conjugates (ASCs) and doses. The “naked” PMO or ASO were dosed via intramuscular injection at the indicated doses. After 4, 7, or 14 days, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated with Trizol and RNeasy Plus 96 Kit (Qiagen, #74192) and reversed transcribed using High Capacity cDNA Reverse transcription Kit (ThermoFisher #4368813). Nested PCR reactions were performed as described. PCR reactions were analyzed in 4% (or 1%) TAE agarose gels which were quantitated by densitometry.

[0352] To confirm exon 23 skipping in treated mice, DNA fragments were isolated from the 4% agarose gels and sequenced.

[0353] To quantitatively determine the skipped *DMD* mRNA copy number, qPCR primer/probe sets were designed to quantify skipped and WT *DMD* mRNA (**Fig. 3**). qPCR quantification standards were designed and produced via PCR using designed PCR primers as seen in **Table 4**. For the qPCR standard for WT and *DMD*, following PCR a 733 base pair fragment was isolated from the agarose gel. For qPCR standard for skipped *DMA*, the nested primers were used.

[0354] The amplification efficiency of the qPCR primer/probes were determined to be within 10% of expected efficiency. qPCR reactions were performed in QuantStudio 7 and TaqmanTM PCR Universal Mastermix II (ThermoFisher #4440041) according to manufacturer’s instructions.

Table 4.

	SEQ ID NO	Primer/Probe	Sequence
DMD Δ-23, for Ex23 skipping	34	Forward Primer	5' GCGCTATCAGGAGACAATGAG
	35	Reverse Primer	5' GTTTTATGTGATTCTGTAATTTCCC
	36	Probe	5' CTCTCTGTACCTTATCTTAGTGTT
DMD Ex22-23, for WT DMD only	37	Forward Primer	5' TGGAGGAGAGACTCGGGAAA
	38	Reverse Primer	5' TTGAAGCCATTTTGTTGCTCTTT
	39	Probe	5' ACAGGCTCTGCAAAGT
DMD Ex20-21, for All DMD	40	Forward Primer	5' AACAGATGACAACTACTGCCGAAA
	41	Reverse Primer	5' TTGGCTCTGATAGGGTGGTAGAC
	42	Probe	5' CTTGTTGAAAACCC
qPCR standard for WT and all DMD	43	Forward Primer	5' TGAGGGTGTTAATGCTGAAAGTA
	44	Reverse Primer	5' CACCAACTGGGAGGAAAGTT

[0355] Example 3: Conjugate Synthesis

[0356] *Analytical and Purification Methods*

[0357] Analytical and purification methods were performed according to **Tables 5-11**.

Table 5. Size exclusion chromatography (SEC) methods

Size Exclusion Chromatography (SEC) Method	Column	Mobile Phase	Flow Rate
method 1	TOSOH Biosciences, TSKgelG3000SW XL, 7.8 X 300 mm, 5 μM	150 mM phosphate buffer	1.0 mL/minute for 20 minutes
method 2	TOSOH Biosciences, TSKgelG3000SW, 21.5 X 600 mm, 5 μM	PBS pH 7.4	1.0 mL/minute for 180 minutes

Table 6. Hydrophobic interaction chromatography (HIC) method 1

Column	Solvent	Gradient		
		Column Volume	%A	%B
GE, HiScreen Butyl HP, 4.7 mL	Solvent A: 50 mM phosphate buffer, 0.8M Ammonium Sulfate, pH 7.0 Solvent B: 80% 50 mM phosphate	1.00	95	5
		30	0	100
		5	0	100

	buffer, 20% IPA, pH 7.0 Flow Rate: 1.0 mL/minute			
--	--	--	--	--

Table 7. Hydrophobic interaction chromatography (HIC) method 2

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, MAbPac HIC-20, 4.6 mm ID X 10 cm, 5 um	Solvent A: 100 mM phosphate buffer, 1.8 M Ammonium Sulfate, pH 7.0 Solvent B: 80% 100 mM phosphate buffer, 20% IPA, pH 7.0 Flow Rate: 0.7 mL/minute	0.00	100	0
		2.00	100	0
		22.00	0	100
		25.00	0	100
		26.00	100	0
		30.00	100	0

Table 8. Hydrophobic interaction chromatography (HIC) method 3

Column	Solvent	Gradient		
		Column Volume	%A	%B
GE, HiScreen Butyl HP, 4.7 mL	Solvent A: 50 mM phosphate buffer, 0.8 M Ammonium Sulfate, pH 7.0 Solvent B: 80% 50 mM phosphate buffer, 20% IPA, pH 7.0 Flow Rate: 1.0 mL/minute	1	100	0
		25	0	80
		1	0	100
		2	0	100

Table 9. Hydrophobic interaction chromatography (HIC) method 4

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, MAbPac HIC-20, 4.6 mm ID X 10 cm, 5 um	Solvent A: 100 mM phosphate buffer, 1.8 M Ammonium Sulfate, pH 7.0 Solvent B: 80% 100 mM phosphate buffer, 20% IPA, pH 7.0 Flow Rate: 0.5 mL/minute	0.00	100	0
		5.00	100	0
		20.00	0	100
		25.00	0	100
		26.00	100	0
		30.00	100	0

Table 10. Strong anion exchange chromatography (SAX) method 1

Column	Solvent	Gradient		
		Column Volume	%A	%B
Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um	Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0 Flow Rate: 6.0 mL/minute	0.5	100	0
		0.5	80	20
		17	20	80
		0.5	0	100
		0.5	0	100

Table 11. Strong anion exchange chromatography (SAX) method 2

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, ProPac™ SAX-10,	Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol	0.0	90	10
		3.00	90	10

Bio LC™, 4 X 250 mm	Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl Flow Rate: 0.75 mL/minute	17.00	0	100
		21.00	0	100
		22.00	90	10
		25.00	90	10

[0358] *Anti-transferrin receptor antibody*

[0359] Anti-mouse transferrin receptor antibody or anti-CD71 mAb that was used was a rat IgG2a subclass monoclonal antibody that binds mouse CD71 or mouse transferrin receptor 1 (mTfR1). The antibody was produced by BioXcell and it is commercially available (Catalog # BE0175).

[0360] **Anti-CD71 antibody morpholino antisense oligonucleotide conjugate (anti-CD71 mAb-PMO)**

[0361] *Anti-CD71 mAb-PMO conjugation*

[0362] Anti-CD71 antibody (10 mg/mL) in borate buffer (25 mM sodium tetraborate, 25 mM NaCl, 1 mM Diethylene triamine pentaacetic acid, pH 8.0) was reduced by adding 4 equivalents of tris(2-carboxyethyl)phosphine (TCEP) in water and incubating at 37 °C for 4 hours. 4(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC) was coupled to the primary amine on the 3' end of the phosphorodiamidate morpholino oligomer (PMO) by incubating the PMO (50 mg/mL) in DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for one hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PMO-SMCC was washed three times with acetate buffer (10 mM sodium acetate, pH 6.0) and used immediately. The reduced antibody was mixed with 2.25 equivalents of PMO-SMCC and incubated overnight at 4 °C. The pH of the reaction mixture was then reduced to 7.5, and 8 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction mixture by hydrophobic interaction chromatography (HIC) method 2 showed antibody-PMO conjugates along with unreacted antibody and PMO (**Fig. 4**). **Fig. 4** shows a chromatogram of anti-CD71 mAb-PMO reaction mixture produced with HIC method 2 showing free antibody peak (1), free PMO (2), DAR 1 (3), DAR 2 (4), DAR 3 (5), DAR > 3 (6). "DAR" refers to a drug-to-antibody ratio. The number in parentheses refers to the peak in the chromatogram.

[0363] *Purification*

[0364] The reaction mixture was purified with an AKTA Explorer FPLC using HIC method 1. Fractions containing conjugates with a drug to antibody ratio of one (DAR 1) and two (DAR 2) were combined and concentrated with Amicon Ultra-15 centrifugal filter units with a MWCO of 50 kDa separately from conjugates with a DAR greater than 2. Concentrated conjugates were buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units prior to analysis.

[0365] *Analysis of the purified conjugate*

[0366] The isolated conjugates were characterized by size exclusion chromatography (SEC) and HIC. SEC method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated PMOs (**Figs. 5A-5C**). **Fig. 5A** shows a chromatogram of anti-CD71 mAb produced using SEC method 1. **Fig. 5B** shows a chromatogram of anti-CD71 mAb-PMO DAR 1,2 produced using SEC method 1. **Fig. 5C** shows a chromatogram of anti-CD71 mAb-PMO DAR greater than 2 produced using SEC method 1. “DAR” refers to a drug-to-antibody ratio.

[0367] The purity of the conjugate was assessed by analytical HPLC using HIC method 2 (**Figs. 6A-6C**). **Fig. 6A** shows a chromatogram of anti-CD71 mAb produced using HIC method 2. **Fig. 6B** shows a chromatogram of purified anti-CD71 mAb-PMO DAR 1,2 conjugate produced using HIC method 2. **Fig. 6C** shows a chromatogram of purified anti-CD71 mAb-PMO DAR >2 conjugate produced using HIC method 2. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of PMO and antibody to confirm DAR. The DAR 1,2 sample had an average DAR of ~1.6 while the DAR greater than 2 sample had an average DAR of ~3.7. “DAR” refers to a drug-to-antibody ratio.

[0368] **Anti-CD71 Fab morpholino antisense oligonucleotide conjugate (anti-CD71 Fab-PMO)****[0369]** *Antibody digestion with pepsin*

[0370] Anti-CD71 antibody (5 mg/mL) in 20 mM acetate buffer (pH 4.0) was incubated with immobilized pepsin for 3 hours at 37 °C. The resin was removed and the reaction mixture was washed with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 30 kDa. The retentate was collected and purified using size exclusion chromatography (SEC) method 2 to isolate the F(ab')₂ fragment.

[0371] *Anti-CD71 (Fab)-PMO conjugation*

[0372] The F(ab')₂ fragment (15 mg/mL) in borate buffer (pH 8.0) was reduced by adding 10 equivalents of TCEP in water and incubating at 37 °C for 2 hours. SMCC was added to the primary amine on the 3' end of the PMO by incubating the PMO (50 mg/mL) in DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for 1 hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PMO-SMCC was washed three times with acetate buffer (pH 6.0) and used immediately. The reduced F(ab')₂ fragment (Fab) was buffer exchanged into borate buffer (pH 8.0) using Amicon Ultra-15 Centrifugal Filter Units with a MWCO of 10 kDa, and 1.75 equivalents of PMO-SMCC was added and incubated overnight at 4 °C. The pH of the reaction mixture was then reduced to 7.5, and 6 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to

quench unreacted cysteines. Analysis of the reaction mixture by hydrophobic interaction chromatography (HIC) method 3 showed anti-CD71 (Fab)-PMO conjugates along with unreacted Fab (**Fig. 7A**). **Fig. 7A** shows a chromatogram of FPLC purification of anti-CD71 Fab-PMO using HIC method 3.

[0373] *Purification*

[0374] The reaction mixture was purified with an AKTA Explorer FPLC using HIC method 3. Fractions containing conjugates with a DAR of one, two and three were combined and concentrated separately. Concentrated conjugates were buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 10 kDa prior to analysis.

[0375] *Analysis of the purified conjugate*

[0376] The isolated conjugates were characterized by SEC, and HIC. SEC method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated PMO. *See Figs. 7B-7E*. **Fig. 7B** shows a chromatogram of anti-CD71 Fab produced using SEC method 1. **Fig. 7C** shows a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using SEC method 1. **Fig. 7D** shows a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using SEC method 1. **Fig. 7E** shows a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using SEC method 1. The purity of the conjugate was assessed by analytical HPLC using HIC method 4. *See Figs. 7F-7I*. **Fig. 7F** shows a chromatogram of anti-CD71 Fab produced using HIC method 4. **Fig. 7G** shows a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using HIC method 4. **Fig. 7H** shows a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using HIC method 4. **Fig. 7I** shows a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using HIC method 4. “DAR” refers to drug-to-antibody ratio. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of PMO and Fab to confirm DAR.

[0377] **Anti-CD71 antibody phosphorothioate antisense oligonucleotide conjugate (anti-CD71 mAb-PS ASO)**

[0378] *Anti-CD71 mAb-PS ASO*

[0379] Anti-CD71 antibody (10 mg/mL) in borate buffer (pH 8.0) was reduced by adding 4 equivalents of TCEP in water and incubating at 37°C for 4 hours. 4(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC) was added to the primary amine on the 5' end of the PS-ASO by incubating the PS ASO (50 mg/mL) in 1:1 mixture of 250 mM PB (pH 7.5) and DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for 1 hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PS ASO-SMCC was washed three times with acetate

buffer (pH 6.0) and used immediately. The reduced antibody was mixed with 1.7 equivalents of PS ASO-SMCC and incubated overnight at 4°C. The pH of the reaction mixture was then reduced to 7.4, and 8 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction mixture by strong anion exchange chromatography (SAX) method 2 showed antibody-PS ASO conjugates along with unreacted antibody and ASO (**Fig. 8A**). **Fig. 8A** shows a chromatogram of anti-CD71 mAb-PS ASO reaction mixture produced with SAX method 2 showing free antibody peak (1), free PS ASO (5), DAR 1 (2), DAR 2 (3), DAR > 2 (4). “DAR” refers to a drug-to-antibody ratio. The number in parentheses refers to the peak.

[0380] *Purification*

[0381] The reaction mixture was purified with an AKTA Explorer FPLC using SAX method 1. Fractions containing conjugates with a drug-to-antibody ratio (DAR) of one, two and three were combined and concentrated separately and buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 50 kDa prior to analysis.

[0382] *Analysis of the purified conjugate*

[0383] The isolated conjugates were characterized by size exclusion chromatography (SEC) and SAX. Size exclusion chromatography method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated ASO. *See Figs. 8B-8E*. **Fig. 8B** shows a chromatogram of anti-CD71 mAb produced using SEC method 1. **Fig. 8C** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SEC method 1. **Fig. 8D** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SEC method 1. **Fig. 8E** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SEC method 1. The purity of the conjugate was assessed by analytical HPLC using SAX method 2. *See Figs. 8F-8H*. **Fig. 8F** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SAX method 2. **Fig. 8G** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SAX method 2. **Fig. 8H** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SAX method 2. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of ASO and antibody to confirm drug-to-antibody ratio (DAR).

[0384] **Example 4: *In vitro* activity of anti-CD71 mAb-PMO conjugate**

[0385] The anti-CD71 mAb-PMO conjugate was made and characterized as described in Example 3. The conjugate was assessed for its ability to mediate exon skipping in vitro in differentiated C2C12 cells using nested PCR using methods similar to Example 2. Briefly, the potency of “naked” morpholino ASO (“PMO”) was compared to an anti-CD71 mAb-PMO

conjugate at multiple concentrations with the relevant vehicle controls. Controls included vehicle (“Veh”), scramble morpholino at 50 uM (“Scr50”), and no antibody (“Neg-Ab”). The concentrations of PMO used included 50 uM, 1 uM, and 0.02 uM. The concentrations of anti-CD71 mAb-PMO DAR 1,2 used included 200 nM, 20 nM, and 2 nM. “DAR” refers to drug-to-antibody ratio.

[0386] Following cDNA synthesis, two rounds of PCR amplification (primary and nested PCR) were used to detect exon-skipping. PCR reactions were analyzed in a 4% TAE agarose gel (**Fig. 9**).

[0387] Referring to **Fig. 9**, anti-CD71 mAb-PMO conjugate produced measurable exon 23 skipping in differentiated C2C12 cells and lower concentrations than the “naked” PMO control. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ 23 of 575 base pairs.

[0388] A second experiment included an anti-CD71 Fab-PMO conjugate and a PMO targeted with an anti-EGFR (“Z-PMO”) as a negative control (**Fig. 10**). The concentrations of PMO used included 10 uM and 2 uM. The concentrations of anti-CD71 mAb-PMO used included 0.2 uM and 0.04 uM. Anti-CD71 mAb-PMO had a DAR of 2. Z-PMO was used at a concentration of 0.2 uM and had a DAR of 2. Concentrations of anti-CD71 Fab-PMO included 0.6 uM and 0.12 uM. DAR of 1, 2, and 3 for anti-CD71 mAb-PMO at 0.6 uM and 0.12 uM were assayed.

[0389] Referring to **Fig. 10**, Receptor mediated uptake utilizing the transferrin receptor, the anti-CD71 mAb-PMO, and anti-CD71 Fab-PMO conjugates resulted in measurable exon 23 skipping in C2C12 cells and lower concentrations than the “naked” PMO control. There was no measurable exon 23 skipping from the Z-PMO at the concentration tested, which produced skipping from the anti-CD71 conjugates.

[0390] **Example 5. *In vitro* activity of anti-CD71-ASO mAb PS conjugate**

[0391] The anti-CD71 mAb-PS ASO conjugate was made and characterized as described in Example 3. The conjugate was assessed for its ability to mediate exon skipping in vitro in differentiated C2C12 cells using nested PCR using similar methods as described in Example 2. Briefly, the potency of “naked” phosphorothioate ASO (PS ASO) was compared to an anti-CD71 mAb-PS ASO conjugate at multiple concentrations, with the relevant vehicle control. Two rounds of PCR amplification (primary and nested PCR) were performed following cDNA synthesis to detect exon-skipping. PCR reactions were analyzed in a 4% TAE agarose gel (**Fig. 11**). **Fig. 11** shows an agarose gel of PMO, ASO, conjugated anti-CD71 mAb-ASO of DAR1 (“ASC-DAR1”), conjugated anti-CD71 mAb-ASO of DAR2 (“ASC-DAR2”), and conjugated anti-CD71 mAb-ASO of DAR3 (“ASC-DAR3”). “PMO” and “ASO” refers to free PMO and ASO, unconjugated to

antibody. “Veh” refers to vehicle only. The concentrations tested included 0.2, 1, and 5 micromolar (μM).

[0392] Referring to **Fig. 11**, the anti-CD71 mAb-PS ASO conjugate produced measurable exon 23 skipping in differentiated C2C12 cells and lower concentrations than the “naked” PS ASO control. The wild-type product had an expected size of 788 base pairs and the skipped DMD $\Delta 23$ of 575 base pairs.

[0393] **Example 6: *In vivo* activity of anti-CD71 mAb-PMO conjugate**

[0394] The anti-CD71 mAb-PMO conjugate was made and characterized as described in Example 3. The conjugate anti-CD71 mAb-PMO DAR_{1,2} anti-CD71 and mAb-PMO DAR_{>2} were assessed for its ability to mediate exon skipping *in vivo* in wild-type CD-1 mice using similar methods as described in Example 2. “DAR” refers to drug-to-antibody ratio.

[0395] Mice were dosed via intravenous (iv) injection with the mAb, vehicle control, and antisense conjugates (ASCs) at the doses as provided in **Table 12**. “DAR” refers to drug-to-antibody ratio. The “naked” PMO was dosed via intramuscular injection into the gastrocnemius muscle at the doses provided in **Table 12**. After 4, 7, or 14 days, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated, reversed transcribed and a nested PCR reactions were performed. PCR reactions were analyzed in 4% TAE agarose gels which were then quantitated by densitometry.

Table 12. *In vivo* study design

Group	Test Article	N	mAb dose (mg/kg)	PMO Dose (mg/kg)	PMO: mAb Ratio (mol/mol)	Harvest Time (h)
1	anti-CD71 mAb-PMO, DAR _{1,2}	3	50	4.8	1.6	96
2	anti-CD71 mAb-PMO, DAR _{1,2}	3	50	4.8	1.6	168
3	anti-CD71 mAb-PMO, DAR _{1,2}	3	50	4.8	1.6	336
4	anti-CD71 mAb-PMO, DAR _{>2}	3	50	10.5	3.7	96
5	anti-CD71 mAb-PMO, DAR _{>2}	3	50	10.5	3.7	168
6	anti-CD71 mAb-PMO, DAR _{>2}	3	50	10.5	3.7	336
7	anti-CD71 mAb	3	50			96
8	anti-CD71 mAb	3	50			168
9	anti-CD71 mAb	3	50			336
10	PMO	3	40 ug/inj.			96
11	PMO	3	40 ug/inj.			168
12	PMO	3	40 ug/inj.			336
13	Vehicle	3				96
14	Vehicle	3				168
15	Vehicle	3				336

[0396] **Fig. 12A** shows a gel electrophoresis of gastrocnemius muscle samples from mice administered anti-CD71 mAb-PMO DAR 1,2, anti-CD71 mAb-PMO DAR>2, anti-CD71 mAb, PMO, and vehicle for 4, 7, or 14 days. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ 23 of 575 base pairs. Anti-CD71 mAb-PMO DAR 1,2 and anti-CD71 mAb-PMO DAR>2 produced measurable exon 23 skipping in gastrocnemius muscle and lower concentrations than the “naked” PMO control. The intensity of the bands on the gel (**FIG. 12A**) was quantitated by densitometry as seen in **Fig. 12B**. **Fig. 12C** shows the quantification of *in vivo* exon skipping in wild-type mice gastrocnemius muscle using Taqman qPCR.

[0397] **Fig. 13A** shows a gel electrophoresis of heart samples from mice administered anti-CD71 mAb-PMO DAR 1,2, anti-CD71 mAb-PMO DAR>2, anti-CD71 mAb, PMO, and vehicle for 4, 7, or 14 days. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ 23 of 575 base pairs. The intensity of the bands on the gel (**FIG. 13A**) was quantitated by densitometry as seen in **Fig. 13B**. Similar results as with the gastrocnemius muscle samples were obtained. Anti-CD71 mAb-PMO DAR 1,2 and anti-CD71 mAb-PMO DAR>2 produced measurable exon 23 skipping in gastrocnemius muscle and lower concentrations than the “naked” PMO control.

[0398] DNA fragments were then isolated from the 4% agarose gels and sequenced. The sequencing data confirmed the correct sequence in the skipped and wild-type products as seen in **Fig. 14**.

[0399] **Example 7. Antisense oligonucleotide sequences and synthesis**

[0400] The sequences in **Table 13** were made targeting different exons in different genes.

Table 13. Sequences

SEQ ID NO.	Target	PMO sequence (5' to 3')
45	Exon 23 in mouse dystrophin	GGCCAAACCTCGGCTTACCTGAAAT
46	Exon 2 in mouse myostatin (MSTN)	AGCCCATCTTCTCCTGGTCCTGGGAAGG
47	Exon 11 in mouse phenylalanine hydroxylase (PAH)	ATCCTCTTTGGTAACCTCACCTCAC
48	KRAS-011 (human cancer)	TCGTCCACAAAATGATTCTGAATTA
49	Scramble	CGGTGTGTGTATCATTCTCTAGTGT

[0401] **Example 8. *In vivo* activity of CD71 mAb-PMO conjugate in multiple tissues**

[0402] The CD71 mAb-PMO conjugates were made and characterized as described in Example 3. The conjugate (DAR3+) was assessed for its ability to mediate exon skipping *in vivo* in wild type CD-1 mice, see example 2 for full experimental details. In brief, mice were dosed via

intravenous (iv) injection with vehicle control and indicated ASCs at the doses indicated, see figure 7A. After 7, 14 or 28 days, diaphragm, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated, reversed transcribed, real-time qPCR and nested PCR reactions were performed as described in Example 2 using the appropriate primer/probe sets. PCR reactions were analyzed in 1% TAE agarose gels.

[0403] *In vivo* study design to assess the ability of the CD71 mAb-PMO conjugate to mediate exon 23 skipping in wild type mice is seen in **Table 14**.

Table 14. *In vivo* study design

Group	Test Article	N	mAb-PMO mAb dose (mg/kg)	PMO Dose (mg/kg)	PMO:mAb Ratio (mol/mol)	Harvest Time (WEEKS)
1	Vehicle	3				1
2	Vehicle	3				4
3	CD71-scr, DAR3+	3	50	10	3.0	2
4	CD71-DMD PMO, DAR3+	3	50	10	3.0	1
5	CD71-DMD PMO, DAR3+	3	50	10	3.0	2
6	CD71-DMD PMO, DAR3+	3	50	10	3.0	4

[0404] Referring to **Fig. 15A**, **Fig. 15C**, and **Fig. 15E**, *in vivo* exon skipping was measured in wild type mice in the gastrocnemius (**Fig. 15A**), diaphragm (**Fig. 15C**) and heart muscle (**Fig. 15E**) using Taqman qPCR. Referring to **Fig. 15B**, **Fig. 15D**, and **Fig. 15F**, the CD71 mAb-PMO conjugates produced measurable exon23 skipping in gastrocnemius (**Fig. 15B**), diaphragm (**Fig. 15D**), and heart muscle (**Fig. 15F**) using nested PCR. The wild type product had an expected size of 788 bp, and the skipped DMD Δ 23 had a size of 575 bp. The intensity of the bands on the gel was quantitated by densitometry, and the data are presented as the % of skipped product compared to wild-type dystrophin.

[0405] **Example 9. *In vivo* activity of CD71 mAb-PMO conjugates against mouse *MSTN***

[0406] The CD71 mAb-PMO conjugate targeting exon 2 of mouse myostatin (5' AGCCCATCTTCTCCTGGTCCTGGGAAGG) (SEQ ID NO: 46) was made and characterized as described in Example 3. The conjugates (DAR1/2 and DAR3+) were assessed for its ability to mediate exon skipping *in vivo* in wild type CD-1 mice using similar methods as described in

Example 2. In brief, mice were dosed via intravenous (iv) injection with the mAb, vehicle control and indicated ASCs at the doses indicated as seen in **Table 15**.

Table 15. *In vivo* study design

Group	Test Article	N	mAb-PMO mAb dose (mg/kg)	PMO Dose (mg/kg)	PMO:mAb Ratio (mol/mol)	Harvest Time (WEEKS)
1	CD71 mAb-PMO, DAR1/2	3	50	5	1.5	1
2	CD71 mAb-PMO, DAR1/2	3	50	5	1.5	2
3	CD71 mAb-PMO, DAR1/2	3	50	5	1.5	4
4	CD71 mAb-PMO, DAR3+	3	50	10	3.0	1
5	CD71 mAb-PMO, DAR3+	3	50	10	3.0	2
6	CD71 mAb-PMO, DAR3+	3	50	10	3.0	4
7	CD71-scr, DAR1/2	3	50	5	1.5	2
8	CD71-scr, DAR3+	3	50	10	3.0	2
9	Vehicle	3				1
10	Vehicle	3				2
11	Vehicle	3				4

[0407] After 7, 14 or 28 days, diaphragm, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated and reversed transcribed. PCR reactions were performed with forward primer (mMSTN-F1: 5' CCTGGAAACAGCTCCTAACATC) (SEQ ID NO: 50) and reverse primer (mMSTN-R1: 5' CAGTCAAGCCCCAAAGTCTCTC) (SEQ ID NO: 51) (hot start: 95 °C for 2 minutes, Denaturation at 95°C for 45 seconds, Annealing of primers at 56°C for 30 seconds, primer extension at 72°C for 40 seconds for 35 cycles). PCR reactions were analyzed in a 1% TAE agarose gel as seen in **Figs. 16A-16C**. The CD71 mAb-PMO conjugates produced measurable exon2 skipping in mouse diaphragm (**Fig. 16A**), heart (**Fig. 16B**) and gastrocnemius (**Fig. 16C**) muscle tissues. The wild type product had an expected size of 622 bp and the skipped *MSTN* Δ2 of 248 bp.

[0408] **Example 10. *In vitro* activity of ASGPR mAb-PMO conjugates against the *PAH* gene**

[0409] The ASGPR mAb-PMO (5' ATCCTCTTTGGTAACCTCACCTCAC) (SEQ ID NO: 47) conjugate targeting exon 11 of mouse *PAH* was made and characterized as described in Example 3. The conjugate was assessed for its ability to mediate exon 11 skipping in the mouse *PAH* gene *in vitro* in primary mouse hepatocytes using PCR (forward primer 5'-

CTAGTGCCCTTGTTTTTCAGA-3' (SEQ ID NO: 52) and reverse primer 5'-AGGATCTACCACTGATGGGT-3') (SEQ ID NO: 53). In brief, the potency of ASGPR mAb-PAH PMO conjugate was compared to ASGPR mAb-scramble PMO at multiple concentrations, with the relevant vehicle control. RNAiMAX was also used to transfect the conjugates as positive controls. PCR reactions were analyzed in a 1% TAE agarose gel as seen in **Fig. 17**. As seen from the gel in **Fig. 17**, the ASGPR mAb-PMO conjugate produced measurable exon11 skipping comparable to the RNAiMAX transfected controls. The wild type product had an expected size of 703 bp and the skipped *PAH* Δ 11 of 569 bp.

[0410] Example 11. *In vivo* activity of ASGPR mAb-PMO conjugates

[0411] The ASGPR mAb-PMO (5' ATCCTCTTTGGTAACCTCACCTCAC) (SEQ ID NO: 47) conjugate targeting exon 11 of mouse *PAH* was made and characterized as described in Example 3. The conjugate (DAR1/2 and DAR3+) was assessed for its ability to mediate exon skipping *in vivo* in wild type CD-1 mice using methods as described in Example 2. In brief, mice were dosed via intravenous (iv) injection with the mAb, vehicle control and indicated ASCs at the doses indicated as seen in **Table 16**.

Table 16. *In vivo* study design

Group	Test Article	N	mAb-ASO mAb dose (mg/kg)	PMO Dose (mg/kg)	PMO:mAb Ratio (mol/mol)	Harvest Time (Weeks)
1	ASGPR mAb-PMO, DAR1/2	3	50	5	1.5	1
2	ASGPR mAb-PMO, DAR1/2	3	50	5	1.5	2
3	ASGPR mAb-PMO, DAR1/2	3	50	5	1.5	4
4	ASGPR mAb-PMO, DAR3+	2	50	10	3.0	1
5	ASGPR mAb-PMO, DAR3+	2	50	10	3.0	2
6	ASGPR mAb-PMO, DAR3+	2	50	10	3.0	4
7	ASGPR-Scr, DAR1/2	3	50		1.5	2
8	ASGPR-Scr, DAR3+	3	50		3.0	2
9	Vehicle	3				1
10	Vehicle	3				2
11	Vehicle	3				4

[0412] RNAs were isolated from harvested liver tissues and reverse transcribed. PCR reactions using forward primer 5'-CTAGTGCCCTTGTTTTTCAGA-3' (SEQ ID NO: 52) and reverse primer 5'-AGGATCTACCACTGATGGGT-3' (SEQ ID NO: 53) were analyzed in a 1% TAE agarose gel as seen in **Fig. 18**. As can be seen from the gel in **Fig. 18**, the ASGPR mAb-PMO conjugates produced measurable exon11 skipping in mouse livers up to two weeks. The wild type product had an expected size of 703 bp and the skipped *PAH* Δ 11 of 569 bp.

[0413] Example 12. Sequences

[0414] **Table 17** illustrates exemplary target sequences to induce insertion, deletion, duplications, or alteration in the *DMD* gene using compositions and methods as described herein. **Table 18** illustrates exemplary nucleotide sequences to induce an insertion, deletion, duplication, or alteration in the *DMD* gene using compositions and methods as described herein. **Table 19** and **Table 20** illustrate exemplary target sequences in several genes for inducing an insertion, deletion, duplications, or alteration in the gene. **Table 21** illustrates exemplary sequences, including sequences in the *DMD* gene to induce an insertion, deletion, duplication, or alteration in the gene using compositions and methods as described herein.

Table 17.

Target Exon	Antisense Sequence	SEQ ID NO.
19	5' GCCUGAGCUGAUCUGCUGGCAUCUUGCAGUU 3'	54
19 or 20	5' GCAGAAUUCGAUCCACCGGCUGUUCAAGCCUG AGCUGAUCUGCUCGCAUCUUGCAGU3'	55
20	5' CAGCAGUAGUUGUCAUCUGCUC 3'	56
21	5' CACAAAGUCUGCAUCCAGGAACAUGGGUC 3'	57
22	5' CUGCAAUUCCCCGAGUCUCUGC 3'	58
51	5' CUCAUACCUUCUGCUUGAUGAUC 3'	59
52	5' UCCAACUGGGGACGCCUCUGUUCCAAAUCC 3'	60

Table 18.

Gene	Target Location	Nucleotide Sequence (5'-3')	SEQ ID NO.
<i>DMD</i>	H8A(-06+18)	GAUAGGUGGUAUCAACAUCUGUAA	61
<i>DMD</i>	H8A(-03+18)	GAUAGGUGGUAUCAACAUCUG	62
<i>DMD</i>	H8A(-07+18)	GAUAGGUGGUAUCAACAUCUGUAAG	63
<i>DMD</i>	H8A(-06+14)	GGUGGUAUCAACAUCUGUAA	64
<i>DMD</i>	H8A(-10+10)	GUAUCAACAUCUGUAAGCAC	65
<i>DMD</i>	H7A(+45+67)	UGCAUGUUCCAGUCGUUGUGUGG	66
<i>DMD</i>	H7A(+02+26)	CACUAUUCCAGUCAAAUAGGUCUGG	67
<i>DMD</i>	H7D(+15-10)	AUUUACCAACCUUCAGGAUCGAGUA	68
<i>DMD</i>	H7A(-18+03)	GGCCUAAAACACAUACACAUA	69
<i>DMD</i>	C6A(-10+10)	CAUUUUUGACCUACAUGUGG	70

<i>DMD</i>	C6A(−14+06)	UUUGACCUACAUGUGGAAAG	71
<i>DMD</i>	C6A(−14+12)	UACAUUUUUGACCUACAUGUGGAAAG	72
<i>DMD</i>	C6A(−13+09)	AUUUUUGACCUACAUGGGAAAG	73
<i>DMD</i>	CH6A(+69+91)	UACGAGUUGAUUGUCGGACCCAG	74
<i>DMD</i>	C6D(+12−13)	GUGGUCUCCUUACCUAUGACUGUGG	75
<i>DMD</i>	C6D(+06−11)	GGUCUCCUUACCUAUGA	76
<i>DMD</i>	H6D(+04−21)	UGUCUCAGUAAUCUUCUUAACCUAU	77
<i>DMD</i>	H6D(+18−04)	UCUUAACCUAUGACUAUGGAUGAGA	78
<i>DMD</i>	H4A(+13+32)	GCAUGAACUCUUGUGGAUCC	79
<i>DMD</i>	H4D(+04−16)	CCAGGGUACUACUUAACAUUA	80
<i>DMD</i>	H4D(−24−44)	AUCGUGUGUCACAGCAUCCAG	81
<i>DMD</i>	H4A(+11+40)	UGUUCAGGGCAUGAACUCUUGUGGAUCCUU	82
<i>DMD</i>	H3A(+30+60)	UAGGAGGCGCCUCCCAUCCUGUAGGUCACUG	83
<i>DMD</i>	H3A(+35+65)	AGGUCUAGGAGGCGCCUCCCAUCCUGUAGGU	84
<i>DMD</i>	H3A(+30+54)	GCGCCUCCCAUCCUGUAGGUCACUG	85
<i>DMD</i>	H3D(+46−21)	CUUCGAGGAGGUCUAGGAGGCGCCUC	86
<i>DMD</i>	H3A(+30+50)	CUCCCAUCCUGUAGGUCACUG	87
<i>DMD</i>	H3D(+19−03)	UACCAGUUUUUGCCCUGUCAGG	88
<i>DMD</i>	H3A(−06+20)	UCAAUAUGCUGCUUCCCAAACUGAAA	89
<i>DMD</i>	H3A(+37+61)	CUAGGAGGCGCCUCCCAUCCUGUAG	90
<i>DMD</i>	H5A(+20+50)	UUAUGAUUUCCAUCUACGAUGUCAGUACUUC	91
<i>DMD</i>	H5D(+25−05)	CUUACCUGCCAGUGGAGGAUUAUAUUCCAA	92
<i>DMD</i>	H5D(+10−15)	CAUCAGGAUUCUUAACUGCCAGUGG	93
<i>DMD</i>	H5A(+10+34)	CGAUGUCAGUACUCCAAUAUUCAC	94
<i>DMD</i>	H5D(−04−21)	ACCAUUCAUCAGGAUUCU	95
<i>DMD</i>	H5D(+16−02)	ACCUGCCAGUGGAGGAUU	96
<i>DMD</i>	H5A(−07+20)	CCAAUAUUCACUAAAUCAACCUGUUA	97
<i>DMD</i>	H5D(+18−12)	CAGGAUUGUUACCUGCCAGUGGAGGAUUAU	98
<i>DMD</i>	H5A(+05+35)	ACGAUGUCAGUACUCCAAUAUUCACUAAAU	99
<i>DMD</i>	H5A(+15+45)	AUUUCCAUCUACGAUGUCAGUACUUCCAAUA	100
<i>DMD</i>	H10A(−05+16)	CAGGAGCUUCCAAAUGCUGCA	101
<i>DMD</i>	H10A(−05+24)	CUUGUCUUCAGGAGCUUCCAAAUGCUGCA	102
<i>DMD</i>	H10A(+98+119)	UCCUCAGCAGAAAGAAGCCACG	103
<i>DMD</i>	H10A(+130+149)	UUAGAAAUCUCUCCUUGUGC	104
<i>DMD</i>	H10A(−33−14)	UAAAUUGGGUGUUACACAAU	105
<i>DMD</i>	H11D(+26+49)	CCCUGAGGCAUUCCCAUCUUGAAU	106
<i>DMD</i>	H11D(+11−09)	AGGACUUACUUGC UUUGUUU	107
<i>DMD</i>	H11A(+118+140)	CUUGAAUUUAGGAGAUUCAUCUG	108
<i>DMD</i>	H11A(+75+97)	CAUCUUCUGAUAAUUUUCCUGUU	109
<i>DMD</i>	H12A(+52+75)	UCUUCUGUUUUUGUUAGCCAGUCA	110
<i>DMD</i>	H12A(−10+10)	UCUAUGUAAACUGAAAAUUU	111
<i>DMD</i>	H12A(+11+30)	UUCUGGAGAUCCAUAUAAAAC	112
<i>DMD</i>	H13A(+77+100)	CAGCAGUUGCGUGAUCUCCACUAG	113
<i>DMD</i>	H13A(+55+75)	UUCAUCAACUACCACCACCAU	114
<i>DMD</i>	H13D(+06−19)	CUAAGCAAAAUAUUCUGACCUUAAG	115

<i>DMD</i>	H14A(+37+64)	CUUGUAAAAGAACCCAGCGGUCUUCUGU	116
<i>DMD</i>	H14A(+14+35)	CAUCUACAGAUGUUUGCCCAUC	117
<i>DMD</i>	H14A(+51+73)	GAAGGAUGUCUUGUAAAAGAACC	118
<i>DMD</i>	H14D(-02+18)	ACCUGUUCUUCAGUAAGACG	119
<i>DMD</i>	H14D(+14-10)	CAUGACACACCUGUUCUUCAGUAA	120
<i>DMD</i>	H14A(+61+80)	CAUUUGAGAAGGAUGUCUUG	121
<i>DMD</i>	H14A(-12+12)	AUCUCCCAAUACCUGGAGAAGAGA	122
<i>DMD</i>	H15A(-12+19)	GCCAUGCACUAAAAAGGCACUGCAAGACA UU	123
<i>DMD</i>	H15A(+48+71)	UCUUUAAAGCCAGUUGUGUGAAUC	124
<i>DMD</i>	H15A(+08+28)	UUUCUGAAAGCCAUGCACUAA	125
<i>DMD</i>	H15D(+17-08)	GUACAUACGGCCAGUUUUUGAAGAC	126
<i>DMD</i>	H16A(-12+19)	CUAGA UCCGCUUUUAAAACCUGUUAAAACAA	127
<i>DMD</i>	H16A(-06+25)	UCUUUUCUAGA UCCGCUUUUAAAACCUGUUA	128
<i>DMD</i>	H16A(-06+19)	CUAGA UCCGCUUUUAAAACCUGUUA	129
<i>DMD</i>	H16A(+87+109)	CCGUCUUCUGGGUCACUGACUUA	130
<i>DMD</i>	H16A(-07+19)	CUAGA UCCGCUUUUAAAACCUGUUAA	131
<i>DMD</i>	H16A(-07+13)	CCGCUUUUAAAACCUGUUAA	132
<i>DMD</i>	H16A(+12+37)	UGGAUUGCUUUUUCUUUUCUAGA UCC	133
<i>DMD</i>	H16A(+92+116)	CAUGCUUCCGUCUUCUGGGUCACUG	134
<i>DMD</i>	H16A(+45+67)	GAUCUUGUUUGAGUGAAUACAGU	135
<i>DMD</i>	H16A(+105+126)	GUUAUCCAGCCAUGCUUCCGUC	136
<i>DMD</i>	H16D(+05-20)	UGAUAAUUGGUAUCACUAACCUGUG	137
<i>DMD</i>	H16D(+12-11)	GUAUCACUAACCUGUGCUGUAC	138
<i>DMD</i>	H19A(+35+53)	CUGCUGGCAUCUUGCAGUU	139
<i>DMD</i>	H19A(+35+65)	GCCUGAGCUGAUCUGCUGGCAUCUUGCAGUU	140
<i>DMD</i>	H20A(+44+71)	CUGGCAGAAUUCGAUCCACCGGCUGUUC	141
<i>DMD</i>	H20A(+147+168)	CAGCAGUAGUUGUCAUCUGCUC	142
<i>DMD</i>	H20A(+185+203)	UGAUGGGGUGGUGGGGUUGG	143
<i>DMD</i>	H20A(-08+17)	AUCUGCAUUAACACCCUCUAGAAAG	144
<i>DMD</i>	H20A(+30+53)	CCGGCUGUUCAGUUGUUCUGAGGC	145
<i>DMD</i>	H20A(-11+17)	AUCUGCAUUAACACCCUCUAGAAAGAAA	146
<i>DMD</i>	H20D(+08-20)	GAAGGAGAAGAGAUUCUUACCUUACAAA	147
<i>DMD</i>	H20A(+44+63)	AUUCGAUCCACCGGCUGUUC	148
<i>DMD</i>	H20A(+149+168)	CAGCAGUAGUUGUCAUCUGC	149
<i>DMD</i>	H21A(-06+16)	GCCGGUUGACUUCAUCCUGUGC	150
<i>DMD</i>	H21A(+85+106)	CUGCAUCCAGGAACAUGGGUCC	151
<i>DMD</i>	H21A(+85+108)	GUCUGCAUCCAGGAACAUGGGUC	152
<i>DMD</i>	H21A(+08+31)	GUUGAAGAUCUGAUAGCCGGUUGA	153
<i>DMD</i>	H21D(+18-07)	UACUUACUGUCUGUAGCUCUUUCU	154
<i>DMD</i>	H22A(+22+45)	CACUCAUGGUCUCCUGAUAGCGCA	155
<i>DMD</i>	H22A(+125+106)	CUGCAAUUC CCCGAGUCUCUGC	156
<i>DMD</i>	H22A(+47+69)	ACUGCUGGACCCAUGUCCUGAUG	157
<i>DMD</i>	H22A(+80+101)	CUAAGUUGAGGUAUGGAGAGU	158
<i>DMD</i>	H22D(+13-11)	UAUUCACAGACCUGCAAUUC CCC	159
<i>DMD</i>	H23A(+34+59)	ACAGUGGUGCUGAGAUAGUAUAGGCC	160

<i>DMD</i>	H23A(+18+39)	UAGGCCACUUUGUUGCUCUUGC	161
<i>DMD</i>	H23A(+72+90)	UUCAGAGGGCGCUUUCUUC	162
<i>DMD</i>	H24A(+48+70)	GGGCAGGCCAUUCCUCCUUCAGA	163
<i>DMD</i>	H24A(-02+22)	UCUUCAGGGUUUGUAUGUGAUUCU	164
<i>DMD</i>	H25A(+9+36)	CUGGGCUGAAUUGUCUGAAUAUCACUG	165
<i>DMD</i>	H25A(+131+156)	CUGUUGGCACAUGUGAUCCCACUGAG	166
<i>DMD</i>	H25D(+16-08)	GUCUAUACCUGUUGGCACAUGUGA	167
<i>DMD</i>	H26A(+132+156)	UGC UUUCUGUAAUUCAUCUGGAGUU	168
<i>DMD</i>	H26A(-07+19)	CCUCCUUUCUGGCAUAGACCUUCCAC	169
<i>DMD</i>	H26A(+68+92)	UGUGUCAUCCAUCGUGCAUCUCUG	170
<i>DMD</i>	H27A(+82+106)	UUAAGGCCUCUUGUGCUACAGGUGG	171
<i>DMD</i>	H27A(-4+19)	GGGGCUCUUCUUUAGCUCUCUGA	172
<i>DMD</i>	H27D(+19-03)	GACUCCAAAGUCUUGCAUUUC	173
<i>DMD</i>	H28A(-05+19)	GCCAACAUGCCCAAACUUCCUAAG	174
<i>DMD</i>	H28A(+99+124)	CAGAGAUUUCUCAGCUCCGCCAGGA	175
<i>DMD</i>	H28D(+16-05)	CUUACAUCUAGCACCUCAGAG	176
<i>DMD</i>	H29A(+57+81)	UCCGCCAUCUGUUAGGGUCUGUGCC	177
<i>DMD</i>	H29A(+18+42)	AUUUGGGUUAUCCUCUGAAUGUCGC	178
<i>DMD</i>	H29D(+17-05)	CAUACCUCUUCAUGUAGUUCCC	179
<i>DMD</i>	H30A(+122+147)	CAUUUGAGCUGCGUCCACCUUGUCUG	180
<i>DMD</i>	H30A(+25+50)	UCCUGGGCAGACUGGAUGCUCUGUUC	181
<i>DMD</i>	H30D(+19-04)	UUGCCUGGGCUUCCUGAGGCAUU	182
<i>DMD</i>	H31D(+06-18)	UUCUGAAAUAAACAUAUACCUGUGC	183
<i>DMD</i>	H31D(+03-22)	UAGUUUCUGAAAUAAACAUAUACCUG	184
<i>DMD</i>	H31A(+05+25)	GACUUGUCAAAUCAGAUUGGA	185
<i>DMD</i>	H31D(+04-20)	GUUUCUGAAAUAAACAUAUACCUGU	186
<i>DMD</i>	H32D(+04-16)	CACCAGAAAUACAUACCACA	187
<i>DMD</i>	H32A(+151+170)	CAAUGAUUUAGCUGUGACUG	188
<i>DMD</i>	H32A(+10+32)	CGAAACUUCAUGGAGACAUCUUG	189
<i>DMD</i>	H32A(+49+73)	CUUGUAGACGCUGCUCAAAAUUGGC	190
<i>DMD</i>	H33D(+09-11)	CAUGCACACACCUUUGCUC	191
<i>DMD</i>	H33A(+53+76)	UCUGUACAAUCUGACGUCCAGUCU	192
<i>DMD</i>	H33A(+30+56)	GUCUUUAUCACCAUUUCCACUUCAGAC	193
<i>DMD</i>	H33A(+64+88)	CCGUCUGCUUUUUCUGUACAAUCUG	194
<i>DMD</i>	H34A(+83+104)	UCCAUAUCUGUAGCUGCCAGCC	195
<i>DMD</i>	H34A(+143+165)	CCAGGCAACUUCAGAAUCCAAAU	196
<i>DMD</i>	H34A(-20+10)	UUUCUGUUACCUGAAAAGAAUUAUAUGAA	197
<i>DMD</i>	H34A(+46+70)	CAUUCAUUUCCUUUCGCAUCUUACG	198
<i>DMD</i>	H34A(+95+120)	UGAUCUCUUUGUCAAUUCCAUAUCUG	199
<i>DMD</i>	H34D(+10-20)	UUCAGUGAUAUAGGUUUUACCUUCCCCAG	200
<i>DMD</i>	H34A(+72+96)	CUG UAG CUG CCA GCC AUU CUG UCA AG	201
<i>DMD</i>	H35A(+141+161)	UCU UCU GCU CGG GAG GUG ACA	202
<i>DMD</i>	H35A(+116+135)	CCA GUU ACU AUU CAG AAG AC	203
<i>DMD</i>	H35A(+24+43)	UCU UCA GGU GCA CCU UCU GU	204
<i>DMD</i>	H36A(+26+50)	UGUGAUGUGGUCCACAUCUGGUCA	205

<i>DMD</i>	H36A(−02+18)	CCAUGUGUUUCUGGUAUUCC	206
<i>DMD</i>	H37A(+26+50)	CGUGUAGAGUCCACCUUUGGGCGUA	207
<i>DMD</i>	H37A(+82+105)	UACUAAUUUCCUGCAGUGGUCACC	208
<i>DMD</i>	H37A(+134+157)	UUCUGUGUGAAAUGGCUGCAAUUC	209
<i>DMD</i>	H38A(−01+19)	CCUUCAAAGGAAUGGAGGCC	210
<i>DMD</i>	H38A(+59+83)	UGCUGAAUUUCAGCCUCCAGUGGUU	211
<i>DMD</i>	H38A(+88+112)	UGAAGUCUUCCUCUUUCAGAUUCAC	212
<i>DMD</i>	H39A(+62+85)	CUGGCUUUCUCUCAUCUGUGAUUC	213
<i>DMD</i>	H39A(+39+58)	GUUGUAAGUUGUCUCCUCUU	214
<i>DMD</i>	H39A(+102+121)	UUGUCUGUAACAGCUGCUGU	215
<i>DMD</i>	H39D(+10−10)	GCUCUAAUACCUUGAGAGCA	216
<i>DMD</i>	H40A(−05+17)	CUUUGAGACCUCAAAUCCUGUU	217
<i>DMD</i>	H40A(+129+153)	CUUUAUUUUCUUCUUCUUCUGGGC	218
<i>DMD</i>	H42A(−04+23)	AUCGUUUCUUCACGGACAGUGUGCUGG	219
<i>DMD</i>	H42A(+86+109)	GGGCUUGUGAGACAUGAGUGAUUU	220
<i>DMD</i>	H42D(+19−02)	ACCUUCAGAGGACUCCUCUUGC	221
<i>DMD</i>	H43D(+10−15)	UAUGUGUUACCUACCCUUGUCGGUC	222
<i>DMD</i>	H43A(+101+120)	GGAGAGAGCUUCCUGUAGCU	223
<i>DMD</i>	H43A(+78+100)	UCACCCUUUCCACAGGCGUUGCA	224
<i>DMD</i>	H44A(+85+104)	UUUGUGUCUUUCUGAGAAAC	225
<i>DMD</i>	H44D(+10−10)	AAAGACUUACCUUAAGAUAC	226
<i>DMD</i>	H44A(−06+14)	AUCUGUCAAAUCGCCUGCAG	227
<i>DMD</i>	H46D(+16−04)	UUACCUUGACUUGCUCUAGC	228
<i>DMD</i>	H46A(+90+109)	UCCAGGUUCAAGUGGGAUAC	229
<i>DMD</i>	H47A(+76+100)	GCUCUUCUGGGCUUAUGGGAGCACU	230
<i>DMD</i>	H47D(+25−02)	ACCUUUAUCCACUGGAGAUUUGUCUGC	231
<i>DMD</i>	H47A(−9+12)	UUCCACCAGUAACUGAAACAG	232
<i>DMD</i>	H50A(+02+30)	CCACUCAGAGCUCAGAUCUUCUAACUUC	233
<i>DMD</i>	H50A(+07+33)	CUUCCACUCAGAGCUCAGAUCUUCUAA	234
<i>DMD</i>	H50D(+07−18)	GGGAUCCAGUAUACUUACAGGCUC	235
<i>DMD</i>	H51A(−01+25)	ACCAGAGUAACAGUCUGAGUAGGAGC	236
<i>DMD</i>	H51D(+16−07)	CUCAUACCUUCUGCUUGAUGAUC	237
<i>DMD</i>	H51A(+111+134)	UUCUGUCCAAGCCCGGUUGAAAUC	238
<i>DMD</i>	H51A(+61+90)	ACAUCAAGGAAGAUGGCAUUUCUAGUUUGG	239
<i>DMD</i>	H51A(+66+90)	ACAUCAAGGAAGAUGGCAUUUCUAG	240
<i>DMD</i>	H51A(+66+95)	CUCCAACAUCAAGGAAGAUGGCAUUUCUAG	241
<i>DMD</i>	H51D(+08−17)	AUCAUUUUUUCUCAUACCUUCUGCU	242
<i>DMD</i>	H51A/D(+08−17) &(−15+)	AUCAUUUUUUCUCAUACCUUCUGCUAG GAGCUAAAA	243
<i>DMD</i>	H51A(+175+195)	CACCCACCAUCACCCUCUGUG	245
<i>DMD</i>	H51A(+199+220)	AUCAUCUCGUUGAUUAUCCUCAA	246
<i>DMD</i>	H52A(−07+14)	UCCUGCAUUGUUGCCUGUAAG	247
<i>DMD</i>	H52A(+12+41)	UCCAACUGGGGACGCCUCUGUUCCAAUCC	248
<i>DMD</i>	H52A(+17+37)	ACUGGGGACGCCUCUGUCCA	249
<i>DMD</i>	H52A(+93+112)	CCGUAAUGAUUGUUCUAGCC	250

<i>DMD</i>	H52D(+05-15)	UGUUAAAAAACUUACUUCGA	251
<i>DMD</i>	H53A(+45+69)	CAUUCAACUGUUGCCUCCGGUUCUG	252
<i>DMD</i>	H53A(+39+62)	CUGUUGCCUCCGGUUCUGAAGGUG	253
<i>DMD</i>	H53A(+39+69)	CAUUCAACUGUUGCCUCCGGUUCUGAAGGUG	254
<i>DMD</i>	H53D(+14-07)	UACUAACCUUGGUUUCUGUGA	255
<i>DMD</i>	H53A(+23+47)	CUGAAGGUGUUCUUGUACUUCAUCC	256
<i>DMD</i>	H53A(+150+176)	UGUAUAGGGACCCUCCUCCAUGACUC	257
<i>DMD</i>	H53D(+20-05)	CUAACCUUGGUUUCUGUGAUUUUCU	258
<i>DMD</i>	H53D(+09-18)	GGUAUCUUUGAUACUAACCUUGGUUUC	259
<i>DMD</i>	H53A(-12+10)	AUUCUUUCAACUAGAAUAAAAG	260
<i>DMD</i>	H53A(-07+18)	GAUUCUGAAUUCUUUCAACUAGAAU	261
<i>DMD</i>	H53A(+07+26)	AUCCCACUGAUUCUGAAUUC	262
<i>DMD</i>	H53A(+124+145)	UUGGCUCUGGCCUGUCCUAAGA	263
<i>DMD</i>	H46A(+86+115)	CUCUUUUCAGGUUCAAGUGGGAUACUAGC	264
<i>DMD</i>	H46A(+107+137)	CAAGCUUUUCUUUAGUUGCUGCUCUUUUC	265
<i>DMD</i>	H46A(-10+20)	UAUUCUUUUGUUCUUCUAGCCUGGAGAAAG	266
<i>DMD</i>	H46A(+50+77)	CUGCUUCCUCCAACCAUAAAACAAAUUC	267
<i>DMD</i>	H45A(-06+20)	CCAAUGCCAUCCUGGAGUCCUGUAA	268
<i>DMD</i>	H45A(+91+110)	UCCUGUAGAAUACUGGCAUC	269
<i>DMD</i>	H45A(+125+151)	UGCAGACCUCUGCCACCGCAGAUUCA	270
<i>DMD</i>	H45D(+16-04)	CUACCUCUUUUUUCUGUCUG	271
<i>DMD</i>	H45A(+71+90)	UGUUUUUGAGGAUUGCUGAA	272

* The first letter designates the species (e.g. H: human, M: murine, C: canine). “#” designates target *DMD* exon number. “A/D” indicates acceptor or donor splice site at the beginning and end of the exon, respectively. (x y) represents the annealing coordinates where “-” or “+” indicate intronic or exonic sequences respectively.

Table 19.

Gene	Nucleotide Sequence (5' - 3')	SEQ ID NO.
Bcl-x	TGGTTCTTACCCAGCCGCCG	273
β-globin 623	GTTATTCTTTAGAATGGTGC	274
β-globin 654	TGCTATTACCTTAACCCAGA	275
c-myc	CTGTGCTTACCGGGTTTTCCACCTCCC	276
c-myc	ATCGTCGTGACTGTCTGTTGGAGGG	277
c-myc	GCTCACGTTGAGGGGGCATCG	278
c-myc	ACGTTGAGGGGGCATCGTCGC	279
c-myc	GGGGCAUCGUCGUGACUGU/CUGUUGGAGGG	280
c-myc	CGUCGUGACUGUCUGUUGGAGG	281
c-myc	CGTCGTGACTGTCTGTTGGAGG	282
c-myc	GGCAUCGUCGCGGGAGGCUGCUGGAGCG	283
c-myc	CCGCGACAUAGGACGGAGAGCAGAGCCC	284
c-myc	ACTGTGAGGGGCGATCGCTGC	285
c-myc	ACGATGAGTGGCATAGTCGC	286

c-myc	GGCATCGTCGCGGGAGGCTG	287
c-myc	GGGCATCGTCGCGGGAGGCT	288
c-myc	GGGGCATCGTCGCGGGAGGC	289
c-myc	AGGGGCATCGTCGCGGGAGG	290
c-myc	GAGGGGCATCGTCGCGGGAG	291
c-myc	TGAGGGGCATCGTCGCGGGA	292
c-myc	TTGAGGGGCATCGTCGCGGG	293
c-myc	GTTGAGGGGCATCGTCGCGG	294
c-myc	CGTTGAGGGGCATCGTCGCG	295
c-myc	ACGTTGAGGGGCATCGTCGC	296
c-myc	AACGTTGAGGGGCATCGTCG	297
c-myc	TAACGTTGAGGGGCATCGTC	298
c-myc	CTAACGTTGAGGGGCATCGT	299
c-myc	GCTAACGTTGAGGGGCATCG	300
c-myc	AGCTAACGTTGAGGGGCATC	301
c-myc	AAGCTAACGTTGAGGGGCAT	302
c-myc	GAAGCTAACGTTGAGGGGCA	303
BCL-2 (rat)	CTCCGCAATGCTGAAAGGTG	304
PCNA-1 (rat)	GGCGUGCCUCAAACAUGGUGGCGG	305

Table 20.

Gene	Target Location	Nucleotide Sequence (5'-3')	SEQ ID NO.
Rat c-myc	2553-79	CTGTGCTTACCGGGTTTTCCACCTCCC	306
Rat c-myc	4140-64	ATCGTCGTGACTGTCTGTTGGAGGG	307
Rat c-myc	4161-80	GCTCACGTTGAGGGGCATCG	308
Rat CYP3A2	1155-74	GGTCACTCACCGGTAGAGAA	309
Rat CYP3A2	1526-45	GGGTTCCAAGTCTATAAAGG	310
Human androgen receptor exon 2	31-44	TGTGTCTTTTCCAG	311
Human androgen receptor exon 2	45-67	TTTGGAGACTGCCAGGGACCATG	312
Human androgen receptor exon 2	48-67	CATGGTCCCTGGCAGTCTCC	313
Human androgen receptor exon 2	45-80	TCAATGGGCAAAACATGGTCCCTGGCAGTCTCCAAA	314
Human androgen receptor exon 3	28-43	TTTGTGTTCTCCCAG	315
Human androgen receptor exon 3	44-66	GGAAACAGAAGTACCTGTGCGCC	316

Human androgen receptor exon 3	49-66	GGCGCACAGGTACTTCTG	317
Human androgen receptor exon 3	44-79	AATCATTTCTGCTGGCGCACAGGTACTTCTGTTTCC	318
Human HCG- β subunit	1321-38	CCCCTGCAGCACGCGGGT	319
Human HCG- β subunit	1321-57	GAGGCAGGGCCGGCAGGACCCCCTGCAGCACGCGGGT	320
Human c-myc	4506-25	GGCATCGTCGCGGGAGGCTG	321
Human c-myc	4507-26	GGGCATCGTCGCGGGAGGCT	322
Human c-myc	4508-27	GGGGCATCGTCGCGGGAGGC	323
Human c-myc	4509-28	AGGGGCATCGTCGCGGGAGG	324
Human c-myc	4510-29	GAGGGGCATCGTCGCGGGAG	325
Human c-myc	4511-30	TGAGGGGCATCGTCGCGGGA	326
Human c-myc	4512-31	TTGAGGGGCATCGTCGCGGG	327
Human c-myc	4513-32	GTTGAGGGGCATCGTCGCGG	328
Human c-myc	4514-33	CGTTGAGGGGCATCGTCGCG	329
Human c-myc	4515-34	ACGTTGAGGGGCATCGTCGC	330
Human c-myc	4516-35	AACGTTGAGGGGCATCGTCG	331
Human c-myc	4517-36	TAACGTTGAGGGGCATCGTC	332
Human c-myc	4518-37	CTAACGTTGAGGGGCATCGT	333
Human c-myc	4519-38	GCTAACGTTGAGGGGCATCG	334
Human c-myc	4520-39	AGCTAACGTTGAGGGGCATC	335
Human c-myc	4521-40	AAGCTAACGTTGAGGGGCAT	336
Human c-myc	4522-41	GAAGCTAACGTTGAGGGGCA	337
Human c-myc	6656-75	TCCTCATCTTCTTGTTCTC	338
Human c-myc	6656-91	AACAACATCGATTTCTTCCTCATCTTCTTGTTCTC	339
Human p53	11691-708	CCCGGAAGGCAGTCTGGC	340
Human p53	11689-724	TCCTCCATGGCAGTGACCCGGAAGGCAGTCTGGCTG	341
Human abl (ds of bcr-abl fusion point)	376-94	CTACTGGCCGCTGAAGGGC	342
Human abl (ds of bcr-abl fusion point)	374-409	GCTCAAAGTCAGATGCTACTGGCCGCTGAAGGGCTT	343
HW-1 rev	5517-43	TCGTCGGTCTCTCCGCTTCTTCTTGCC	344
HW-1 rev	7885-7904	CTCTGGTGGTGGGTAAGGGT	345
HW-1 rev	7885-7921	CGGGTCTGTCGGGTTCCTCTGGTGGTGGGTAAGGGT	346
Rat c-myc	4140-69	GGGGCAUCGUCGUGACUGUCUGUUGGAGGG	347
Rat c-myc	4141-62	CGUCGUGACUGUCUGUUGGAGG	348
Rat c-myc	4141-62	CGTCGTGACTGTCTGTTGGAGG	349
Human c-myc	4498-	GGCAUCGUCGCGGGAGGCUG/CUGGAGCG	350

	4505		
Rat c-myc	4364-91	CCGCGACAUAGGACGGAGAGCAGAGCCC	351

Table 21.

Target	Nucleotide Sequence (5' - 3')	SEQ ID NO.
Hu.DMD.Exon44.25.001	CTGCAGGTAAAAGCATATGGATCAA	352
Hu.DMD.Exon44.25.002	ATCGCCTGCAGGTAAAAGCATATGG	353
Hu.DMD.Exon44.25.003	GTCAAATCGCCTGCAGGTAAAAGCA	354
Hu.DMD.Exon44.25.004	GATCTGTCAAATCGCCTGCAGGTAA	355
Hu.DMD.Exon44.25.005	CAACAGATCTGTCAAATCGCCTGCA	356
Hu.DMD.Exon44.25.006	TTTCTCAACAGATCTGTCAAATCGC	357
Hu.DMD.Exon44.25.007	CCATTTCTCAACAGATCTGTCAAAT	358
Hu.DMD.Exon44.25.008	ATAATGAAAACGCCGCCATTTCTCA	359
Hu.DMD.Exon44.25.009	AAATATCTTTATATCATAATGAAAA	360
Hu.DMD.Exon44.25.010	TGTTAGCCACTGATTAAATATCTTT	361
Hu.DMD.Exon44.25.011	AAACTGTTTCAGCTTCTGTTAGCCAC	362
Hu.DMD.Exon44.25.012	TTGTGTCTTTCTGAGAAACTGTTCA	363
Hu.DMD.Exon44.25.013	CCAATTCTCAGGAATTTGTGTCTTT	364
Hu.DMD.Exon44.25.014	GTATTTAGCATGTTCCCAATTCTCA	365
Hu.DMD.Exon44.25.015	CTTAAGATACCATTTGTATTTAGCA	366
Hu.DMD.Exon44.25.016	CTTACCTTAAGATACCATTTGTATT	367
Hu.DMD.Exon44.25.017	AAAGACTTACCTTAAGATACCATTT	368
Hu.DMD.Exon44.25.018	AAATCAAAGACTTACCTTAAGATAC	369
Hu.DMD.Exon44.25.019	AAAACAAATCAAAGACTTACCTTAA	370
Hu.DMD.Exon44.25.020	TCGAAAAAACAATCAAAGACTTAC	371
Hu.DMD.Exon45.25.001	CTGTAAGATACCAAAAAGGCAAAAC	372
Hu.DMD.Exon45.25.002	CCTGTAAGATACCAAAAAGGCAAAA	373
Hu.DMD.Exon45.25.002.2	AGTTCCTGTAAGATACCAAAAAGGC	374
Hu.DMD.Exon45.25.003	GAGTTCCTGTAAGATACCAAAAAGG	375
Hu.DMD.Exon45.25.003.2	CCTGGAGTTCCTGTAAGATACCAAA	376
Hu.DMD.Exon45.25.004	TCCTGGAGTTCCTGTAAGATACCAA	377
Hu.DMD.Exon45.25.004.2	GCCATCCTGGAGTTCCTGTAAGATA	378
Hu.DMD.Exon45.25.005	TGCCATCCTGGAGTTCCTGTAAGAT	379
Hu.DMD.Exon45.25.005.2	CCAATGCCATCCTGGAGTTCCTGTA	380
Hu.DMD.Exon45.25.006	CCCAATGCCATCCTGGAGTTCCTGT	381
Hu.DMD.Exon45.25.006.2	GCTGCCCAATGCCATCCTGGAGTTC	382
Hu.DMD.Exon45.25.007	CGCTGCCCAATGCCATCCTGGAGTT	383
Hu.DMD.Exon45.25.008	AACAGTTTGCCGCTGCCCAATGCCA	384
Hu.DMD.Exon45.25.008.2	CTGACAACAGTTTGCCGCTGCCCAA	385
Hu.DMD.Exon45.25.009	GTTGCATTCAATGTTCTGACAACAG	386
Hu.DMD.Exon45.25.010	GCTGAATTATTTCTTCCCCAGTTGC	387
Hu.DMD.Exon45.25.010.2	ATTATTTCTTCCCCAGTTGCATTCA	388
Hu.DMD.Exon45.25.011	GGCATCTGTTTTTGAGGATTGCTGA	389
Hu.DMD.Exon45.25.011.2	TTTGAGGATTGCTGAATTATTTCTT	390

Hu.DMD.Exon45.25.012	AATTTTTCCTGTAGAATACTGGCAT	391
Hu.DMD.Exon45.25.012.2	ATACTGGCATCTGTTTTTGAGGATT	392
Hu.DMD.Exon45.25.013	ACCGCAGATTCAGGCTTCCCAATTT	393
Hu.DMD.Exon45.25.013.2	AATTTTTCCTGTAGAATACTGGCAT	394
Hu.DMD.Exon45.25.014	CTGTTTGCAGACCTCCTGCCACCGC	395
Hu.DMD.Exon45.25.014.2	AGATTCAGGCTTCCCAATTTTTCCT	396
Hu.DMD.Exon45.25.015	CTCTTTTTTCTGTCTGACAGCTGTT	397
Hu.DMD.Exon45.25.015.2	ACCTCCTGCCACCGCAGATTCAGGC	398
Hu.DMD.Exon45.25.016	CCTACCTCTTTTTTCTGTCTGACAG	399
Hu.DMD.Exon45.25.016.2	GACAGCTGTTTGCAGACCTCCTGCC	400
Hu.DMD.Exon45.25.017	GTCGCCCTACCTCTTTTTTCTGTCT	401
Hu.DMD.Exon45.25.018	GATCTGTCGCCCTACCTCTTTTTTC	402
Hu.DMD.Exon45.25.019	TATTAGATCTGTCGCCCTACCTCTT	403
Hu.DMD.Exon45.25.020	ATTCCTATTAGATCTGTCGCCCTAC	404
Hu.DMD.Exon45.20.001	AGATACCAAAAAGGCCAAAAC	405
Hu.DMD.Exon45.20.002	AAGATACCAAAAAGGCCAAA	406
Hu.DMD.Exon45.20.003	CCTGTAAGATACCAAAAAGG	407
Hu.DMD.Exon45.20.004	GAGTTCCTGTAAGATACCAA	408
Hu.DMD.Exon45.20.005	TCCTGGAGTTCCTGTAAGAT	409
Hu.DMD.Exon45.20.006	TGCCATCCTGGAGTTCCTGT	410
Hu.DMD.Exon45.20.007	CCCAATGCCATCCTGGAGTT	411
Hu.DMD.Exon45.20.008	CGCTGCCCAATGCCATCCTG	412
Hu.DMD.Exon45.20.009	CTGACAACAGTTTGCCGCTG	413
Hu.DMD.Exon45.20.010	GTTGCATTCAATGTTCTGAC	414
Hu.DMD.Exon45.20.011	ATTATTTCTTCCCCAGTTGC	415
Hu.DMD.Exon45.20.012	TTTGAGGATTGCTGAATTAT	416
Hu.DMD.Exon45.20.013	ATACTGGCATCTGTTTTTGA	417
Hu.DMD.Exon45.20.014	AATTTTTCCTGTAGAATACT	418
Hu.DMD.Exon45.20.015	AGATTCAGGCTTCCCAATTT	419
Hu.DMD.Exon45.20.016	ACCTCCTGCCACCGCAGATT	420
Hu.DMD.Exon45.20.017	GACAGCTGTTTGCAGACCTC	421
Hu.DMD.Exon45.20.018	CTCTTTTTTCTGTCTGACAG	422
Hu.DMD.Exon45.20.019	CCTACCTCTTTTTTCTGTCT	423
Hu.DMD.Exon45.20.020	GTCGCCCTACCTCTTTTTTC	424
Hu.DMD.Exon45.20.021	GATCTGTCGCCCTACCTCTT	425
Hu.DMD.Exon45.20.022	TATTAGATCTGTCGCCCTAC	426
Hu.DMD.Exon45.20.023	ATTCCTATTAGATCTGTCGC	427
Hu.DMD.Exon46.25.001	GGGGGATTTGAGAAAATAAAATTAC	428
Hu.DMD.Exon46.25.002	ATTTGAGAAAATAAAATTACCTTGA	429
Hu.DMD.Exon46.25.002.2	CTAGCCTGGAGAAAGAATAAAA	430
Hu.DMD.Exon46.25.003	AGAAAATAAAATTACCTTGACTTGC	431
Hu.DMD.Exon46.25.003.2	TTCTTCTAGCCTGGAGAAAGAAGAA	432
Hu.DMD.Exon46.25.004	ATAAAATTACCTTGACTTGCTCAAG	433
Hu.DMD.Exon46.25.004.2	TTTTGTTCTTCTAGCCTGGAGAAAG	434
Hu.DMD.Exon46.25.005	ATTACCTTGACTTGCTCAAGCTTTT	435

Hu.DMD.Exon46.25.005.2	TATTCTTTTGTTCCTTCTAGCCTGGA	436
Hu.DMD.Exon46.25.006	CTTGACTTGCTCAAGCTTTTCTTTT	437
Hu.DMD.Exon46.25.006.2	CAAGATATTCTTTTGTTCCTTCTAGC	438
Hu.DMD.Exon46.25.007	CTTTTAGTTGCTGCTCTTTTCCAGG	439
Hu.DMD.Exon46.25.008	CCAGGTTCAAGTGGGATACTAGCAA	440
Hu.DMD.Exon46.25.008.2	ATCTCTTTGAAATTCTGACAAGATA	441
Hu.DMD.Exon46.25.009	AGCAATGTTATCTGCTTCCTCCAAC	442
Hu.DMD.Exon46.25.009.2	AACAAATTCATTAAATCTCTTTGA	443
Hu.DMD.Exon46.25.010	CCAACCATAAAACAAATTCATTAA	444
Hu.DMD.Exon46.25.010.2	TTCCTCCAACCATAAAACAAATTCA	445
Hu.DMD.Exon46.25.011	TTTAAATCTCTTTGAAATTCTGACA	446
Hu.DMD.Exon46.25.012	TGACAAGATATTCTTTTGTTCCTTCT	447
Hu.DMD.Exon46.25.012.2	TTCAAGTGGGATACTAGCAATGTTA	448
Hu.DMD.Exon46.25.013	AGATATTCTTTTGTTCCTTCTAGCCT	449
Hu.DMD.Exon46.25.013.2	CTGCTCTTTTCCAGGTTCAAGTGGG	450
Hu.DMD.Exon46.25.014	TTCTTTTGTTCCTTCTAGCCTGGAGA	451
Hu.DMD.Exon46.25.014.2	CTTTTCTTTTAGTTGCTGCTCTTTT	452
Hu.DMD.Exon46.25.015	TTGTTCTTCTAGCCTGGAGAAAGAA	453
Hu.DMD.Exon46.25.016	CTTCTAGCCTGGAGAAAGAAGAATA	454
Hu.DMD.Exon46.25.017	AGCCTGGAGAAAGAAGAATAAAATT	455
Hu.DMD.Exon46.25.018	CTGGAGAAAGAAGAATAAAATTGTT	456
Hu.DMD.Exon46.20.001	GAAAGAAGAATAAAATTGTT	457
Hu.DMD.Exon46.20.002	GGAGAAAGAAGAATAAAATT	458
Hu.DMD.Exon46.20.003	AGCCTGGAGAAAGAAGAATA	459
Hu.DMD.Exon46.20.004	CTTCTAGCCTGGAGAAAGAA	460
Hu.DMD.Exon46.20.005	TTGTTCTTCTAGCCTGGAGA	461
Hu.DMD.Exon46.20.006	TTCTTTTGTTCCTTCTAGCCT	462
Hu.DMD.Exon46.20.007	TGACAAGATATTCTTTTGTTC	463
Hu.DMD.Exon46.20.008	ATCTCTTTGAAATTCTGACA	464
Hu.DMD.Exon46.20.009	AACAAATTCATTAAATCTC	465
Hu.DMD.Exon46.20.010	TTCCTCCAACCATAAAACAA	466
Hu.DMD.Exon46.20.011	AGCAATGTTATCTGCTTCCT	467
Hu.DMD.Exon46.20.012	TTCAAGTGGGATACTAGCAA	468
Hu.DMD.Exon46.20.013	CTGCTCTTTTCCAGGTTCAA	469
Hu.DMD.Exon46.20.014	CTTTTCTTTTAGTTGCTGCT	470
Hu.DMD.Exon46.20.015	CTTGACTTGCTCAAGCTTTT	471
Hu.DMD.Exon46.20.016	ATTACCTTGACTTGCTCAAG	472
Hu.DMD.Exon46.20.017	ATAAAATTACCTTGACTTGC	473
Hu.DMD.Exon46.20.018	AGAAAATAAAATTACCTTGA	474
Hu.DMD.Exon46.20.019	ATTTGAGAAAATAAAATTAC	475
Hu.DMD.Exon46.20.020	GGGGGATTTGAGAAAATAAA	476
Hu.DMD.Exon47.25.001	CTGAAACAGACAAATGCAACAACGT	477
Hu.DMD.Exon47.25.002	AGTAACTGAAACAGACAAATGCAAC	478
Hu.DMD.Exon47.25.003	CCACCAGTAACTGAAACAGACAAAT	479
Hu.DMD.Exon47.25.004	CTCTTCCACCAGTAACTGAAACAGA	480

Hu.DMD.Exon47.25.005	GGCAACTCTTCCACCAGTAACTGAA	481
Hu.DMD.Exon47.25.006	GCAGGGGCAACTCTTCCACCAGTAA	482
Hu.DMD.Exon47.25.007	CTGGCGCAGGGGGCAACTCTTCCACC	483
Hu.DMD.Exon47.25.008	TTTAATTGTTTGAGAATTCCCTGGC	484
Hu.DMD.Exon47.25.008.2	TTGTTTGAGAATTCCCTGGCGCAGG	485
Hu.DMD.Exon47.25.009	GCACGGGTCCTCCAGTTTCATTTAA	486
Hu.DMD.Exon47.25.009.2	TCCAGTTTCATTTAATTGTTTGAGA	487
Hu.DMD.Exon47.25.010	GCTTATGGGAGCACTTACAAGCACG	488
Hu.DMD.Exon47.25.010.2	TACAAGCACGGGTCCTCCAGTTTCA	489
Hu.DMD.Exon47.25.011	AGTTTATCTTGCTCTTCTGGGCTTA	490
Hu.DMD.Exon47.25.012	TCTGCTTGAGCTTATTTTCAAGTTT	491
Hu.DMD.Exon47.25.012.2	ATCTTGCTCTTCTGGGCTTATGGGA	492
Hu.DMD.Exon47.25.013	CTTTATCCACTGGAGATTTGTCTGC	493
Hu.DMD.Exon47.25.013.2	CTTATTTTCAAGTTTATCTTGCTCT	494
Hu.DMD.Exon47.25.014	CTAACCTTTATCCACTGGAGATTTG	495
Hu.DMD.Exon47.25.014.2	ATTTGTCTGCTTGAGCTTATTTTCA	496
Hu.DMD.Exon47.25.015	AATGTCTAACCTTTATCCACTGGAG	497
Hu.DMD.Exon47.25.016	TGGTTAATGTCTAACCTTTATCCAC	498
Hu.DMD.Exon47.25.017	AGAGATGGTTAATGTCTAACCTTTA	499
Hu.DMD.Exon47.25.018	ACGGAAGAGATGGTTAATGTCTAAC	500
Hu.DMD.Exon47.20.001	ACAGACAAATGCAACAACGT	501
Hu.DMD.Exon47.20.002	CTGAAACAGACAAATGCAAC	502
Hu.DMD.Exon47.20.003	AGTAACTGAAACAGACAAAT	503
Hu.DMD.Exon47.20.004	CCACCAGTAACTGAAACAGA	504
Hu.DMD.Exon47.20.005	CTCTTCCACCAGTAACTGAA	505
Hu.DMD.Exon47.20.006	GGCAACTCTTCCACCAGTAA	506
Hu.DMD.Exon47.20.007	CTGGCGCAGGGGGCAACTCTT	507
Hu.DMD.Exon47.20.008	TTGTTTGAGAATTCCCTGGC	508
Hu.DMD.Exon47.20.009	TCCAGTTTCATTTAATTGTT	509
Hu.DMD.Exon47.20.010	TACAAGCACGGGTCCTCCAG	510
Hu.DMD.Exon47.20.011	GCTTATGGGAGCACTTACAA	511
Hu.DMD.Exon47.20.012	ATCTTGCTCTTCTGGGCTTA	512
Hu.DMD.Exon47.20.013	CTTATTTTCAAGTTTATCTT	513
Hu.DMD.Exon47.20.014	ATTTGTCTGCTTGAGCTTAT	514
Hu.DMD.Exon47.20.015	CTTTATCCACTGGAGATTTG	515
Hu.DMD.Exon47.20.016	CTAACCTTTATCCACTGGAG	516
Hu.DMD.Exon47.20.017	AATGTCTAACCTTTATCCAC	517
Hu.DMD.Exon47.20.018	TGGTTAATGTCTAACCTTTA	518
Hu.DMD.Exon47.20.019	AGAGATGGTTAATGTCTAAC	519
Hu.DMD.Exon47.20.020	ACGGAAGAGATGGTTAATGT	520
Hu.DMD.Exon48.25.001	CTGAAAGGAAAATACATTTTAAAAA	521
Hu.DMD.Exon48.25.002	CCTGAAAGGAAAATACATTTTAAAAA	522
Hu.DMD.Exon48.25.002.2	GAAACCTGAAAGGAAAATACATTTT	523
Hu.DMD.Exon48.25.003	GGAAACCTGAAAGGAAAATACATTT	524
Hu.DMD.Exon48.25.003.2	CTCTGGAAACCTGAAAGGAAAATAC	525

Hu.DMD.Exon48.25.004	GCTCTGGAAACCTGAAAGGAAAATA	526
Hu.DMD.Exon48.25.004.2	TAAAGCTCTGGAAACCTGAAAGGAA	527
Hu.DMD.Exon48.25.005	GTAAAGCTCTGGAAACCTGAAAGGA	528
Hu.DMD.Exon48.25.005.2	TCAGGTAAAGCTCTGGAAACCTGAA	529
Hu.DMD.Exon48.25.006	CTCAGGTAAAGCTCTGGAAACCTGA	530
Hu.DMD.Exon48.25.006.2	GTTTCTCAGGTAAAGCTCTGGAAAC	531
Hu.DMD.Exon48.25.007	TGTTTCTCAGGTAAAGCTCTGGAAA	532
Hu.DMD.Exon48.25.007.2	AATTTCTCCTTGTTTCTCAGGTAAA	533
Hu.DMD.Exon48.25.008	TTTGAGCTTCAATTTCTCCTTGTTT	534
Hu.DMD.Exon48.25.008	TTTTATTTGAGCTTCAATTTCTCCT	535
Hu.DMD.Exon48.25.009	AAGCTGCCCAAGGTCTTTTATTTGA	536
Hu.DMD.Exon48.25.010	AGGTCTTCAAGCTTTTTTTTCAAGCT	537
Hu.DMD.Exon48.25.010.2	TTCAAGCTTTTTTTTCAAGCTGCCCA	538
Hu.DMD.Exon48.25.011	GATGATTTAACTGCTCTTCAAGGTC	539
Hu.DMD.Exon48.25.011.2	CTGCTCTTCAAGGTCTTCAAGCTTT	540
Hu.DMD.Exon48.25.012	AGGAGATAACCACAGCAGCAGATGA	541
Hu.DMD.Exon48.25.012.2	CAGCAGATGATTTAACTGCTCTTCA	542
Hu.DMD.Exon48.25.013	ATTTCCAACCTGATTCCTAATAGGAG	543
Hu.DMD.Exon48.25.014	CTTGGTTTGGTTGGTTATAAATTTC	544
Hu.DMD.Exon48.25.014.2	CAACTGATTCCTAATAGGAGATAAC	545
Hu.DMD.Exon48.25.015	CTTAACGTCAAATGGTCCTTCTTGG	546
Hu.DMD.Exon48.25.015.2	TTGGTTATAAATTTCCAACCTGATTC	547
Hu.DMD.Exon48.25.016	CCTACCTTAACGTCAAATGGTCCTT	548
Hu.DMD.Exon48.25.016.2	TCCTTCTTGGTTTGGTTGGTTATAA	549
Hu.DMD.Exon48.25.017	AGTTCCCTACCTTAACGTCAAATGG	550
Hu.DMD.Exon48.25.018	CAAAAAGTTCCCTACCTTAACGTCA	551
Hu.DMD.Exon48.25.019	TAAAGCAAAAAGTTCCCTACCTTAA	552
Hu.DMD.Exon48.25.020	ATATTTAAAGCAAAAAGTTCCCTAC	553
Hu.DMD.Exon48.20.001	AGGAAAATACATTTTAAAAA	554
Hu.DMD.Exon48.20.002	AAGGAAAATACATTTTAAAAA	555
Hu.DMD.Exon48.20.003	CCTGAAAGGAAAATACATTT	556
Hu.DMD.Exon48.20.004	GGAAACCTGAAAGGAAAATA	557
Hu.DMD.Exon48.20.005	GCTCTGGAAACCTGAAAGGA	558
Hu.DMD.Exon48.20.006	GTAAAGCTCTGGAAACCTGA	559
Hu.DMD.Exon48.20.007	CTCAGGTAAAGCTCTGGAAA	560
Hu.DMD.Exon48.20.008	AATTTCTCCTTGTTTCTCAG	561
Hu.DMD.Exon48.20.009	TTTTATTTGAGCTTCAATTT	562
Hu.DMD.Exon48.20.010	AAGCTGCCCAAGGTCTTTTA	563
Hu.DMD.Exon48.20.011	TTCAAGCTTTTTTTTCAAGCT	564
Hu.DMD.Exon48.20.012	CTGCTCTTCAAGGTCTTCAA	565
Hu.DMD.Exon48.20.013	CAGCAGATGATTTAACTGCT	566
Hu.DMD.Exon48.20.014	AGGAGATAACCACAGCAGCA	567
Hu.DMD.Exon48.20.015	CAACTGATTCCTAATAGGAG	568
Hu.DMD.Exon48.20.016	TTGGTTATAAATTTCCAACCT	569
Hu.DMD.Exon48.20.017	TCCTTCTTGGTTTGGTTGGT	570

Hu.DMD.Exon48.20.018	CTTAACGTCAAATGGTCCTT	571
Hu.DMD.Exon48.20.019	CCTACCTTAACGTCAAATGG	572
Hu.DMD.Exon48.20.020	AGTTCCCTACCTTAACGTCA	573
Hu.DMD.Exon48.20.021	CAAAAAGTTCCCTACCTTAA	574
Hu.DMD.Exon48.20.022	TAAAGCAAAAAGTTCCCTAC	575
Hu.DMD.Exon48.20.023	ATATTTAAAGCAAAAAGTTC	576
Hu.DMD.Exon49.25.001	CTGGGGAAAAGAACCCATATAGTGC	577
Hu.DMD.Exon49.25.002	TCCTGGGGAAAAGAACCCATATAGT	578
Hu.DMD.Exon49.25.002.2	GTTTCCTGGGGAAAAGAACCCATAT	579
Hu.DMD.Exon49.25.003	CAGTTTCCTGGGGAAAAGAACCCAT	580
Hu.DMD.Exon49.25.003.2	TTTCAGTTTCCTGGGGAAAAGAACC	581
Hu.DMD.Exon49.25.004	TATTTTCAGTTTCCTGGGGAAAAGAA	582
Hu.DMD.Exon49.25.004.2	TGCTATTTTCAGTTTCCTGGGGAAAA	583
Hu.DMD.Exon49.25.005	ACTGCTATTTTCAGTTTCCTGGGGAA	584
Hu.DMD.Exon49.25.005.2	TGAACTGCTATTTTCAGTTTCCTGGG	585
Hu.DMD.Exon49.25.006	CTTGAAGCTGCTATTTTCAGTTTCCTG	586
Hu.DMD.Exon49.25.006.2	TAGCTTGAAGCTGCTATTTTCAGTTTC	587
Hu.DMD.Exon49.25.007	TTTAGCTTGAAGCTGCTATTTTCAGTT	588
Hu.DMD.Exon49.25.008	TTCCACATCCGGTTGTTTAGCTTGA	589
Hu.DMD.Exon49.25.009	TGCCCTTTAGACAAAATCTCTTCCA	590
Hu.DMD.Exon49.25.009.2	TTTAGACAAAATCTCTTCCACATCC	591
Hu.DMD.Exon49.25.010	GTTTTTCCTTGTACAAATGCTGCCC	592
Hu.DMD.Exon49.25.010.2	GTACAAATGCTGCCCTTTAGACAAA	593
Hu.DMD.Exon49.25.011	CTTCACTGGCTGAGTGGCTGGTTTTT	594
Hu.DMD.Exon49.25.011.2	GGCTGGTTTTTTCCTTGTACAAATGC	595
Hu.DMD.Exon49.25.012	ATTACCTTCACTGGCTGAGTGGCTG	596
Hu.DMD.Exon49.25.013	GCTTCATTACCTTCACTGGCTGAGT	597
Hu.DMD.Exon49.25.014	AGGTTGCTTCATTACCTTCACTGGC	598
Hu.DMD.Exon49.25.015	GCTAGAGGTTGCTTCATTACCTTCA	599
Hu.DMD.Exon49.25.016	ATATTGCTAGAGGTTGCTTCATTAC	600
Hu.DMD.Exon49.20.001	GAAAAGAACCCATATAGTGC	601
Hu.DMD.Exon49.20.002	GGGAAAAGAACCCATATAGT	602
Hu.DMD.Exon49.20.003	TCCTGGGGAAAAGAACCCAT	603
Hu.DMD.Exon49.20.004	CAGTTTCCTGGGGAAAAGAA	604
Hu.DMD.Exon49.20.005	TATTTTCAGTTTCCTGGGGAA	605
Hu.DMD.Exon49.20.006	ACTGCTATTTTCAGTTTCCTG	606
Hu.DMD.Exon49.20.007	CTTGAAGCTGCTATTTTCAGTT	607
Hu.DMD.Exon49.20.008	TTTAGCTTGAAGCTGCTATTT	608
Hu.DMD.Exon49.20.009	TTCCACATCCGGTTGTTTAG	609
Hu.DMD.Exon49.20.010	TTTAGACAAAATCTCTTCCA	610
Hu.DMD.Exon49.20.011	GTACAAATGCTGCCCTTTAG	611
Hu.DMD.Exon49.20.012	GGCTGGTTTTTTCCTTGTACA	612
Hu.DMD.Exon49.20.013	CTTCACTGGCTGAGTGGCTG	613
Hu.DMD.Exon49.20.014	ATTACCTTCACTGGCTGAGT	614
Hu.DMD.Exon49.20.015	GCTTCATTACCTTCACTGGC	615

Hu.DMD.Exon49.20.016	AGGTTGCTTCATTACCTTCA	616
Hu.DMD.Exon49.20.017	GCTAGAGGTTGCTTCATTAC	617
Hu.DMD.Exon49.20.018	ATATTGCTAGAGGTTGCTTC	618
Hu.DMD.Exon50.25.001	CTTTAACAGAAAAGCATAACATTA	619
Hu.DMD.Exon50.25.002	TCCTCTTTAACAGAAAAGCATAAC	620
Hu.DMD.Exon50.25.002.2	TTCCTCTTTAACAGAAAAGCATACA	621
Hu.DMD.Exon50.25.003	TAACTTCCTCTTTAACAGAAAAGCA	622
Hu.DMD.Exon50.25.003.2	CTAACTTCCTCTTTAACAGAAAAGC	623
Hu.DMD.Exon50.25.004	TCTTCTAACTTCCTCTTTAACAGAA	624
Hu.DMD.Exon50.25.004.2	ATCTTCTAACTTCCTCTTTAACAGA	625
Hu.DMD.Exon50.25.005	TCAGATCTTCTAACTTCCTCTTTAA	626
Hu.DMD.Exon50.25.005.2	CTCAGATCTTCTAACTTCCTCTTTA	627
Hu.DMD.Exon50.25.006	AGAGCTCAGATCTTCTAACTTCCTC	628
Hu.DMD.Exon50.25.006.2 NG-08-0731	CAGAGCTCAGATCTTCTAACTTCCT	629
Hu.DMD.Exon50.25.007	CACTCAGAGCTCAGATCTTCTACT	630
Hu.DMD.Exon50.25.007.2	CCTTCCACTCAGAGCTCAGATCTTC	631
Hu.DMD.Exon50.25.008	GTAAACGGTTTACCGCCTTCCACTC	632
Hu.DMD.Exon50.25.009	CTTTGCCCTCAGCTCTTGAAGTAAA	633
Hu.DMD.Exon50.25.009.2	CCCTCAGCTCTTGAAGTAAACGGTT	634
Hu.DMD.Exon50.25.010	CCAGGAGCTAGGTCAGGCTGCTTTG	635
Hu.DMD.Exon50.25.010.2	GGTCAGGCTGCTTTGCCCTCAGCTC	636
Hu.DMD.Exon50.25.011	AGGCTCCAATAGTGGTCAGTCCAGG	637
Hu.DMD.Exon50.25.011.2	TCAGTCCAGGAGCTAGGTCAGGCTG	638
Hu.DMD.Exon50.25.012 AVI-5038	CTTACAGGCTCCAATAGTGGTCAGT	639
Hu.DMD.Exon50.25.013	GTATACTTACAGGCTCCAATAGTGG	640
Hu.DMD.Exon50.25.014	ATCCAGTATACTTACAGGCTCCAAT	641
Hu.DMD.Exon50.25.015 NG-08-0741	ATGGGATCCAGTATACTTACAGGCT	642
Hu.DMD.Exon50.25.016 NG-08-0742	AGAGAATGGGATCCAGTATACTTAC	643
Hu.DMD.Exon50.20.001	ACAGAAAAGCATAACATTA	644
Hu.DMD.Exon50.20.002	TTTAACAGAAAAGCATAAC	645
Hu.DMD.Exon50.20.003	TCCTCTTTAACAGAAAAGCA	646
Hu.DMD.Exon50.20.004	TAACTTCCTCTTTAACAGAA	647
Hu.DMD.Exon50.20.005	TCTTCTAACTTCCTCTTTAA	648
Hu.DMD.Exon50.20.006	TCAGATCTTCTAACTTCCTC	649
Hu.DMD.Exon50.20.007	CCTTCCACTCAGAGCTCAGA	650
Hu.DMD.Exon50.20.008	GTAAACGGTTTACCGCCTTC	651
Hu.DMD.Exon50.20.009	CCCTCAGCTCTTGAAGTAAA	652
Hu.DMD.Exon50.20.010	GGTCAGGCTGCTTTGCCCTC	653
Hu.DMD.Exon50.20.011	TCAGTCCAGGAGCTAGGTCA	654
Hu.DMD.Exon50.20.012	AGGCTCCAATAGTGGTCAGT	655
Hu.DMD.Exon50.20.013	CTTACAGGCTCCAATAGTGG	656
Hu.DMD.Exon50.20.014	GTATACTTACAGGCTCCAAT	657

Hu.DMD.Exon50.20.015	ATCCAGTATACTTACAGGCT	658
Hu.DMD.Exon50.20.016	ATGGGATCCAGTATACTTAC	659
Hu.DMD.Exon50.20.017	AGAGAATGGGATCCAGTATA	660
Hu.DMD.Exon51.25.001-44	CTAAAATATTTTGGGTTTTTGCAAAA	661
Hu.DMD.Exon51.25.002-45	GCTAAAATATTTTGGGTTTTTGCAAA	662
Hu.DMD.Exon51.25.002.2-46	TAGGAGCTAAAATATTTTGGGTTTTT	663
Hu.DMD.Exon51.25.003	AGTAGGAGCTAAAATATTTTGGGTT	664
Hu.DMD.Exon51.25.003.2	TGAGTAGGAGCTAAAATATTTTGGG	665
Hu.DMD.Exon51.25.004	CTGAGTAGGAGCTAAAATATTTTGGG	666
Hu.DMD.Exon51.25.004.2	CAGTCTGAGTAGGAGCTAAAATATT	667
Hu.DMD.Exon51.25.005	ACAGTCTGAGTAGGAGCTAAAATATT	668
Hu.DMD.Exon51.25.005.2	GAGTAACAGTCTGAGTAGGAGCTAAA	669
Hu.DMD.Exon51.25.006	CAGAGTAACAGTCTGAGTAGGAGCT	670
Hu.DMD.Exon51.25.006.2	CACCAGAGTAACAGTCTGAGTAGGAG	671
Hu.DMD.Exon51.25.007	GTCACCAGAGTAACAGTCTGAGTAG	672
Hu.DMD.Exon51.25.007.2	AACCACAGGTTGTGTCACCAGAGTAA	673
Hu.DMD.Exon51.25.008	GTTGTGTCACCAGAGTAACAGTCTG	674
Hu.DMD.Exon51.25.009	TGGCAGTTTCCTTAGTAACCACAGGT	675
Hu.DMD.Exon51.25.010	ATTTCTAGTTTGGAGATGGCAGTTTC	676
Hu.DMD.Exon51.25.010.2	GGAAGATGGCATTTCCTAGTTTGGAG	677
Hu.DMD.Exon51.25.011	CATCAAGGAAGATGGCATTTCCTAGTT	678
Hu.DMD.Exon51.25.011.2	GAGCAGGTACCTCCAACATCAAGGAA	679
Hu.DMD.Exon51.25.012	ATCTGCCAGAGCAGGTACCTCCAAC	680
Hu.DMD.Exon51.25.013	AAGTTCTGTCCAAGCCCGGTTGAAAT	681
Hu.DMD.Exon51.25.013.2	CGGTTGAAATCTGCCAGAGCAGGTAC	682
Hu.DMD.Exon51.25.014	GAGAAAGCCAGTCGGTAAGTTCTGTC	683
Hu.DMD.Exon51.25.014.2	GTCGGTAAGTTCTGTCCAAGCCCGG	684
Hu.DMD.Exon51.25.015	ATAACTTGATCAAGCAGAGAAAGCCA	685
Hu.DMD.Exon51.25.015.2	AAGCAGAGAAAGCCAGTCGGTAAGT	686
Hu.DMD.Exon51.25.016	CACCCTCTGTGATTTTATAACTTGAT	687
Hu.DMD.Exon51.25.017	CAAGGTCACCCACCATCACCTCTGT	688
Hu.DMD.Exon51.25.017.2	CATCACCTCTGTGATTTTATAACT	689
Hu.DMD.Exon51.25.018	CTTCTGCTTGATGATCATCTCGTTGA	690
Hu.DMD.Exon51.25.019	CCTTCTGCTTGATGATCATCTCGTTG	691
Hu.DMD.Exon51.25.019.2	ATCTCGTTGATATCCTCAAGGTCACC	692
Hu.DMD.Exon51.25.020	TCATACCTTCTGCTTGATGATCATCT	693
Hu.DMD.Exon51.25.020.2	TCATTTTTTCTCATACCTTCTGCTTG	694
Hu.DMD.Exon51.25.021	TTTTCTCATACCTTCTGCTTGATGAT	695
Hu.DMD.Exon51.25.022	TTTTATCATTTTTTCTCATACCTTCT	696
Hu.DMD.Exon51.25.023	CCAACCTTTATCATTTTTTCTCATAC	697
Hu.DMD.Exon51.20.001	ATATTTTGGGTTTTTGCAAA	698
Hu.DMD.Exon51.20.002	AAAATATTTTGGGTTTTTGCA	699
Hu.DMD.Exon51.20.003	GAGCTAAAATATTTTGGGTT	700

Hu.DMD.Exon51.20.004	AGTAGGAGCTAAAATATTTT	701
Hu.DMD.Exon51.20.005	GTCTGAGTAGGAGCTAAAAT	702
Hu.DMD.Exon51.20.006	TAACAGTCTGAGTAGGAGCT	703
Hu.DMD.Exon51.20.007	CAGAGTAACAGTCTGAGTAG	704
Hu.DMD.Exon51.20.008	CACAGGTTGTGTCACCAGAG	705
Hu.DMD.Exon51.20.009	AGTTTCCTTAGTAACCACAG	706
Hu.DMD.Exon51.20.010	TAGTTTGGAGATGGCAGTTT	707
Hu.DMD.Exon51.20.011	GGAAGATGGCATTCTAGTT	708
Hu.DMD.Exon51.20.012	TACCTCCAACATCAAGGAAG	709
Hu.DMD.Exon51.20.013	ATCTGCCAGAGCAGGTACCT	710
Hu.DMD.Exon51.20.014	CCAAGCCCGGTTGAAATCTG	711
Hu.DMD.Exon51.20.015	GTCGGTAAGTTCTGTCCAAG	712
Hu.DMD.Exon51.20.016	AAGCAGAGAAAGCCAGTCGG	713
Hu.DMD.Exon51.20.017	TTTTATAACTTGATCAAGCA	714
Hu.DMD.Exon51.20.018	CATCACCTCTGTGATTTTA	715
Hu.DMD.Exon51.20.019	CTCAAGGTCACCCACCATCA	716
Hu.DMD.Exon51.20.020	CATCTCGTTGATATCCTCAA	717
Hu.DMD.Exon51.20.021	CTTCTGCTTGATGATCATCT	718
Hu.DMD.Exon51.20.022	CATACCTTCTGCTTGATGAT	719
Hu.DMD.Exon51.20.023	TTTCTCATACCTTCTGCTTG	720
Hu.DMD.Exon51.20.024	CATTTTTTCTCATACCTTCT	721
Hu.DMD.Exon51.20.025	TTTATCATTTTTTCTCATAC	722
Hu.DMD.Exon51.20.026	CAACTTTTATCATTTTTTCT	723
Hu.DMD.Exon52.25.001	CTGTAAGAACAAATATCCCTTAGTA	724
Hu.DMD.Exon52.25.002	TGCCTGTAAGAACAAATATCCCTTA	725
Hu.DMD.Exon52.25.002.2	GTTGCCTGTAAGAACAAATATCCCT	726
Hu.DMD.Exon52.25.003	ATTGTTGCCTGTAAGAACAAATATC	727
Hu.DMD.Exon52.25.003.2	GCATTGTTGCCTGTAAGAACAAATA	728
Hu.DMD.Exon52.25.004	CCTGCATTGTTGCCTGTAAGAACAA	729
Hu.DMD.Exon52.25.004.2	ATCCTGCATTGTTGCCTGTAAGAAC	730
Hu.DMD.Exon52.25.005	CAAATCCTGCATTGTTGCCTGTAAG	731
Hu.DMD.Exon52.25.005.2	TCCAAATCCTGCATTGTTGCCTGTA	732
Hu.DMD.Exon52.25.006	TGTTCCAAATCCTGCATTGTTGCCT	733
Hu.DMD.Exon52.25.006.2	TCTGTTCCAAATCCTGCATTGTTGC	734
Hu.DMD.Exon52.25.007	AACTGGGGACGCCTCTGTTCCAAAT	735
Hu.DMD.Exon52.25.007.2	GCCTCTGTTCCAAATCCTGCATTGT	736
Hu.DMD.Exon52.25.008	CAGCGGTAATGAGTTCTTCCAACTG	737
Hu.DMD.Exon52.25.008.2	CTTCCAACCTGGGGACGCCTCTGTTC	738
Hu.DMD.Exon52.25.009	CTTGTTTTTCAAATTTTGGGCAGCG	739
Hu.DMD.Exon52.25.010	CTAGCCTCTTGATTGCTGGTCTTGT	740
Hu.DMD.Exon52.25.010.2	TTTTCAAATTTTGGGCAGCGGTAAT	741
Hu.DMD.Exon52.25.011	TTCGATCCGTAATGATTGTTCTAGC	742
Hu.DMD.Exon52.25.011.2	GATTGCTGGTCTTGTTTTTCAAATT	743
Hu.DMD.Exon52.25.012	CTTACTTCGATCCGTAATGATTGTT	744
Hu.DMD.Exon52.25.012.2	TTGTTCTAGCCTCTTGATTGCTGGT	745

Hu.DMD.Exon52.25.013	AAAAACTTACTTCGATCCGTAATGA	746
Hu.DMD.Exon52.25.014	TGTTAAAAAACTTACTTCGATCCGT	747
Hu.DMD.Exon52.25.015	ATGCTTGTTAAAAAACTTACTTCGA	748
Hu.DMD.Exon52.25.016	GTCCCATGCTTGTTAAAAAACTTAC	749
Hu.DMD.Exon52.20.001	AGAACAAATATCCCTTAGTA	750
Hu.DMD.Exon52.20.002	GTAAGAACAAATATCCCTTA	751
Hu.DMD.Exon52.20.003	TGCCTGTAAGAACAAATATC	752
Hu.DMD.Exon52.20.004	ATTGTTGCCTGTAAGAACAA	753
Hu.DMD.Exon52.20.005	CCTGCATTGTTGCCTGTAAG	754
Hu.DMD.Exon52.20.006	CAAATCCTGCATTGTTGCCT	755
Hu.DMD.Exon52.20.007	GCCTCTGTTCCAAATCCTGC	756
Hu.DMD.Exon52.20.008	CTTCCAACCTGGGGACGCCTC	757
Hu.DMD.Exon52.20.009	CAGCGGTAATGAGTTCTTCC	758
Hu.DMD.Exon52.20.010	TTTTCAAATTTTGGGCAGCG	759
Hu.DMD.Exon52.20.011	GATTGCTGGTCTTGTTTTTC	760
Hu.DMD.Exon52.20.012	TTGTTCTAGCCTCTTGATTG	761
Hu.DMD.Exon52.20.013	TTCGATCCGTAATGATTGTT	762
Hu.DMD.Exon52.20.014	CTTACTTCGATCCGTAATGA	763
Hu.DMD.Exon52.20.015	AAAAACTTACTTCGATCCGT	764
Hu.DMD.Exon52.20.016	TGTTAAAAAACTTACTTCGA	765
Hu.DMD.Exon52.20.017	ATGCTTGTTAAAAAACTTAC	766
Hu.DMD.Exon52.20.018	GTCCCATGCTTGTTAAAAAA	767
Hu.DMD.Exon53.25.001	CTAGAATAAAAGGAAAAATAAATAT	768
Hu.DMD.Exon53.25.002	AACTAGAATAAAAGGAAAAATAAAT	769
Hu.DMD.Exon53.25.002.2	TTCAACTAGAATAAAAGGAAAAATA	770
Hu.DMD.Exon53.25.003	CTTTCAACTAGAATAAAAGGAAAAA	771
Hu.DMD.Exon53.25.003.2	ATTCTTTCAACTAGAATAAAAGGAA	772
Hu.DMD.Exon53.25.004	GAATTCTTTCAACTAGAATAAAAGG	773
Hu.DMD.Exon53.25.004.2	TCTGAATTCTTTCAACTAGAATAAA	774
Hu.DMD.Exon53.25.005	ATTCTGAATTCTTTCAACTAGAATA	775
Hu.DMD.Exon53.25.005.2	CTGATTCTGAATTCTTTCAACTAGA	776
Hu.DMD.Exon53.25.006	CACTGATTCTGAATTCTTTCAACTA	777
Hu.DMD.Exon53.25.006.2	TCCCACCTGATTCTGAATTCTTTCAA	778
Hu.DMD.Exon53.25.007	CATCCCACCTGATTCTGAATTCTTTC	779
Hu.DMD.Exon53.25.008	TACTTCATCCCACCTGATTCTGAATT	780
Hu.DMD.Exon53.25.008.2	CTGAAGGTGTTCTTGTACTTCATCC	781
Hu.DMD.Exon53.25.009	CGGTTCTGAAGGTGTTCTTGTACT	782
Hu.DMD.Exon53.25.009.2	CTGTTGCCTCCGGTTCTGAAGGTGT	783
Hu.DMD.Exon53.25.010	TTTCATTCAACTGTTGCCTCCGGTT	784
Hu.DMD.Exon53.25.010.2	TAACATTTCATTCAACTGTTGCCTC	785
Hu.DMD.Exon53.25.011	TTGTGTTGAATCCTTTAACATTTC	786
Hu.DMD.Exon53.25.012	TCTTCCTTAGCTTCCAGCCATTGTG	787
Hu.DMD.Exon53.25.012.2	CTTAGCTTCCAGCCATTGTGTTGAA	788
Hu.DMD.Exon53.25.013	GTCCTAAGACCTGCTCAGCTTCTTC	789
Hu.DMD.Exon53.25.013.2	CTGCTCAGCTTCTTCCTTAGCTTCC	790

Hu.DMD.Exon53.25.014	CTCAAGCTTGGCTCTGGCCTGTCCT	791
Hu.DMD.Exon53.25.014.2	GGCCTGTCCTAAGACCTGCTCAGCT	792
Hu.DMD.Exon53.25.015	TAGGGACCCTCCTTCCATGACTCAA	793
Hu.DMD.Exon53.25.016	TTTGGATTGCATCTACTGTATAGGG	794
Hu.DMD.Exon53.25.016.2	ACCCTCCTTCCATGACTCAAGCTTG	795
Hu.DMD.Exon53.25.017	CTTGGTTTCTGTGATTTTCTTTTGG	796
Hu.DMD.Exon53.25.017.2	ATCTACTGTATAGGGACCCTCCTTC	797
Hu.DMD.Exon53.25.018	CTAACCTTGGTTTCTGTGATTTTCT	798
Hu.DMD.Exon53.25.018.2	TTTCTTTTGGATTGCATCTACTGTA	799
Hu.DMD.Exon53.25.019	TGATACTAACCTTGGTTTCTGTGAT	800
Hu.DMD.Exon53.25.020	ATCTTTGATACTAACCTTGGTTTCT	801
Hu.DMD.Exon53.25.021	AAGGTATCTTTGATACTAACCTTGG	802
Hu.DMD.Exon53.25.022	TTAAAAAGGTATCTTTGATACTAAC	803
Hu.DMD.Exon53.20.001	ATAAAAGGAAAAATAAATAT	804
Hu.DMD.Exon53.20.002	GAATAAAAGGAAAAATAAAT	805
Hu.DMD.Exon53.20.003	AACTAGAATAAAAGGAAAAA	806
Hu.DMD.Exon53.20.004	CTTTCAACTAGAATAAAAGG	807
Hu.DMD.Exon53.20.005	GAATTCTTTCAACTAGAATA	808
Hu.DMD.Exon53.20.006	ATTCTGAATTCTTTCAACTA	809
Hu.DMD.Exon53.20.007	TACTTCATCCCCTGATTCT	810
Hu.DMD.Exon53.20.008	CTGAAGGTGTTCTTGTACT	811
Hu.DMD.Exon53.20.009	CTGTTGCCTCCGGTTCTGAA	812
Hu.DMD.Exon53.20.010	TAACATTTCAATTCAACTGTT	813
Hu.DMD.Exon53.20.011	TTGTGTTGAATCCTTTAACA	814
Hu.DMD.Exon53.20.012	CTTAGCTTCCAGCCATTGTG	815
Hu.DMD.Exon53.20.013	CTGCTCAGCTTCTTCCTTAG	816
Hu.DMD.Exon53.20.014	GGCCTGTCCTAAGACCTGCT	817
Hu.DMD.Exon53.20.015	CTCAAGCTTGGCTCTGGCCT	818
Hu.DMD.Exon53.20.016	ACCCTCCTTCCATGACTCAA	819
Hu.DMD.Exon53.20.017	ATCTACTGTATAGGGACCCT	820
Hu.DMD.Exon53.20.018	TTTCTTTTGGATTGCATCTA	821
Hu.DMD.Exon53.20.019	CTTGGTTTCTGTGATTTTCT	822
Hu.DMD.Exon53.20.020	CTAACCTTGGTTTCTGTGAT	823
Hu.DMD.Exon53.20.021	TGATACTAACCTTGGTTTCT	824
Hu.DMD.Exon53.20.022	ATCTTTGATACTAACCTTGG	825
Hu.DMD.Exon53.20.023	AAGGTATCTTTGATACTAAC	826
Hu.DMD.Exon53.20.024	TTAAAAAGGTATCTTTGATA	827
Hu.DMD.Exon54.25.001	CTATAGATTTTTATGAGAAAGAGA	828
Hu.DMD.Exon54.25.002	AACTGCTATAGATTTTTATGAGAAA	829
Hu.DMD.Exon54.25.003	TGGCCAAGTCTATAGATTTTTATG	830
Hu.DMD.Exon54.25.004	GTCTTTGGCCAAGTCTATAGATTT	831
Hu.DMD.Exon54.25.005	CGGAGGTCTTTGGCCAAGTCTATA	832
Hu.DMD.Exon54.25.006	ACTGGCGGAGGTCTTTGGCCAAGTG	833
Hu.DMD.Exon54.25.007	TTTGTCTGCCACTGGCGGAGGTCTT	834
Hu.DMD.Exon54.25.008	AGTCATTTGCCACATCTACATTTGT	835

Hu.DMD.Exon54.25.008.2	TTTGCCACATCTACATTTGTCTGCC	836
Hu.DMD.Exon54.25.009	CCGGAGAAGTTTCAGGGCCAAGTCA	837
Hu.DMD.Exon54.25.010	GTATCATCTGCAGAATAATCCCGGA	838
Hu.DMD.Exon54.25.010.2	TAATCCCGGAGAAGTTTCAGGGCCA	839
Hu.DMD.Exon54.25.011	TTATCATGTGGACTTTTCTGGTATC	840
Hu.DMD.Exon54.25.012	AGAGGCATTGATATTCTCTGTTATC	841
Hu.DMD.Exon54.25.012.2	ATGTGGACTTTTCTGGTATCATCTG	842
Hu.DMD.Exon54.25.013	CTTTTATGAATGCTTCTCCAAGAGG	843
Hu.DMD.Exon54.25.013.2	ATATTCTCTGTTATCATGTGGACTT	844
Hu.DMD.Exon54.25.014	CATACCTTTTATGAATGCTTCTCCA	845
Hu.DMD.Exon54.25.014.2	CTCCAAGAGGCATTGATATTCTCTG	846
Hu.DMD.Exon54.25.015	TAATTCATACCTTTTATGAATGCTT	847
Hu.DMD.Exon54.25.015.2	CTTTTATGAATGCTTCTCCAAGAGG	848
Hu.DMD.Exon54.25.016	TAATGTAATTCATACCTTTTATGAA	849
Hu.DMD.Exon54.25.017	AGAAATAATGTAATTCATACCTTTT	850
Hu.DMD.Exon54.25.018	GTTTTAGAAATAATGTAATTCATAC	851
Hu.DMD.Exon54.20.001	GATTTTTATGAGAAAGAGA	852
Hu.DMD.Exon54.20.002	CTATAGATTTTTTATGAGAAA	853
Hu.DMD.Exon54.20.003	AACTGCTATAGATTTTTTATG	854
Hu.DMD.Exon54.20.004	TGGCCAACTGCTATAGATTT	855
Hu.DMD.Exon54.20.005	GTCTTTGGCCAACTGCTATA	856
Hu.DMD.Exon54.20.006	CGGAGGTCTTTGGCCAACTG	857
Hu.DMD.Exon54.20.007	TTTGTCTGCCACTGGCGGAG	858
Hu.DMD.Exon54.20.008	TTTGCCACATCTACATTTGT	859
Hu.DMD.Exon54.20.009	TTCAGGGCCAAGTCATTTGC	860
Hu.DMD.Exon54.20.010	TAATCCCGGAGAAGTTTCAG	861
Hu.DMD.Exon54.20.011	GTATCATCTGCAGAATAATC	862
Hu.DMD.Exon54.20.012	ATGTGGACTTTTCTGGTATC	863
Hu.DMD.Exon54.20.013	ATATTCTCTGTTATCATGTG	864
Hu.DMD.Exon54.20.014	CTCCAAGAGGCATTGATATT	865
Hu.DMD.Exon54.20.015	CTTTTATGAATGCTTCTCCA	866
Hu.DMD.Exon54.20.016	CATACCTTTTATGAATGCTT	867
Hu.DMD.Exon54.20.017	TAATTCATACCTTTTATGAA	868
Hu.DMD.Exon54.20.018	TAATGTAATTCATACCTTTT	869
Hu.DMD.Exon54.20.019	AGAAATAATGTAATTCATAC	870
Hu.DMD.Exon54.20.020	GTTTTAGAAATAATGTAATT	871
Hu.DMD.Exon55.25.001	CTGCAAAGGACCAAATGTTTCAGATG	872
Hu.DMD.Exon55.25.002	TCACCCTGCAAAGGACCAAATGTTC	873
Hu.DMD.Exon55.25.003	CTCACTCACCCTGCAAAGGACCAAA	874
Hu.DMD.Exon55.25.004	TCTCGCTCACTCACCCTGCAAAGGA	875
Hu.DMD.Exon55.25.005	CAGCCTCTCGCTCACTCACCCTGCA	876
Hu.DMD.Exon55.25.006	CAAAGCAGCCTCTCGCTCACTCACC	877
Hu.DMD.Exon55.25.007	TCTTCCAAAGCAGCCTCTCGCTCAC	878
Hu.DMD.Exon55.25.007.2	TCTATGAGTTTCTTCCAAAGCAGCC	879
Hu.DMD.Exon55.25.008	GTTGCAGTAATCTATGAGTTTCTTC	880

Hu.DMD.Exon55.25.008.2	GAAGTGTTCAGTAATCTATGAGTT	881
Hu.DMD.Exon55.25.009	TTCCAGGTCCAGGGGGAAGTGTTC	882
Hu.DMD.Exon55.25.010	GTAAGCCAGGCAAGAACTTTTCCA	883
Hu.DMD.Exon55.25.010.2	CCAGGCAAGAACTTTTCCAGGTCC	884
Hu.DMD.Exon55.25.011	TGGCAGTTGTTTCAGCTTCTGTAAG	885
Hu.DMD.Exon55.25.011.2	TTCAGCTTCTGTAAGCCAGGCAAGA	886
Hu.DMD.Exon55.25.012	GGTAGCATCCTGTAGGACATTGGCA	887
Hu.DMD.Exon55.25.012.2	GACATTGGCAGTTGTTTCAGCTTCT	888
Hu.DMD.Exon55.25.013	TCTAGGAGCCTTTCCTTACGGGTAG	889
Hu.DMD.Exon55.25.014	CTTTTACTCCCTTGGAGTCTTCTAG	890
Hu.DMD.Exon55.25.014.2	GAGCCTTTCCTTACGGGTAGCATCC	891
Hu.DMD.Exon55.25.015	TTGCCATTGTTTCATCAGCTCTTTT	892
Hu.DMD.Exon55.25.015.2	CTTGGAGTCTTCTAGGAGCCTTTCC	893
Hu.DMD.Exon55.25.016	CTTACTTGCCATTGTTTCATCAGCT	894
Hu.DMD.Exon55.25.016.2	CAGCTCTTTTACTCCCTTGGAGTCT	895
Hu.DMD.Exon55.25.017	CCTGACTTACTTGCCATTGTTTCAT	896
Hu.DMD.Exon55.25.018	AAATGCCTGACTTACTTGCCATTGT	897
Hu.DMD.Exon55.25.019	AGCGGAAATGCCTGACTTACTTGCC	898
Hu.DMD.Exon55.25.020	GCTAAAGCGGAAATGCCTGACTTAC	899
Hu.DMD.Exon55.20.001	AAGGACCAAATGTTTCAGATG	900
Hu.DMD.Exon55.20.002	CTGCAAAGGACCAAATGTTTC	901
Hu.DMD.Exon55.20.003	TCACCCTGCAAAGGACCAAA	902
Hu.DMD.Exon55.20.004	CTCACTCACCCTGCAAAGGA	903
Hu.DMD.Exon55.20.005	TCTCGCTCACTCACCCTGCA	904
Hu.DMD.Exon55.20.006	CAGCCTCTCGCTCACTCACC	905
Hu.DMD.Exon55.20.007	CAAAGCAGCCTCTCGCTCAC	906
Hu.DMD.Exon55.20.008	TCTATGAGTTTCTTCCAAAG	907
Hu.DMD.Exon55.20.009	GAAGTGTTCAGTAATCTAT	908
Hu.DMD.Exon55.20.010	TTCCAGGTCCAGGGGGAAGT	909
Hu.DMD.Exon55.20.011	CCAGGCAAGAACTTTTCCA	910
Hu.DMD.Exon55.20.012	TTCAGCTTCTGTAAGCCAGG	911
Hu.DMD.Exon55.20.013	GACATTGGCAGTTGTTTCAG	912
Hu.DMD.Exon55.20.014	GGTAGCATCCTGTAGGACAT	913
Hu.DMD.Exon55.20.015	GAGCCTTTCCTTACGGGTAG	914
Hu.DMD.Exon55.20.016	CTTGGAGTCTTCTAGGAGCC	915
Hu.DMD.Exon55.20.017	CAGCTCTTTTACTCCCTTGG	916
Hu.DMD.Exon55.20.018	TTGCCATTGTTTCATCAGCT	917
Hu.DMD.Exon55.20.019	CTTACTTGCCATTGTTTCAT	918
Hu.DMD.Exon55.20.020	CCTGACTTACTTGCCATTGT	919
Hu.DMD.Exon55.20.021	AAATGCCTGACTTACTTGCC	920
Hu.DMD.Exon55.20.022	AGCGGAAATGCCTGACTTAC	921
Hu.DMD.Exon55.20.023	GCTAAAGCGGAAATGCCTGA	922
H50A(+02+30)-AVI-5656	CCACTCAGAGCTCAGATCTTCTAACTTCC	923
H50D(+07-18)-AVI-5915	GGGATCCAGTATACTTACAGGCTCC	924
H50A(+07+33)	CTTCCACTCAGAGCTCAGATCTTCTAA	925

H51A(+61+90)-AVI-4657	ACATCAAGGAAGATGGCATTCTAGTTTGG	926
H51A(+66+95)-AVI-4658	CTCCAACATCAAGGAAGATGGCATTCTAG	927
H51A(+111+134)	TTCTGTCCAAGCCCGGTTGAAATC	928
H51A(+175+195)	CACCCACCATCACCTCYGTG	929
H51A(+199+220)	ATCATCTCGTTGATATCCTCAA	930
H51A(+66+90)	ACATCAAGGAAGATGGCATTCTAG	931
H51A(-01+25)	ACCAGAGTAACAGTCTGAGTAGGAGC	932
h51AON1	TCAAGGAAGATGGCATTCT	933
h51AON2	CCTCTGTGATTTTATAACTTGAT	934
H51D(+08-17)	ATCATTTTTTCTCATACCTTCTGCT	935
H51D(+16-07)	CTCATACCTTCTGCTTGATGATC	936
hAON#23	TGGCATTCTAGTTTGG	937
hAON#24	CCAGAGCAGGTACCTCCAACATC	938
H44A(+61+84)	TGTTTCAGCTTCTGTTAGCCACTGA	939
H44A(+85+104)	TTTGTGTCTTTCTGAGAAAC	940
h44AON1	CGCCGCCATTTCTCAACAG	941
H44A(-06+14)	ATCTGTCAAATCGCCTGCAG	942
H45A(+71+90)	TGTTTTTGAGGATTGCTGAA	943
h45AON1	GCTGAATTATTTCTTCCCC	944
h45AON5	GCCCAATGCCATCCTGG	945
H45A(-06+20)	CCAATGCCATCCTGGAGTTCCTGTAA	946
H53A(+39+69)	CATTCAACTGTTGCCTCCGGTTCTGAAGGTG	947
H53A(+23+47)	CTGAAGGTGTTCTTGTACTTCATCC	948
h53AON1	CTGTTGCCTCCGGTTCTG	949
H53A(-12+10)	ATTCTTTCAACTAGATAAAAAG	950
huEx45.30.66	GCCATCCTGGAGTTCCTGTAAGATACCAA	951
huEx45.30.71	CCAATGCCATCCTGGAGTTCCTGTAAGATA	952
huEx45.30.79	GCCGCTGCCCAATGCCATCCTGGAGTTCCT	953
huEx45.30.83	GTTTGCCGCTGCCCAATGCCATCCTGGAGT	954
huEx45.30.88	CAACAGTTTGCCGCTGCCCAATGCCATCCT	955
huEx45.30.92	CTGACAACAGTTTGCCGCTGCCCAATGCCA	956
huEx45.30.96	TGTTCTGACAACAGTTTGCCGCTGCCCAAT	957
huEx45.30.99	CAATGTTCTGACAACAGTTTGCCGCTGCCC	958
huEx45.30.103	CATTCAATGTTCTGACAACAGTTTGCCGCT	959
huEx45.30.120	TATTTCTTCCCCAGTTGCATTCAATGTTCT	960
huEx45.30.127	GCTGAATTATTTCTTCCCCAGTTGCATTCA	961
huEx45.30.132	GGATTGCTGAATTATTTCTTCCCCAGTTGC	962
huEx45.30.137	TTTGAGGATTGCTGAATTATTTCTTCCCCA	963
huEx53.30.84	GTACTIONCATCCCACTGATTCTGAATTCTTT	964
huEx53.30.88	TCTTGTACTTCATCCCACTGATTCTGAATT	965
huEx53.30.91	TGTTCTTGTACTTCATCCCACTGATTCTGA	966
huEx53.30.103	CGGTTCTGAAGGTGTTCTTGTACTTCATCC	967
huEx53.30.106	CTCCGGTTCTGAAGGTGTTCTTGTACTTCA	968
huEx53.30.109	TGCCTCCGGTTCTGAAGGTGTTCTTGTACT	969
huEx53.30.112	TGTTGCCTCCGGTTCTGAAGGTGTTCTTGT	970

huEx53.30.115	AACTGTTGCCTCCGGTTCTGAAGGTGTTCT	971
huEx53.30.118	TTCAACTGTTGCCTCCGGTTCTGAAGGTGT	972

[0415] Step 1: Antibody conjugation with maleimide-PEG-NHS followed by siRNA-DMD conjugates

[0416] Anti-dystrophin antibody is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker or maleimide-PEGxkDa-NHS (x = 1, 5, 10, 20) is added and rotated for 4 hours at room temperature. Unreacted maleimide-PEG is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS pH 7.4. The antibody-PEG-Mal conjugate is collected and transferred into a reaction vessel. Various siRNA conjugates are synthesized using sequences listed in **Tables 13-17**. siRNA-DMD conjugates (2 equivalents) is added at RT to the antibody-PEG-maleimide in PBS and rotated overnight. The reaction mixture is analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA is seen.

[0417] Step 2: Purification

[0418] The crude reaction mixture is purified by AKTA explorer FPLC using anion exchange chromatography. Fractions containing the antibody-PEG-DMD conjugate are pooled, concentrated and buffer exchanged with PBS, pH 7.4. Antibody siRNA conjugates with SMCC linker, PEG1kDa, PEG5kDa and PEG10kDa are separated based on the siRNA loading.

[0419] Step-3: Analysis of the purified conjugate

[0420] The isolated conjugate is characterized by either mass spec or SDS-PAGE. The purity of the conjugate is assessed by analytical HPLC using anion exchange chromatography.

[0421] The examples and embodiments described herein are for illustrative purposes only and various modifications or changes suggested to persons skilled in the art are to be included within the spirit and purview of this application and scope of the appended claims.

CLAIMS**WHAT IS CLAIMED IS:**

1. A method of treating a disease or disorder comprising:
 administering to a subject a polynucleic acid molecule conjugate; wherein the polynucleic acid molecule conjugate comprises a target cell binding moiety and a targeted pre-mRNA specific splice modulating polynucleic acid moiety;
 wherein the target cell binding moiety specifically binds to a targeted cell, and the targeted pre-mRNA specific splice modulating polynucleic acid moiety induces insertion, deletion, duplication, or alteration of a targeted pre-mRNA transcript in the targeted cell to induce a splicing event in the targeted pre-mRNA transcript to generate a mRNA transcript; and wherein the mRNA transcript encodes a protein that is modified when compared to the same protein in untreated target cells, thereby treating the disease or disorder in the subject.
2. The method of claim 1, wherein the splicing event is exon skipping.
3. The method of claim 1, wherein the splicing event is exon inclusion.
4. The method of claim 1, wherein the disease or disorder is further characterized by one or more mutations in the pre-mRNA.
5. The method of claim 1, wherein the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.
6. The method of claim 5, wherein the disease or disorder is muscular dystrophy.
7. The method of claim 5, wherein the disease or disorder is Duchenne muscular dystrophy.
8. The method of claim 1, wherein the splicing event is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of *DMD* gene.
9. The method of claim 1, wherein the splicing event is of exon 23 of *DMD* gene.
10. The method of claim 1, wherein the splicing event is of an exon of *PAH*, *MSTN*, or *K-Ras* gene.
11. The method of claim 1, wherein the polynucleic acid molecule conjugate comprises a structure of Formula (I):

$$A-X-B$$

Formula I

wherein,

A comprises a binding moiety;

B consists of a polynucleotide; and

X consists of a bond or first linker.

12. The method of claim 1, wherein the polynucleic acid molecule conjugate comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

X consists a bond or first linker; and

Y consists of a bond or second linker.

13. The method of claim 1, wherein the polynucleic acid molecule conjugate comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of a polynucleotide;

C consists of a polymer;

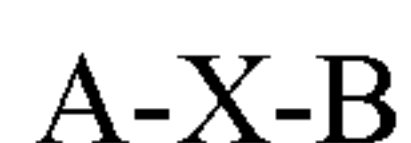
X consists of a bond or first linker; and

Y consists of a bond or second linker.

14. The method of claim 1, wherein the polynucleic acid molecule conjugate optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety.
15. The method of claim 14, wherein the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.
16. The method of claim 14, wherein the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), or a peptide nucleic acid (PNA).
17. The method of claim 14, wherein the at least one 2' modified nucleotide comprises a morpholino.
18. The method of claim 14, wherein the at least one inverted basic moiety is at least one terminus.

19. The method of claim 14, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage.
20. The method of claim 1, wherein the polynucleic acid molecule comprises at least from about 10 to about 30 nucleotides in length.
21. The method of claim 1, wherein the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification.
22. The method of claim 1, wherein the polynucleic acid molecule comprises a single strand.
23. The method of claim 1, wherein the polynucleic acid molecule comprises two or more strands.
24. The method of claim 1, wherein the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule.
25. The method of claim 24, wherein the second polynucleotide comprises at least one modification.
26. The method of claim 24, wherein the first polynucleotide and the second polynucleotide comprise RNA molecules.
27. The method of claim 24, wherein the first polynucleotide and the second polynucleotide comprise siRNA molecules.
28. The method of claim 11, wherein X is a bond.
29. The method of claim 12, wherein X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group.
30. The method of claim 13, wherein X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group.
31. The method of claim 11, wherein X is a C₁-C₆ alkyl group.
32. The method of claim 12, wherein X or Y is a C₁-C₆ alkyl group.
33. The method of claim 13, wherein X or Y is a C₁-C₆ alkyl group.
34. The method of claim 11, wherein the binding moiety is an antibody or binding fragment thereof.
35. The method of claim 12, wherein the binding moiety is an antibody or binding fragment thereof.
36. The method of claim 13, wherein the binding moiety is an antibody or binding fragment thereof.
37. The method of claim 12, wherein C is polyethylene glycol.
38. The method of claim 13, wherein C is polyethylene glycol.

39. The method of claim 12, wherein A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B.
40. The method of claim 12, wherein Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B.
41. The method of claim 12, further comprising D.
42. The method of claim 41, wherein D is conjugated to C or to A.
43. The method of claim 11, further comprising at least a second binding moiety A.
44. The method of claim 12, further comprising at least a second binding moiety A.
45. The method of claim 13, further comprising at least a second binding moiety A.
46. A method of inducing a splicing event in a targeted pre-mRNA transcript, comprising:
 - a) contacting a target cell with a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a target cell binding moiety and a targeted pre-mRNA splice modulating polynucleic acid moiety;
 - b) hybridizing the targeted pre-mRNA splice modulating polynucleic acid moiety to the targeted pre-mRNA transcript within the target cell to induce the splicing event in the targeted pre-mRNA transcript to produce a mRNA transcript; and
 - c) optionally, translating the mRNA transcript of step b) in the target cell to produce a protein.
47. The method of claim 46, wherein the splicing event is exon skipping.
48. The method of claim 46, wherein the splicing event is exon inclusion.
49. The method of claim 46, wherein the targeted pre-mRNA transcript induces a disease or disorder.
50. The method of claim 49, wherein the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.
51. The method of claim 46, wherein the polynucleic acid molecule conjugate:
 - a) comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of the polynucleotide; and

X consists of a bond or first linker;

- b) comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y is a bond or second linker; or

c) comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

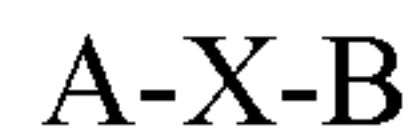
52. The method of claim 46, wherein the polynucleic acid molecule conjugate optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety.
53. The method of claim 52, wherein the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.
54. The method of claim 52, wherein the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), peptide nucleic acid (PNA).
55. The method of claim 52, wherein the at least one 2' modified nucleotide comprises a morpholino.
56. The method of claim 52, wherein the at least one inverted basic moiety is at least one terminus.
57. The method of claim 52, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage.
58. The method of any one of the claims 46-57, wherein the polynucleic acid molecule comprises at least from about 10 to about 30 nucleotides in length.

59. The method of any one of the claims 46-58, wherein the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification.
60. The method of any one of the claims 46-59, wherein the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications.
61. The method of any one of the claims 46-60, wherein X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group.
62. The method of any one of the claims 46-61, wherein X is a bond.
63. The method of any one of the claims 46-61, wherein X is a C₁-C₆ alkyl group.
64. The method of any one of the claims 46-63, wherein Y is a C₁-C₆ alkyl group.
65. The method of any one of the claims 46-63, wherein X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C₁-C₆ alkyl group.
66. The method of any one of the claims 46-63, wherein Y is a homobifunctional linker or a heterobifunctional linker.
67. The method of any one of the claims 46-66, wherein the binding moiety is an antibody or binding fragment thereof.
68. The method of any one of the claims 46-67, wherein C is polyethylene glycol.
69. The method of any one of the claims 46-68, wherein A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B.
70. The method of any one of the claims 46-69, wherein Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B.
71. The method of any one of the claims 46-70, wherein A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group.
72. The method of any one of the claims 46-71, further comprising D.
73. The method of claim 72, wherein D is conjugated to C or to A.
74. The method of any one of the claims 46-73, further comprising at least a second binding moiety A.
75. A polynucleic acid molecule conjugate composition comprising a target cell binding moiety and a targeted pre-mRNA specific splice modulating polynucleic acid moiety wherein the targeted pre-mRNA specific splice modulating polynucleic acid moiety comprises a

sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 54-972.

76. The composition of claim 75, wherein the polynucleic acid molecule conjugate:

a) comprises a structure of Formula (I):



Formula I

wherein,

A comprises a binding moiety;

B consists of the polynucleotide; and

X consists of a bond or first linker;

b) comprises a structure of Formula (II):



Formula II

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker; or

c) comprises a structure of Formula (III):



Formula III

wherein,

A comprises a binding moiety;

B consists of the polynucleotide;

C consists of a polymer;

X consists of a bond or first linker; and

Y consists of a bond or second linker.

77. The composition of any one of claim 75 or 76, wherein the pharmaceutical composition is formulated as a nanoparticle formulation.

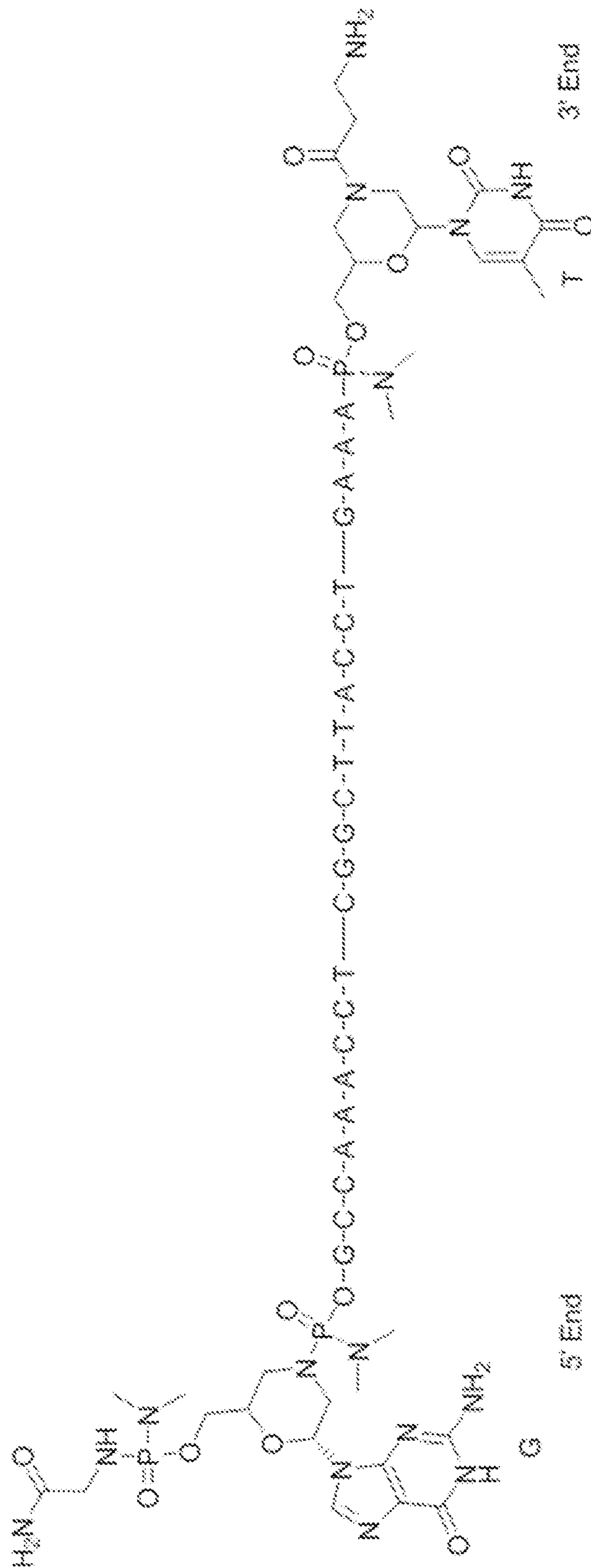


Fig. 1

2/40

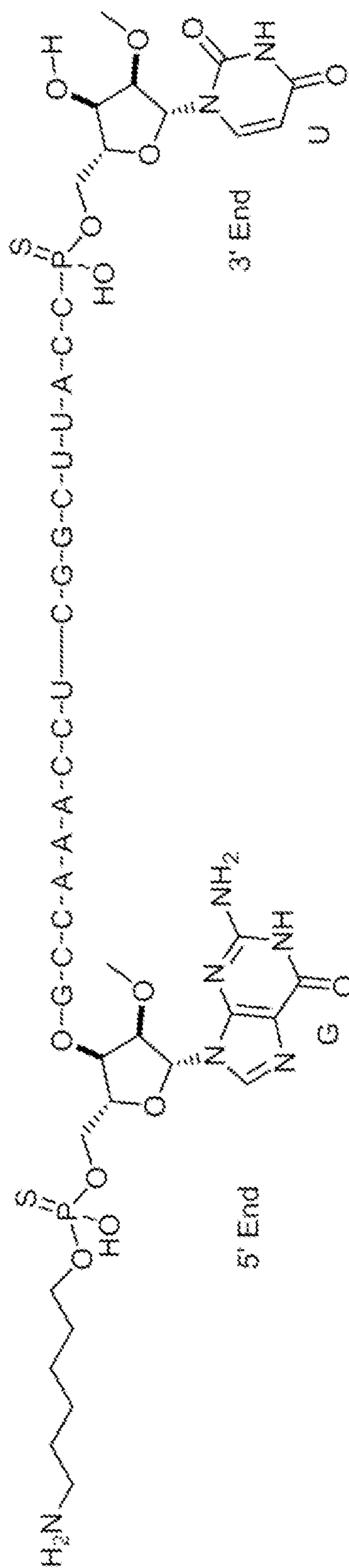


Fig. 2A

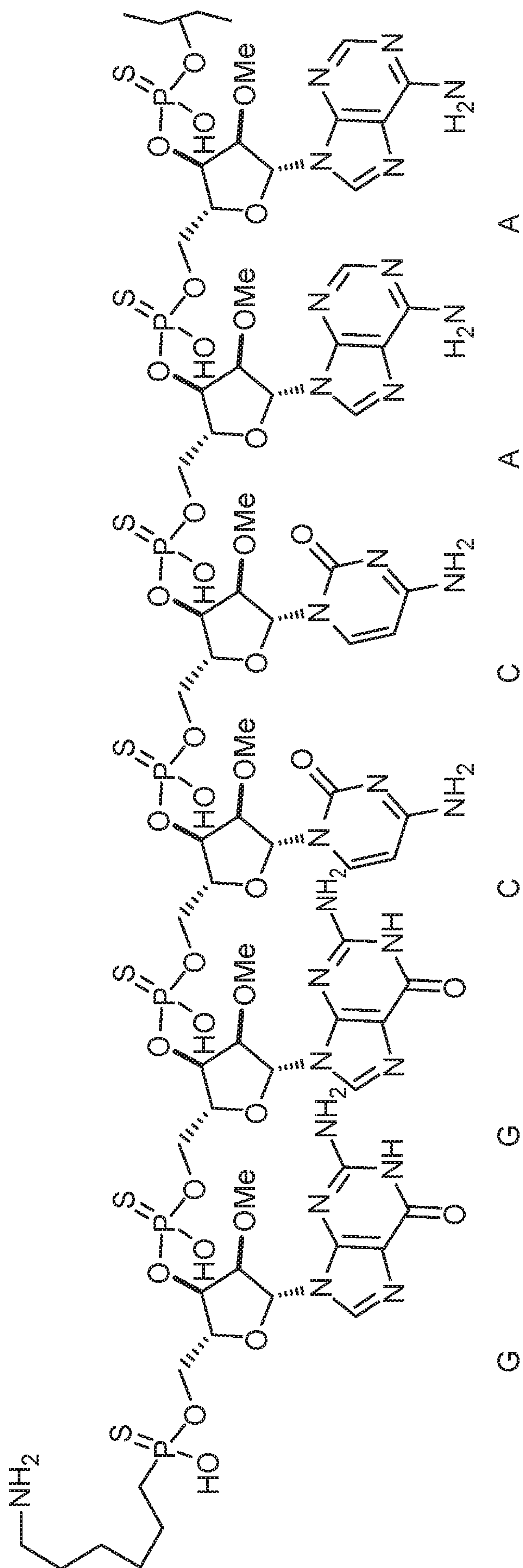
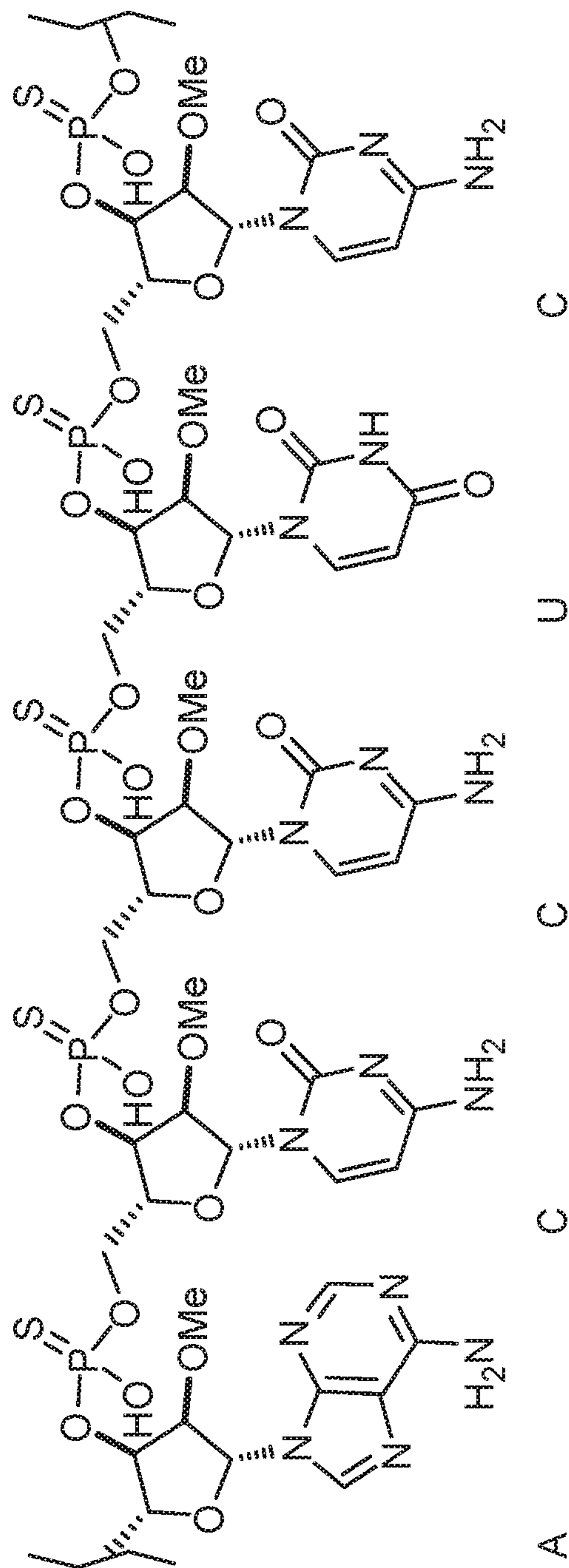


FIG. 2B



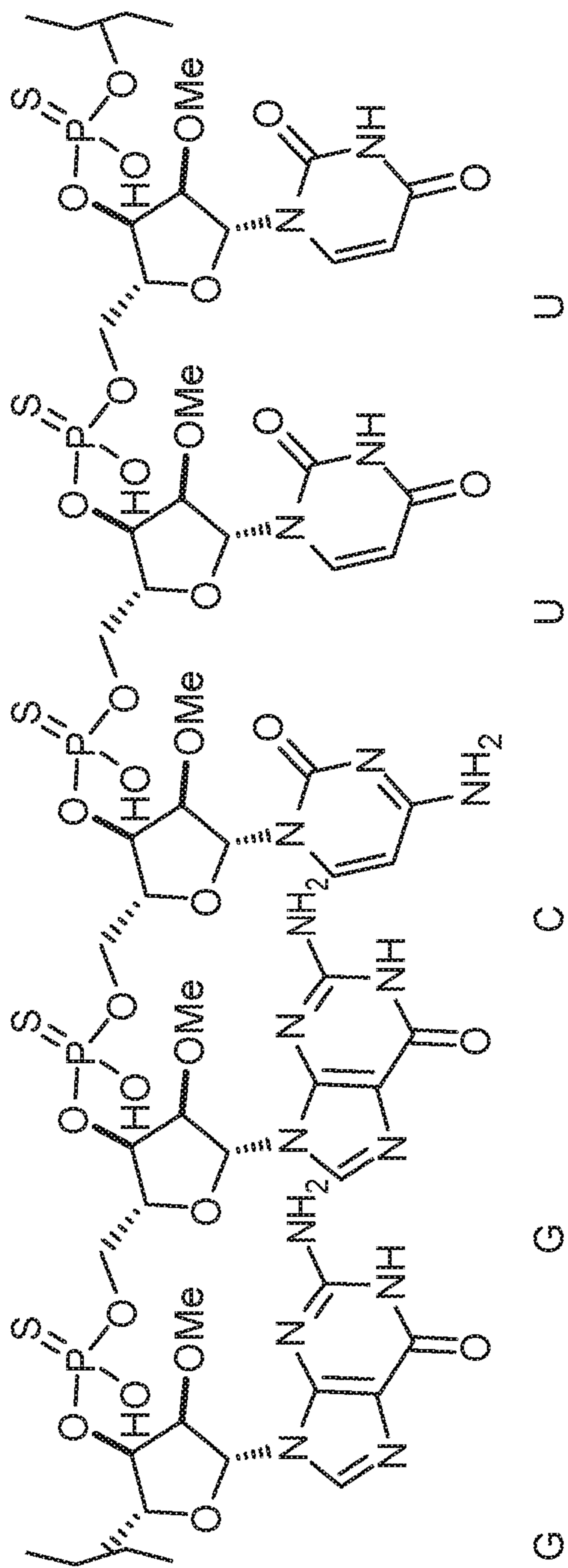


FIG. 2B (Cont.2)

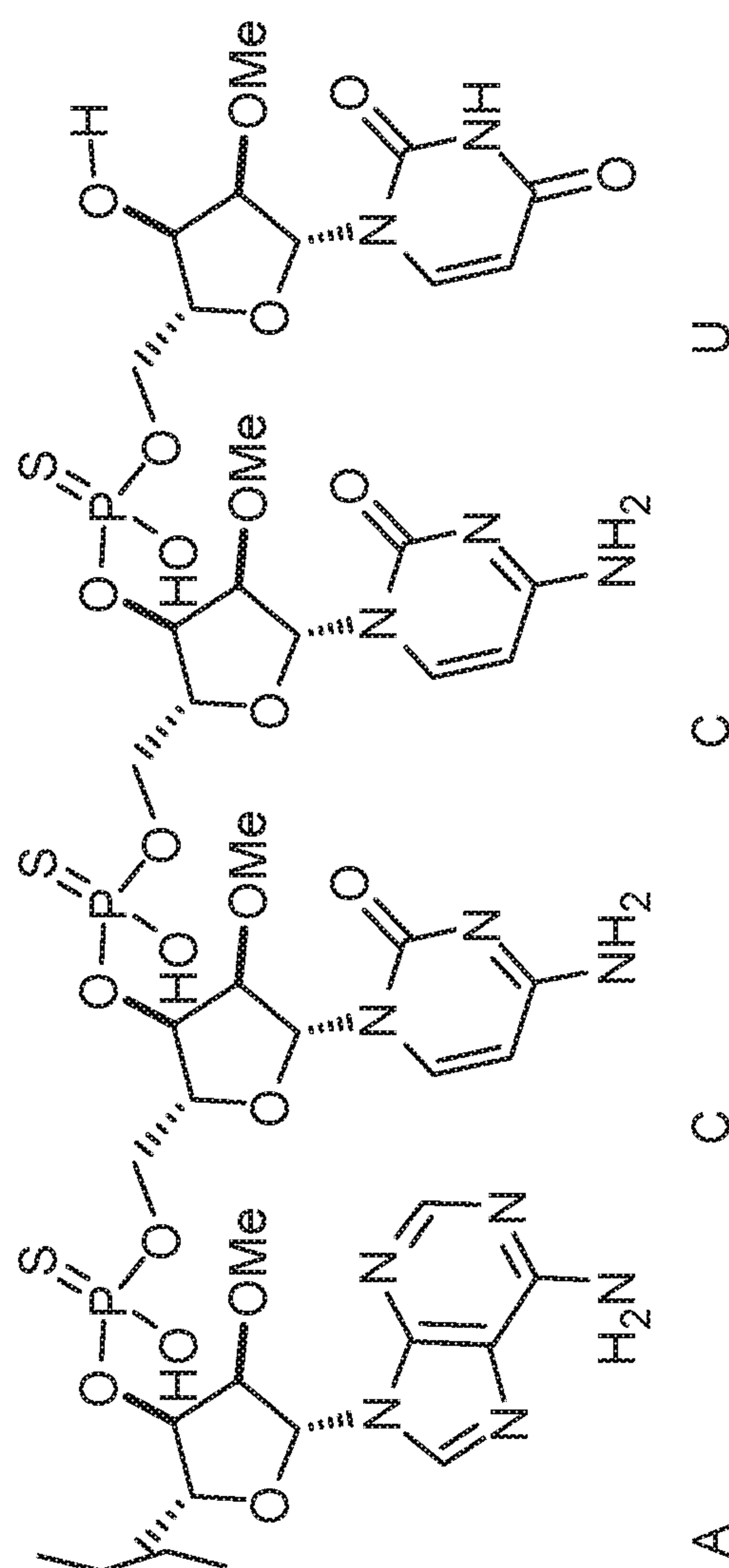


FIG. 2B (Cont.3)

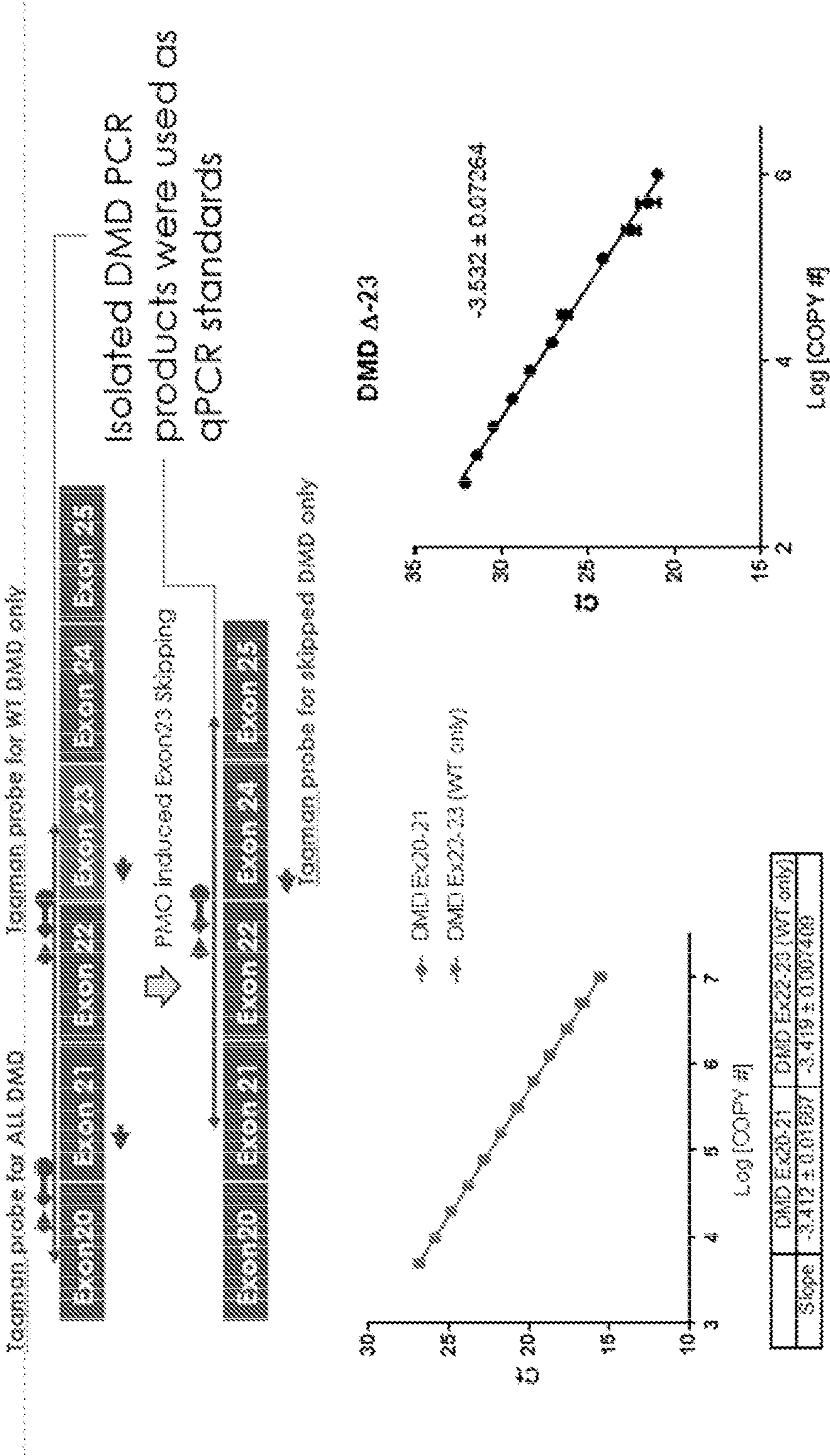


Fig. 3

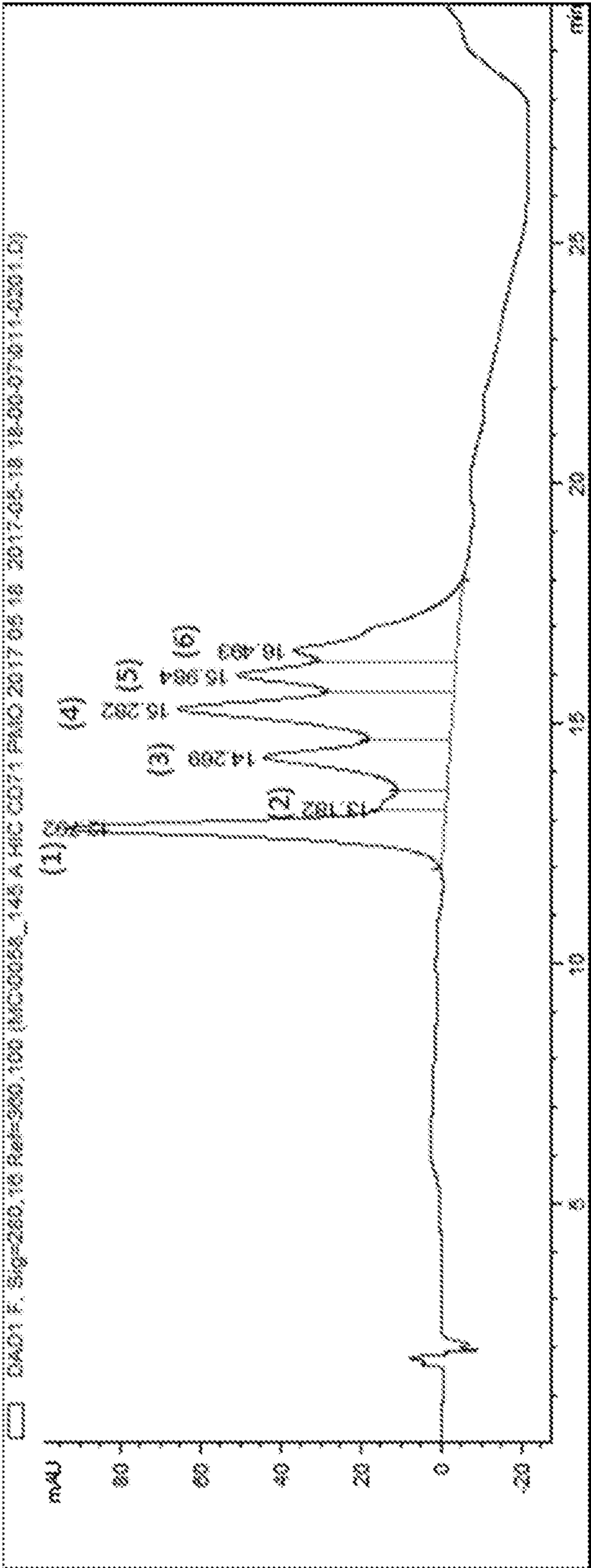


Fig. 4

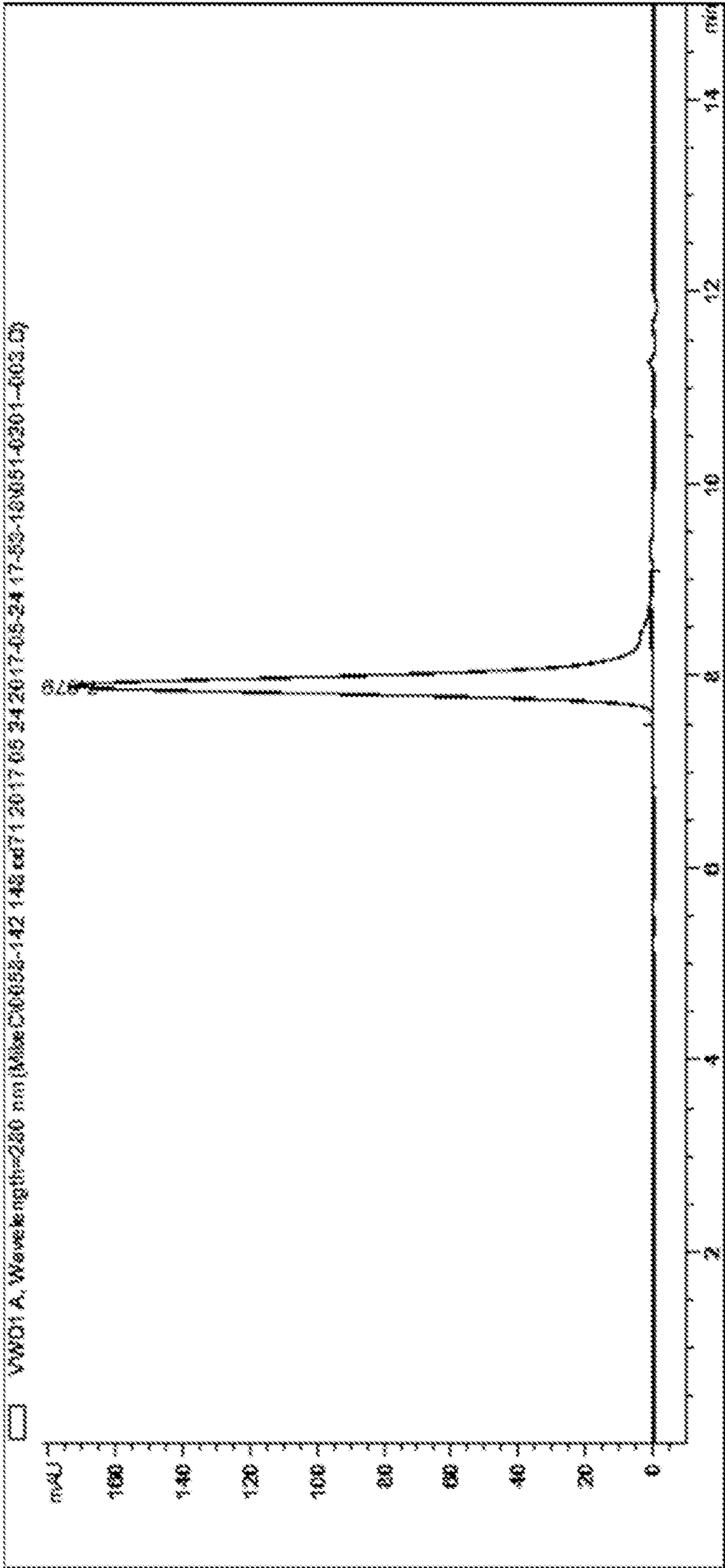


Fig. 5A

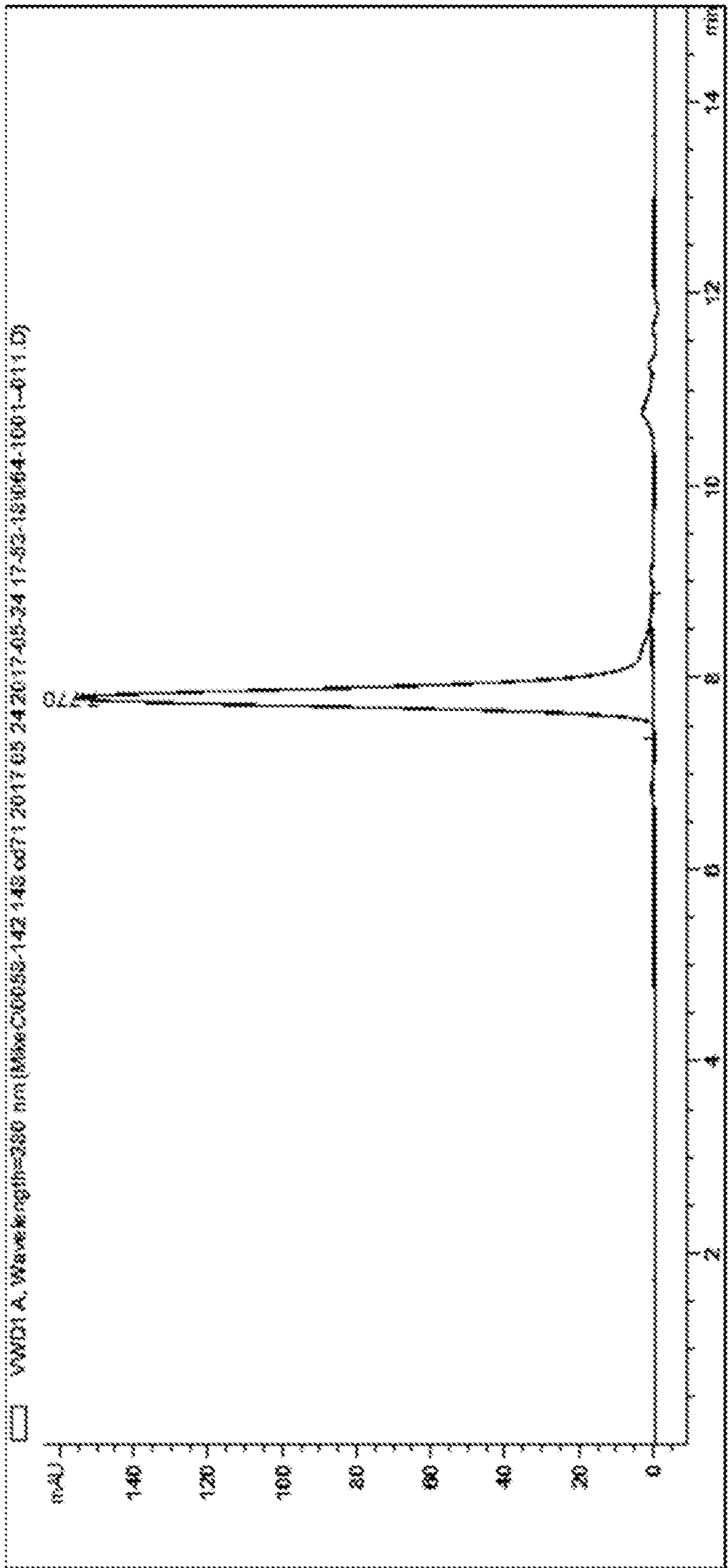


Fig. 5B

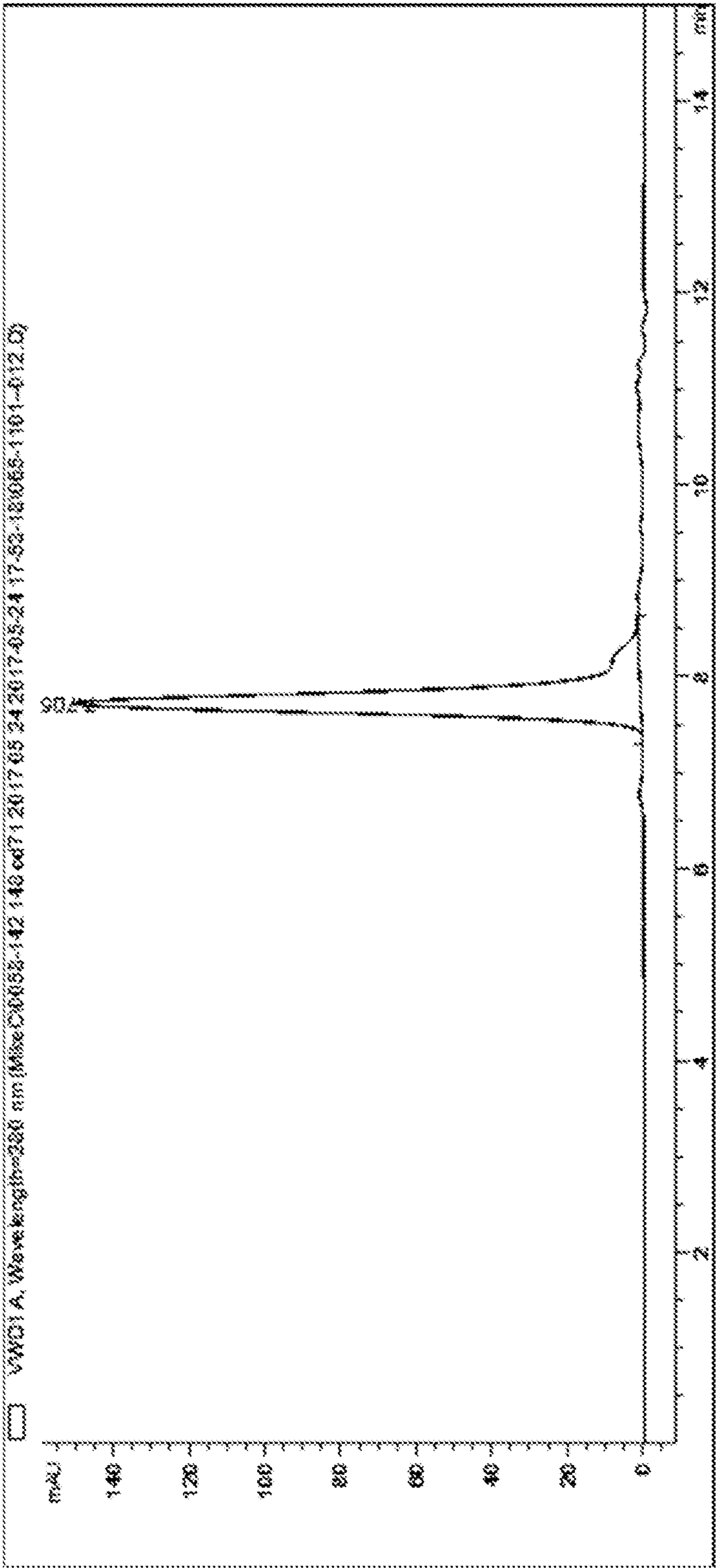


Fig. 5C

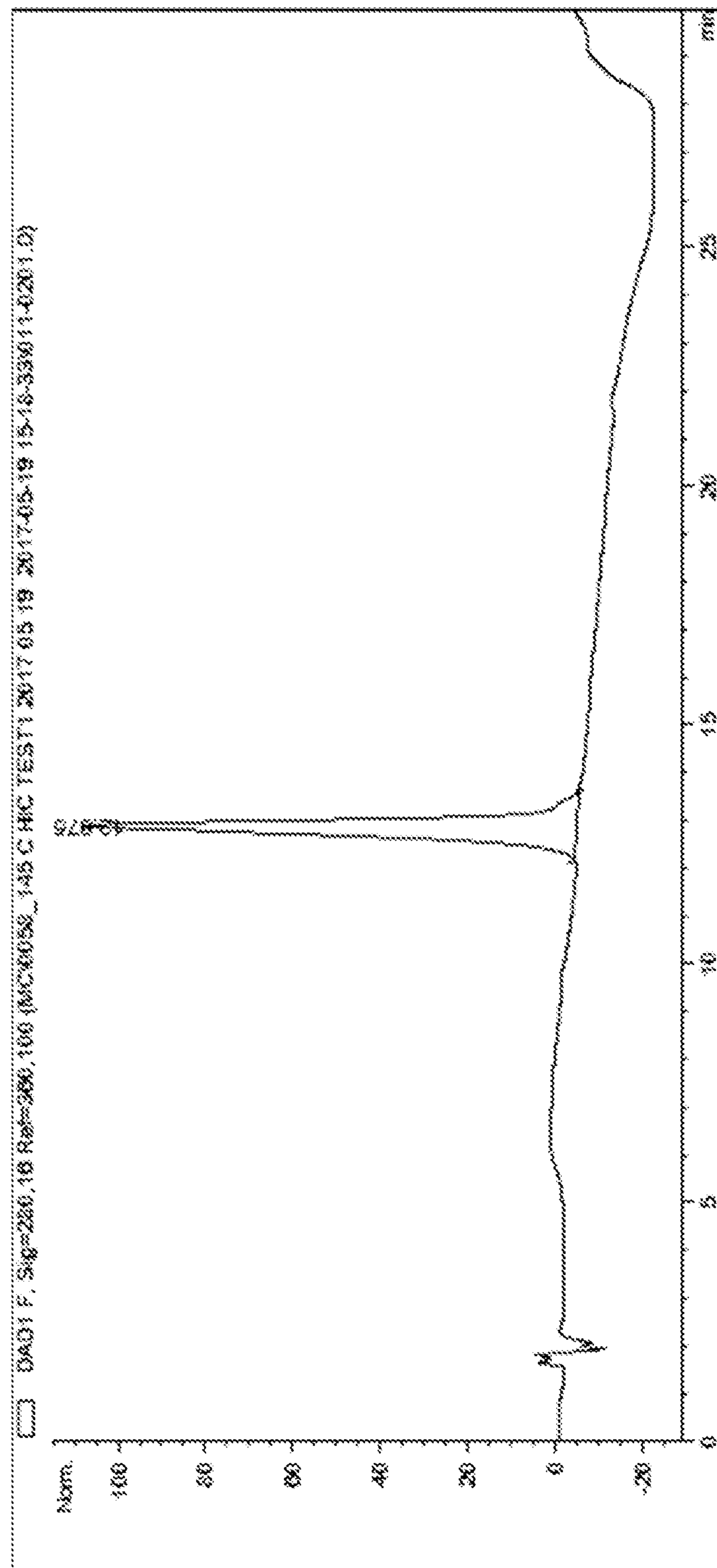


Fig. 6A

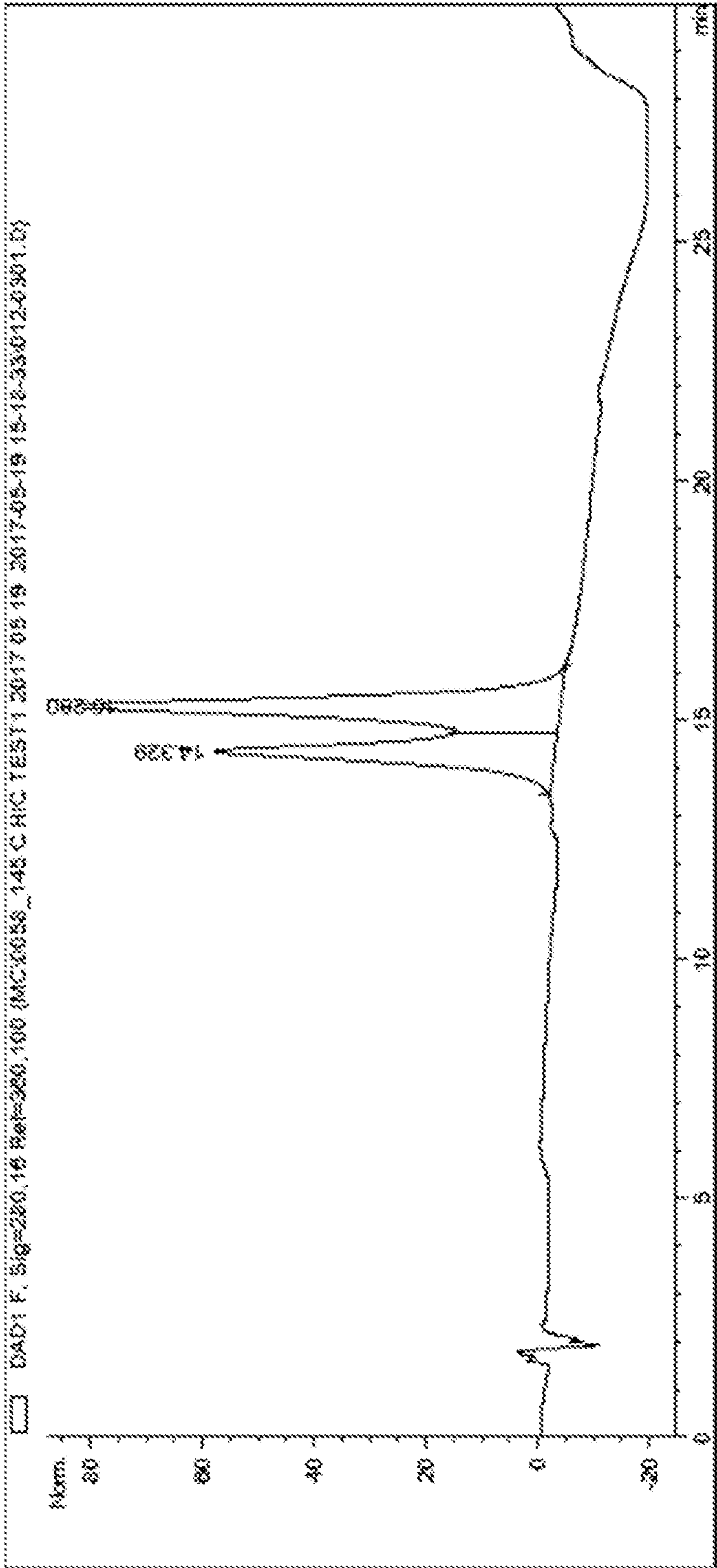


Fig. 6B

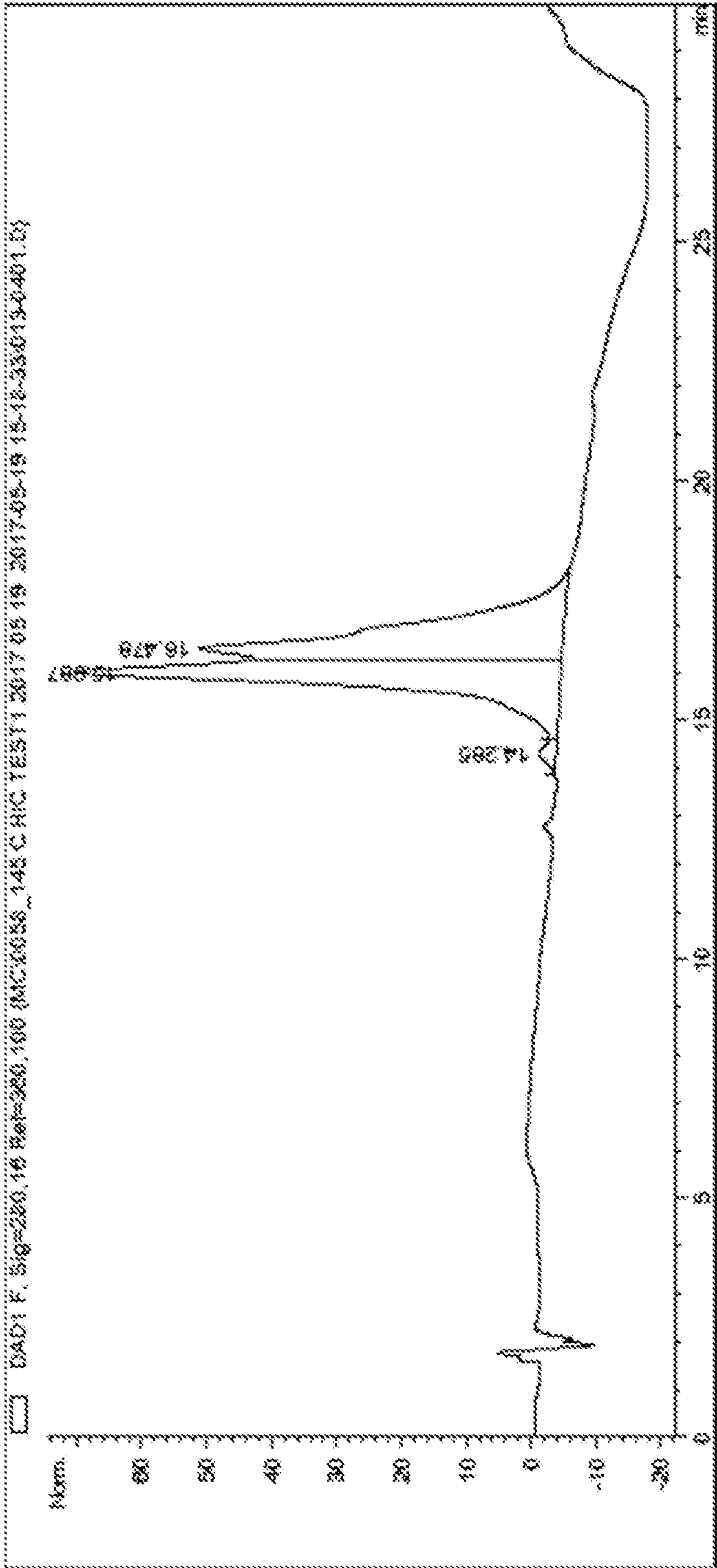


Fig. 6C

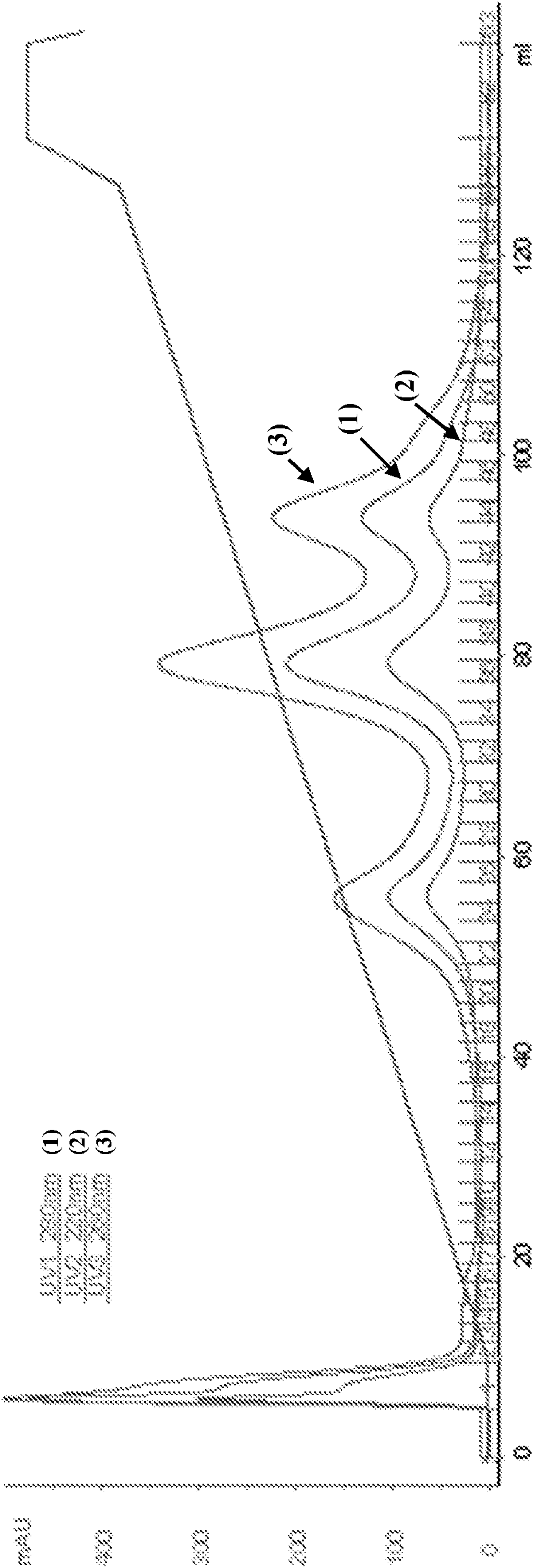


Fig. 7A

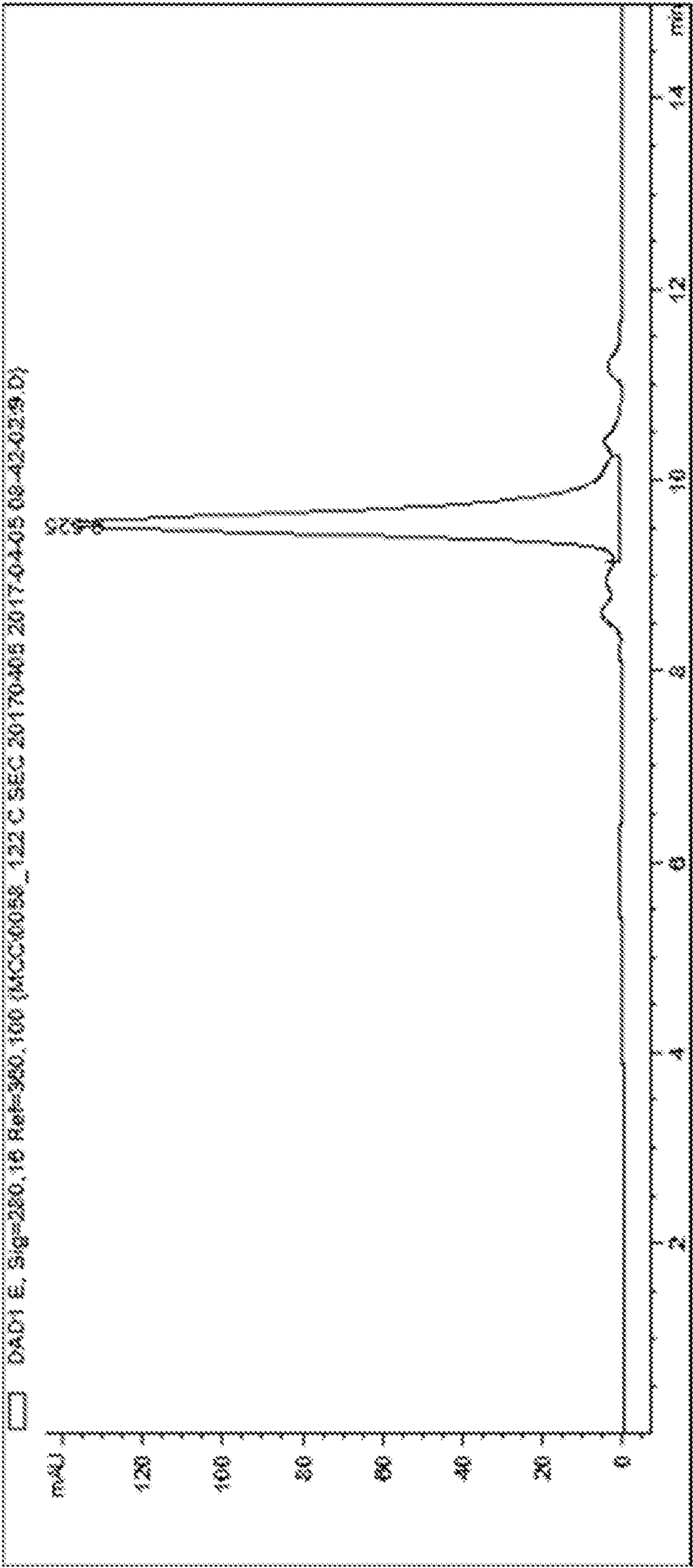


Fig. 7B

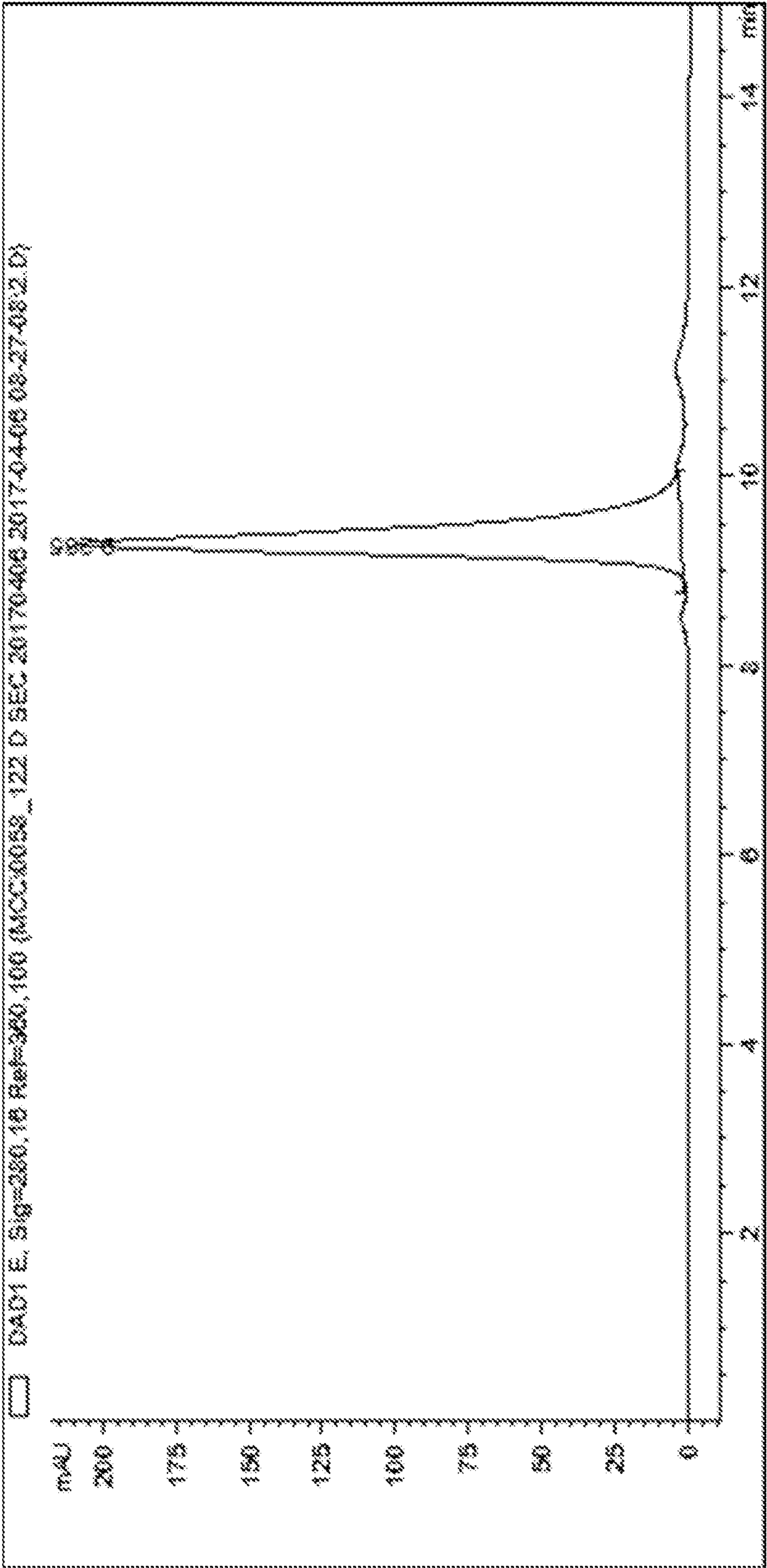


Fig. 7C

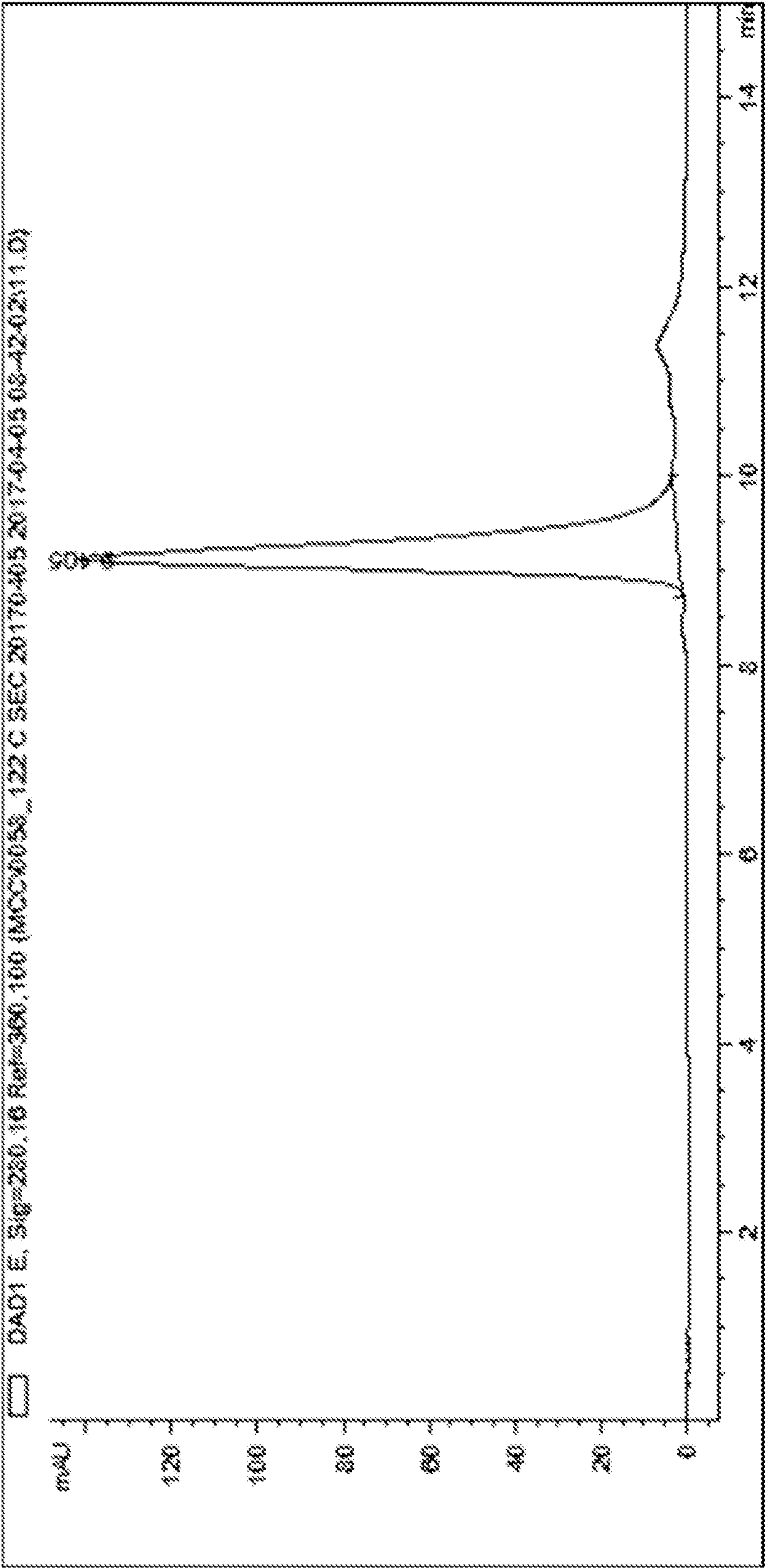


Fig. 7D

16/40

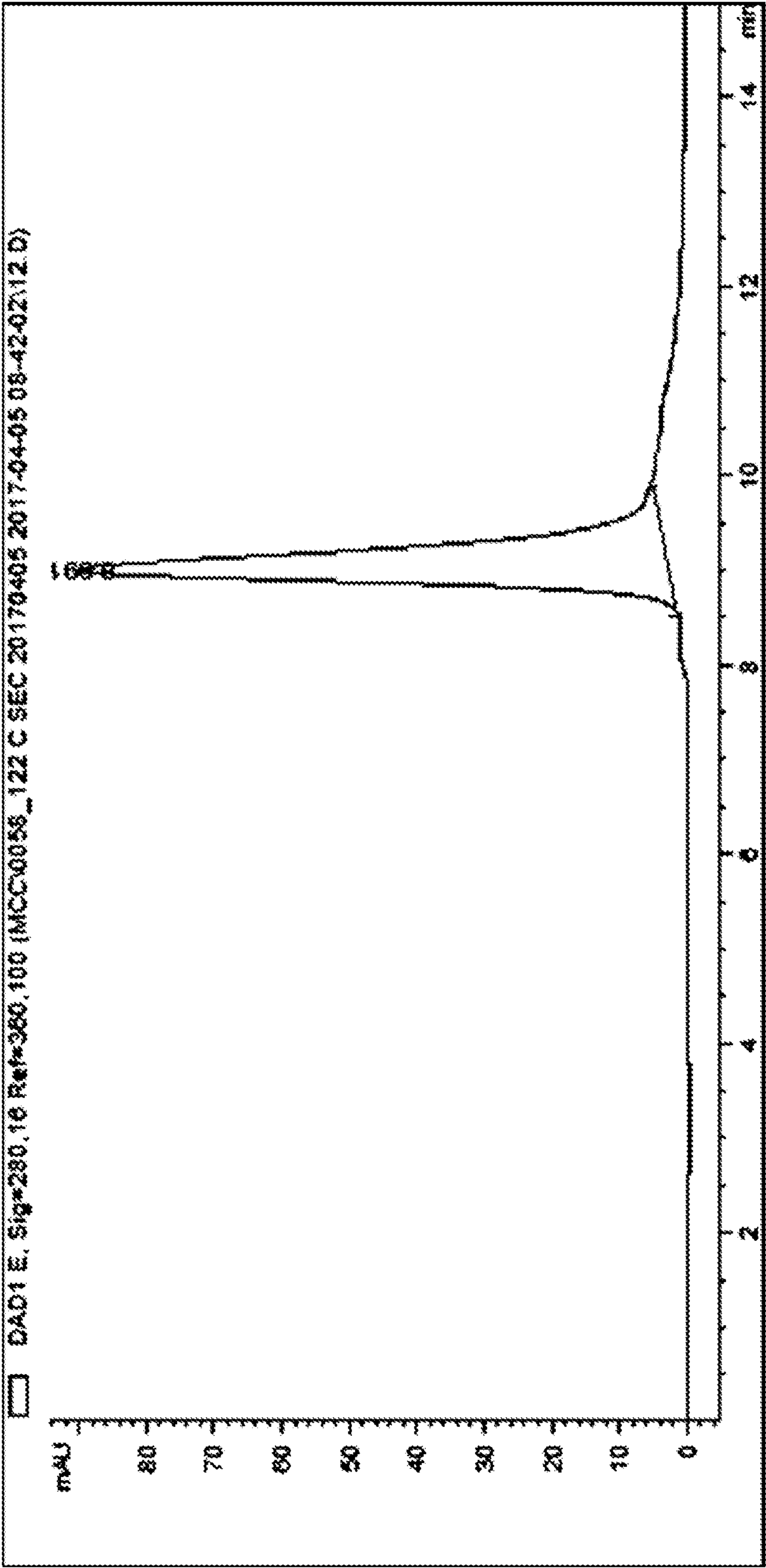


Fig. 7E

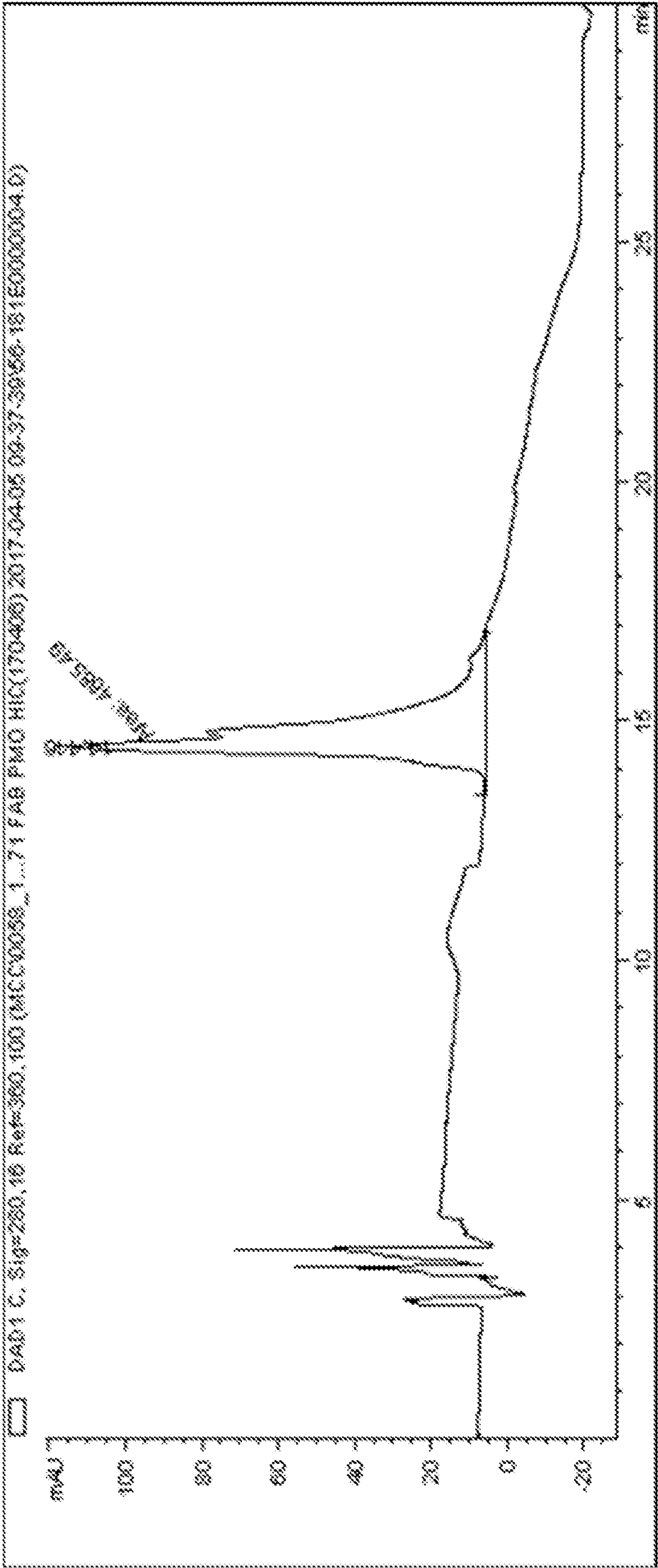


Fig. 7F

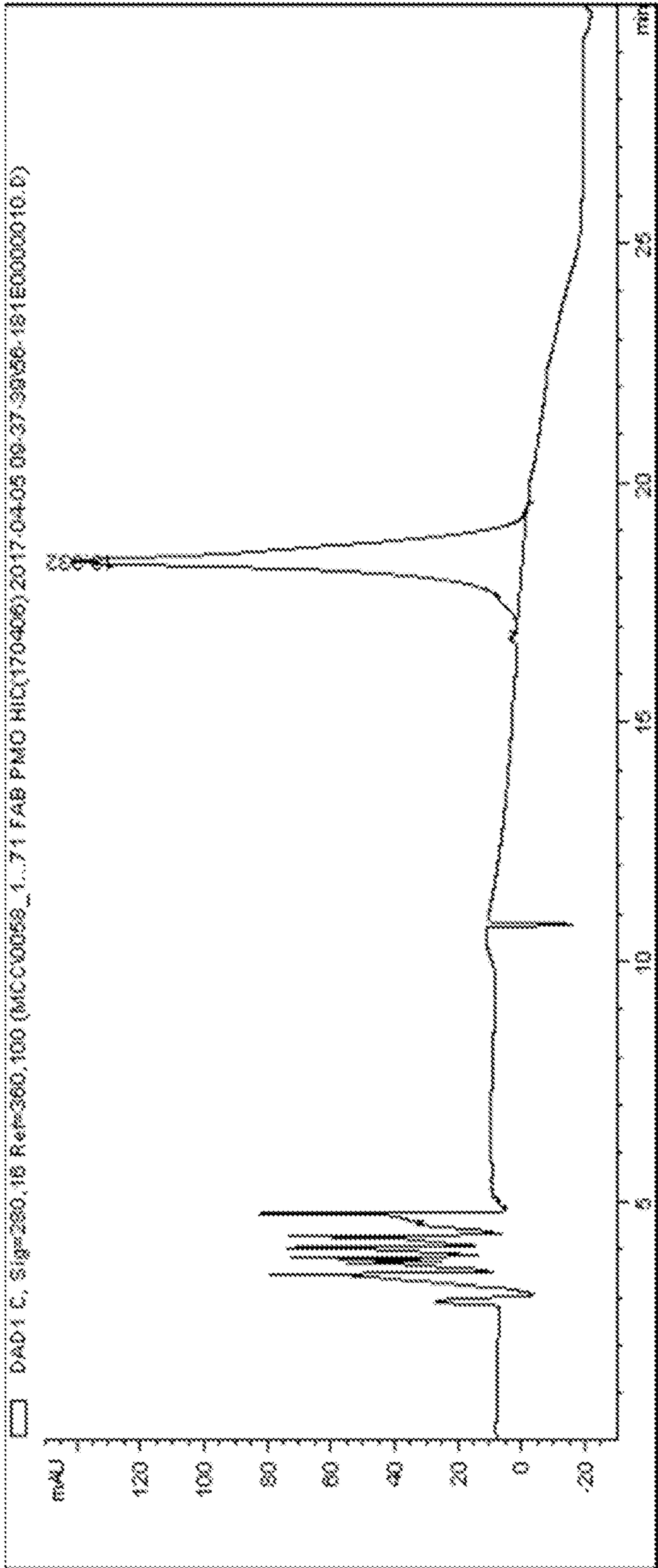


Fig. 7G

19/40

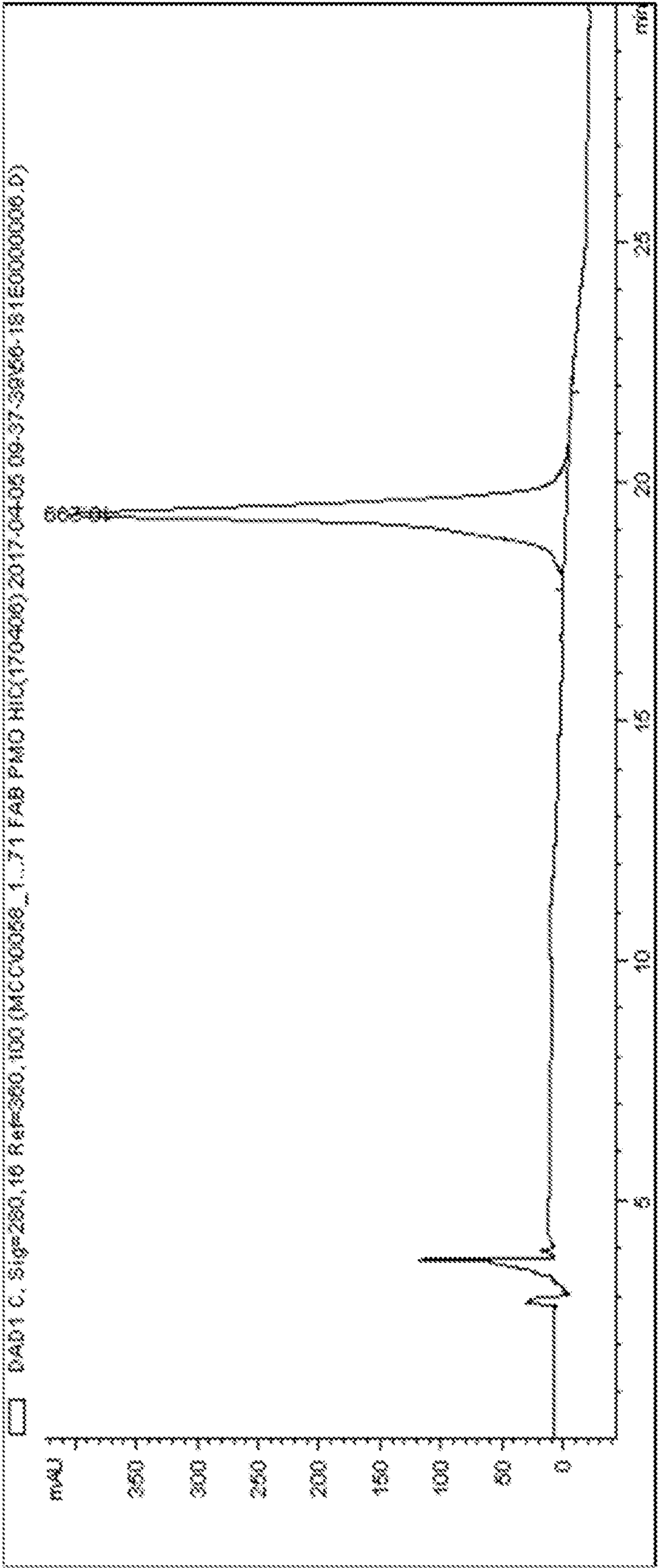


Fig. 7H

20/40

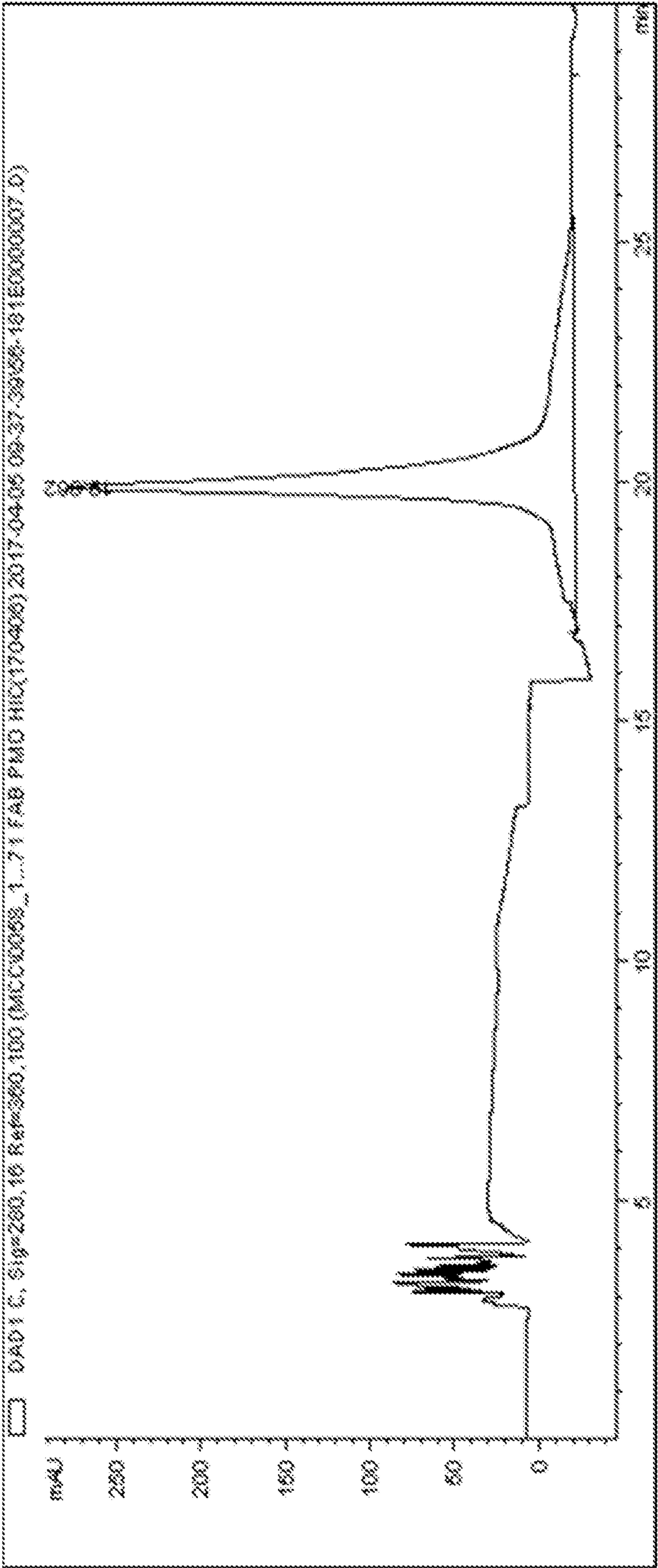


Fig. 7I

21/40

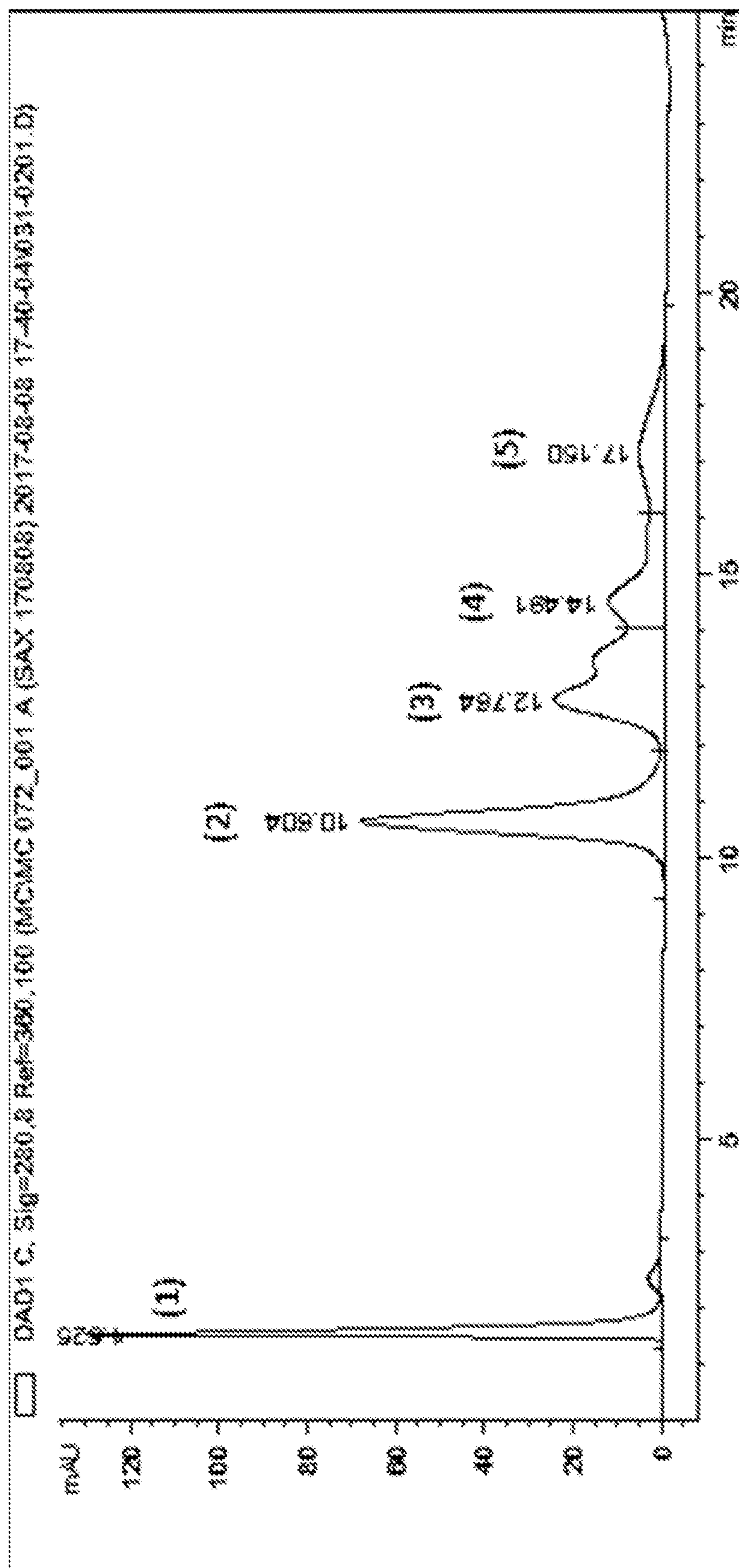


Fig. 8A

22/40

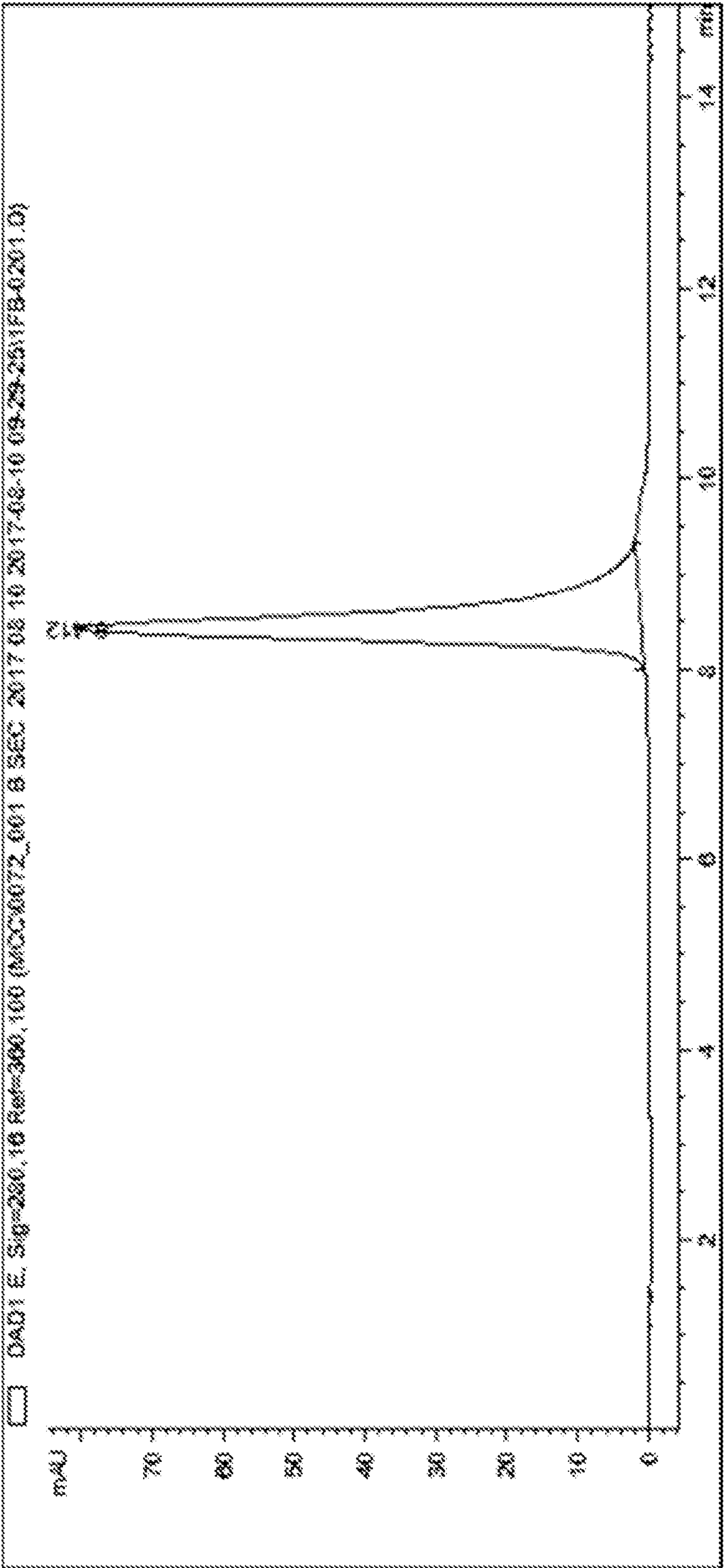


Fig. 8B

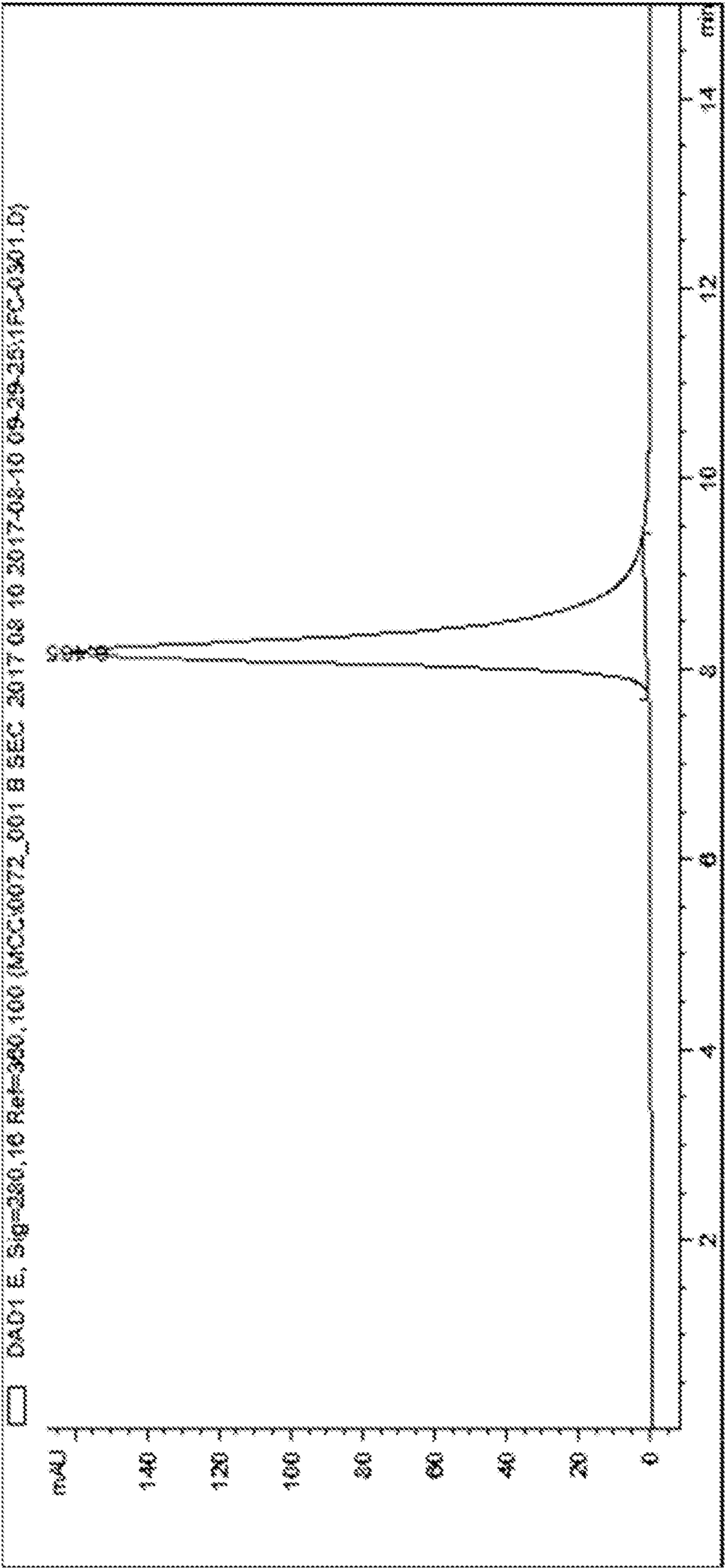


Fig. 8C

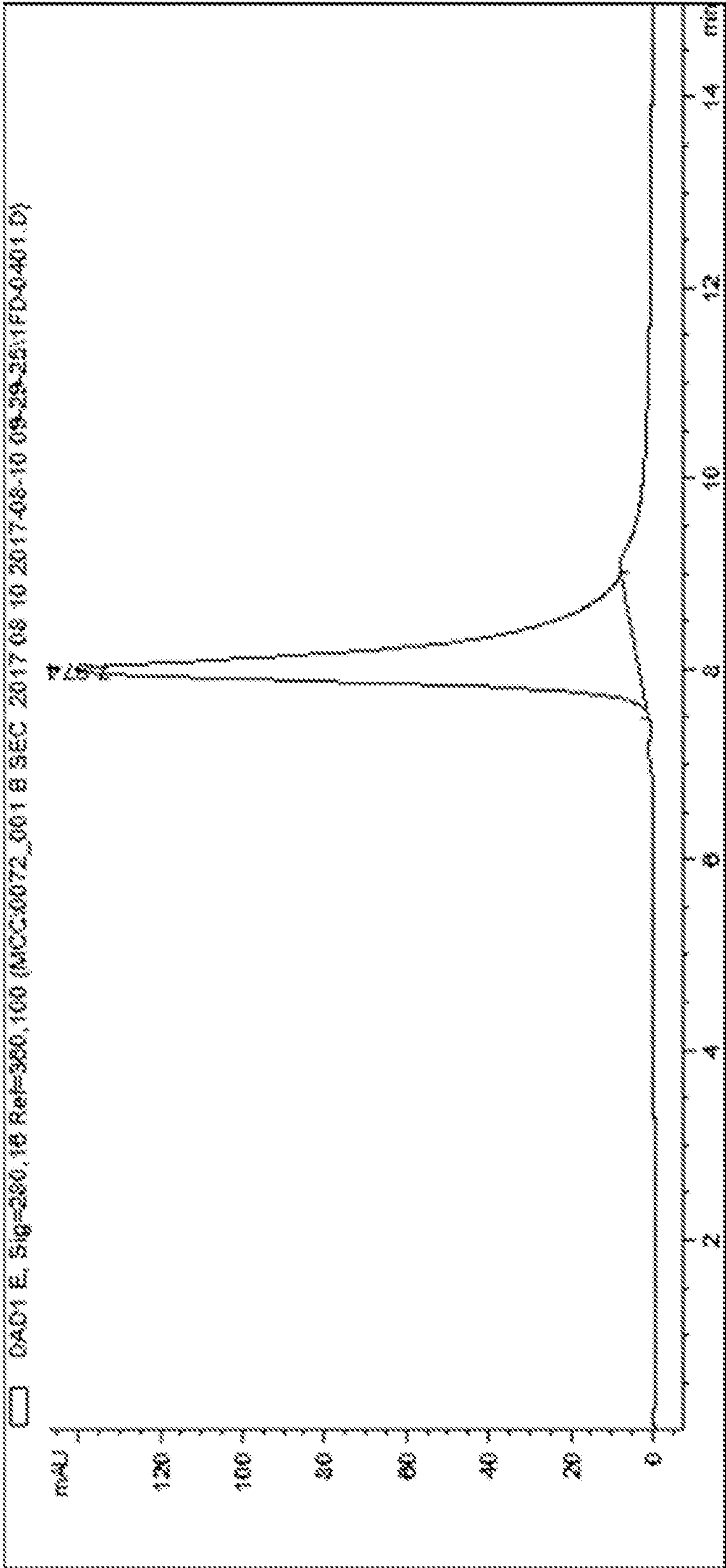


Fig. 8D

25/40

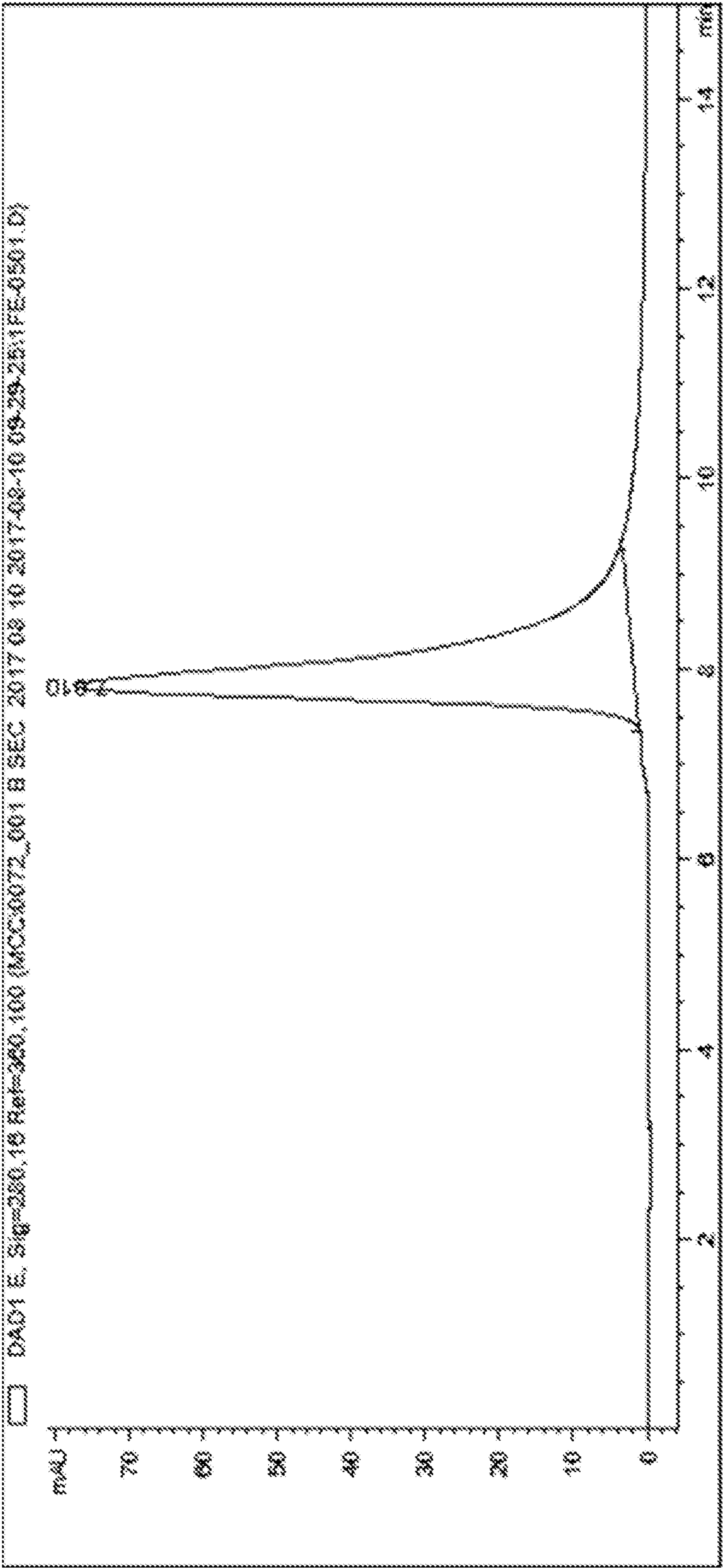


Fig. 8E

26/40

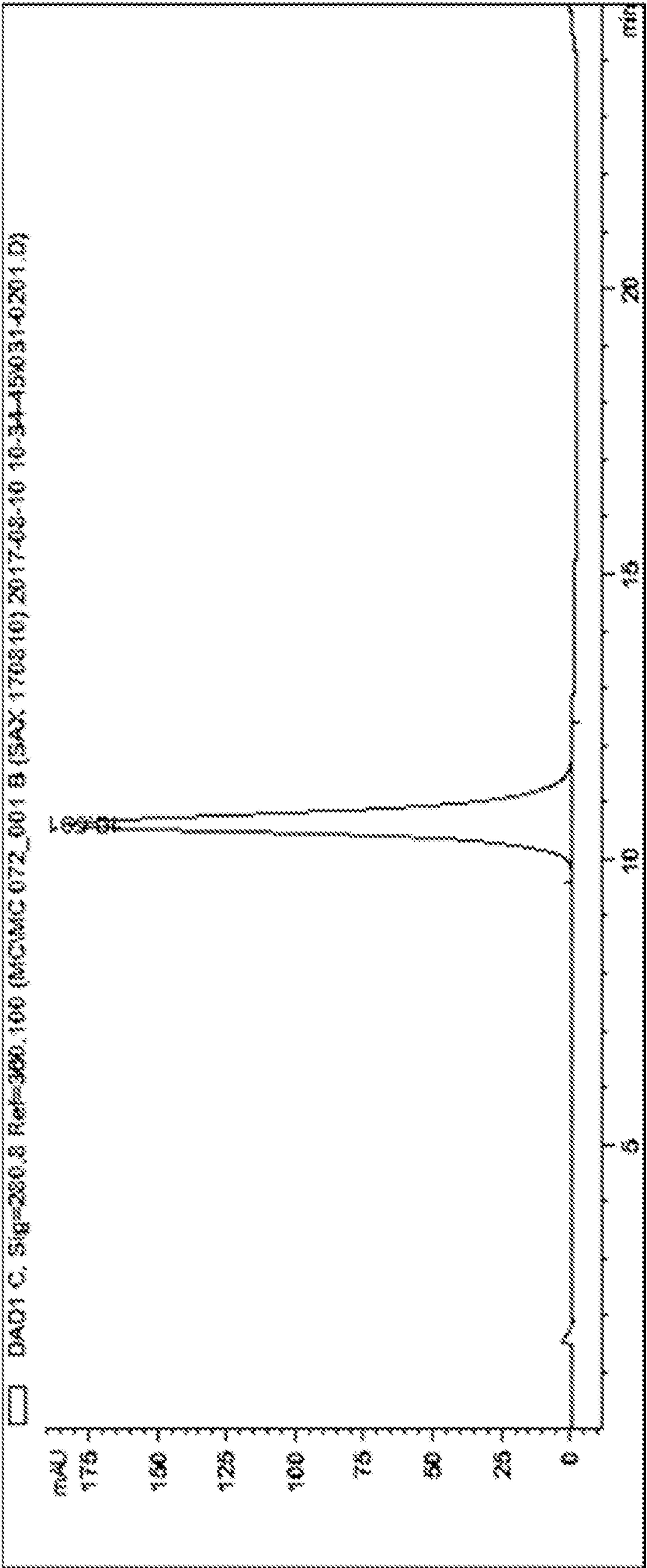


Fig. 8F

27/40

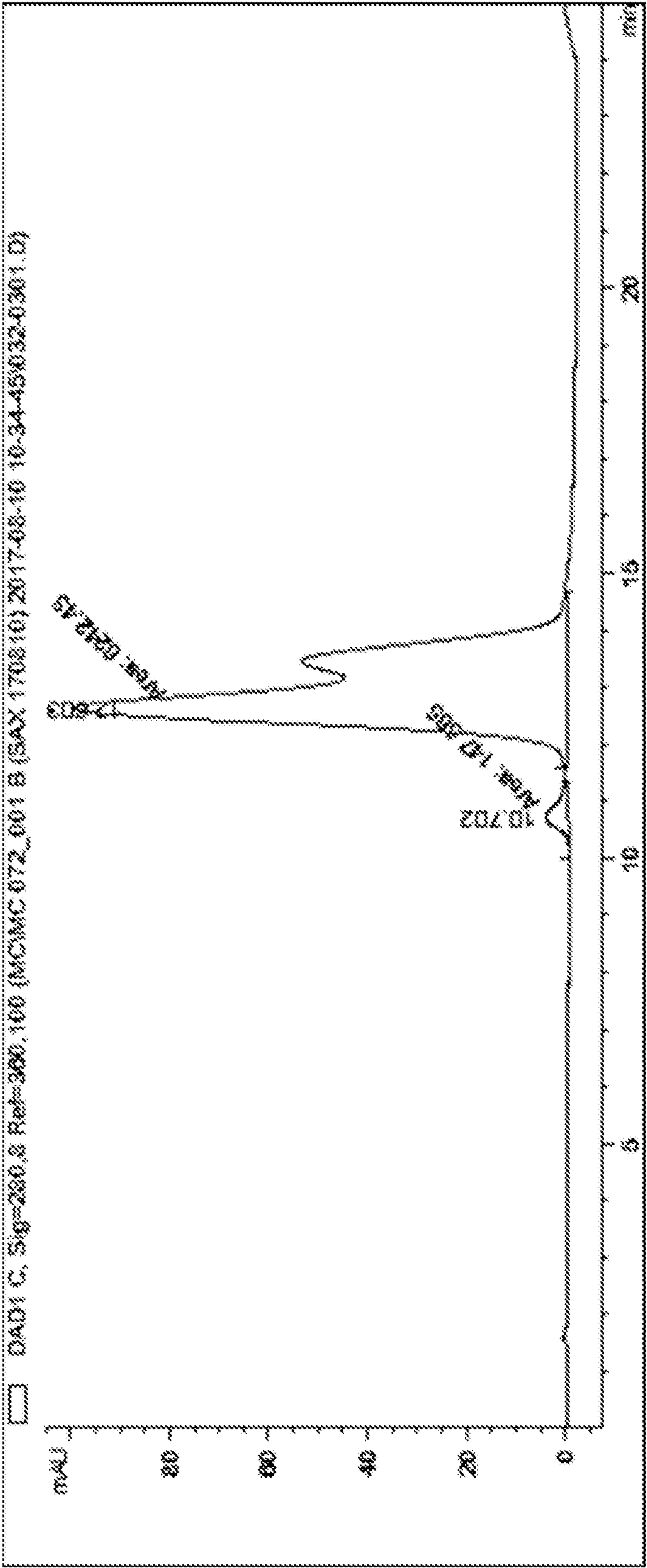
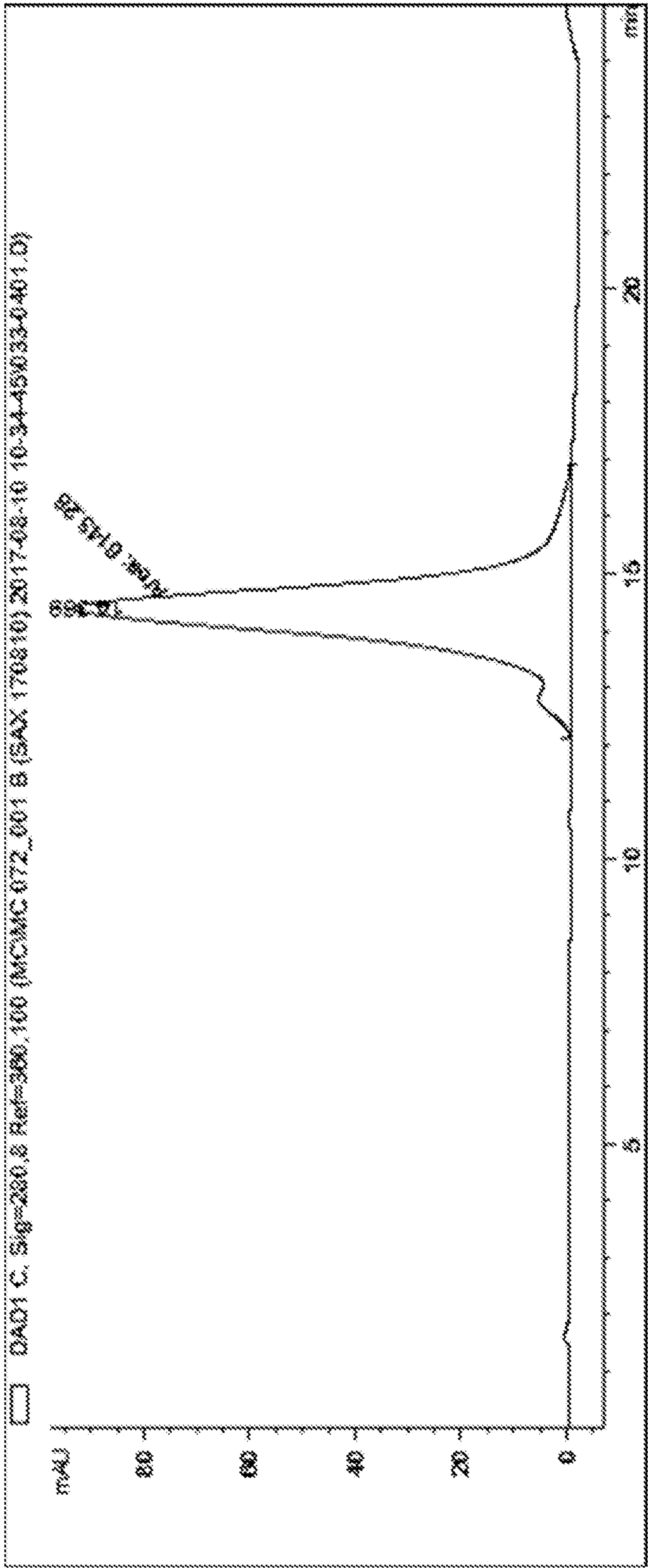


Fig. 8G



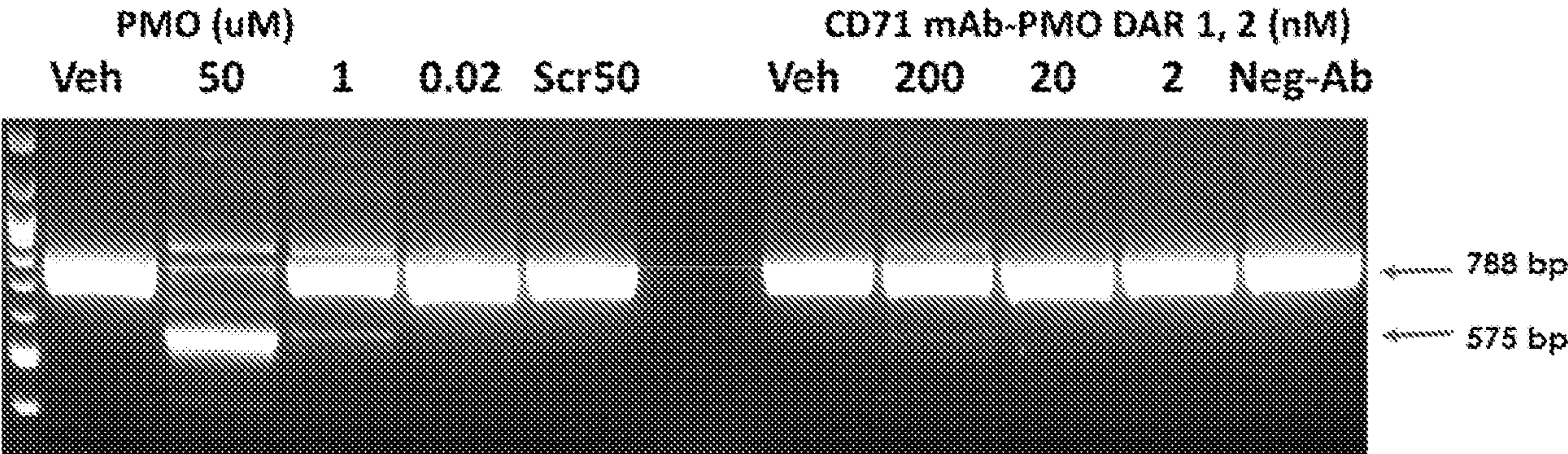


Fig. 9

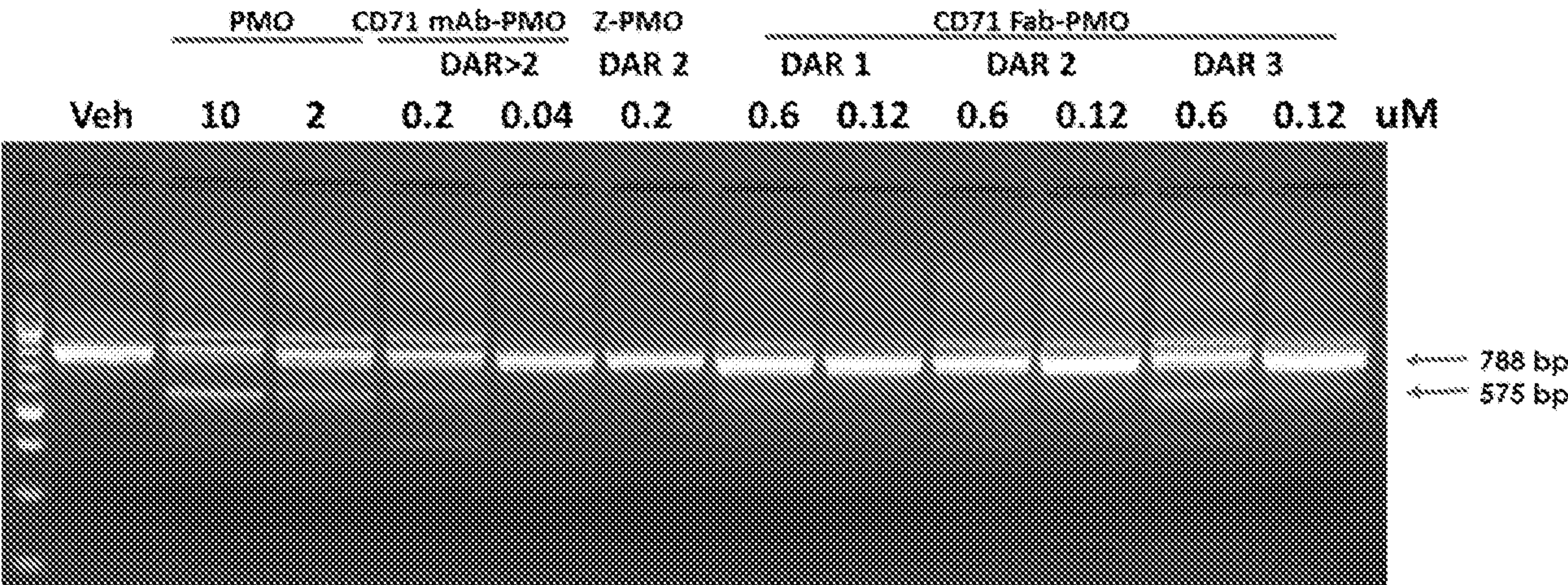


Fig. 10

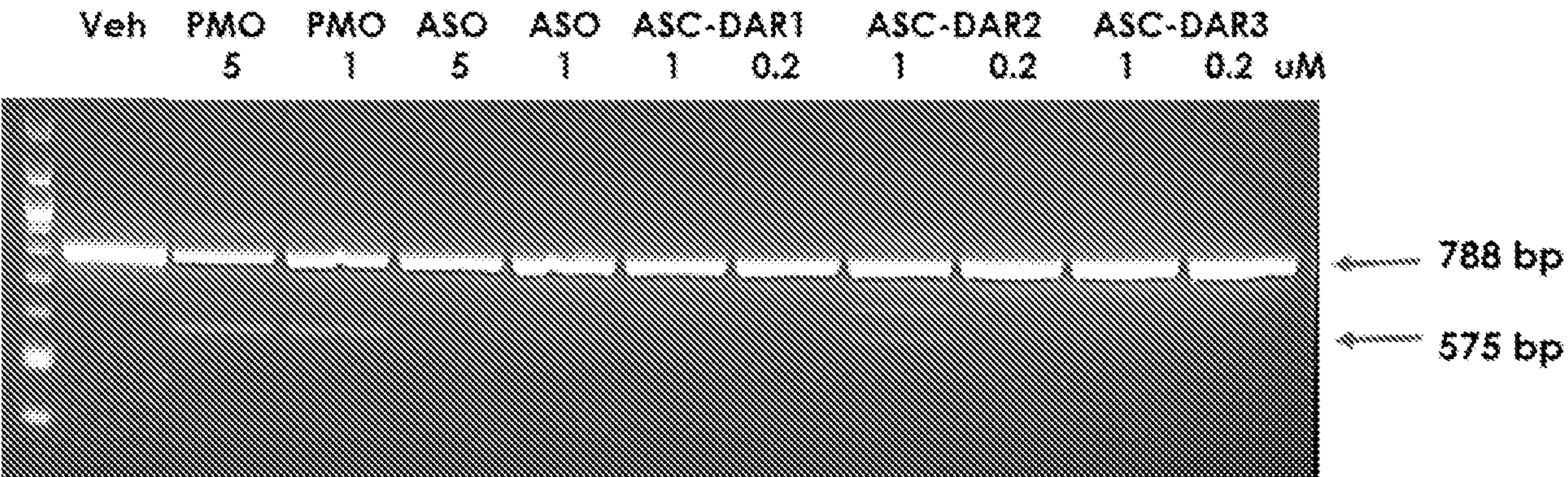


Fig. 11

30/40

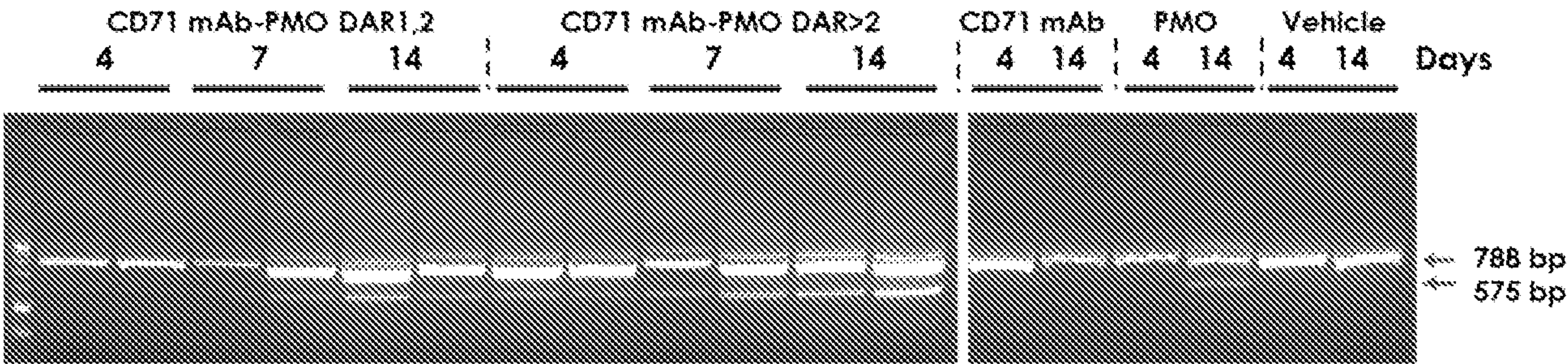


Fig. 12A

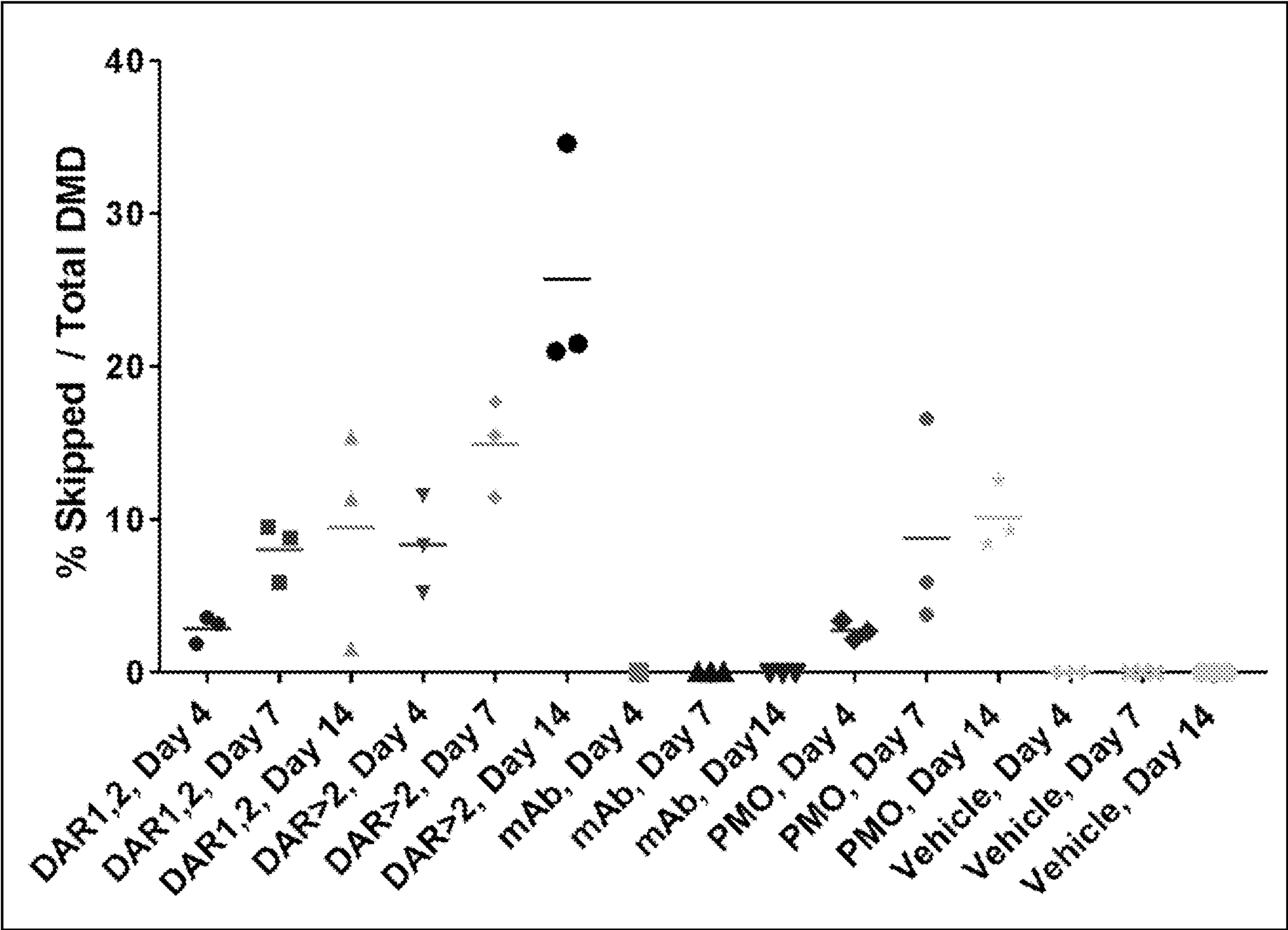


Fig. 12B

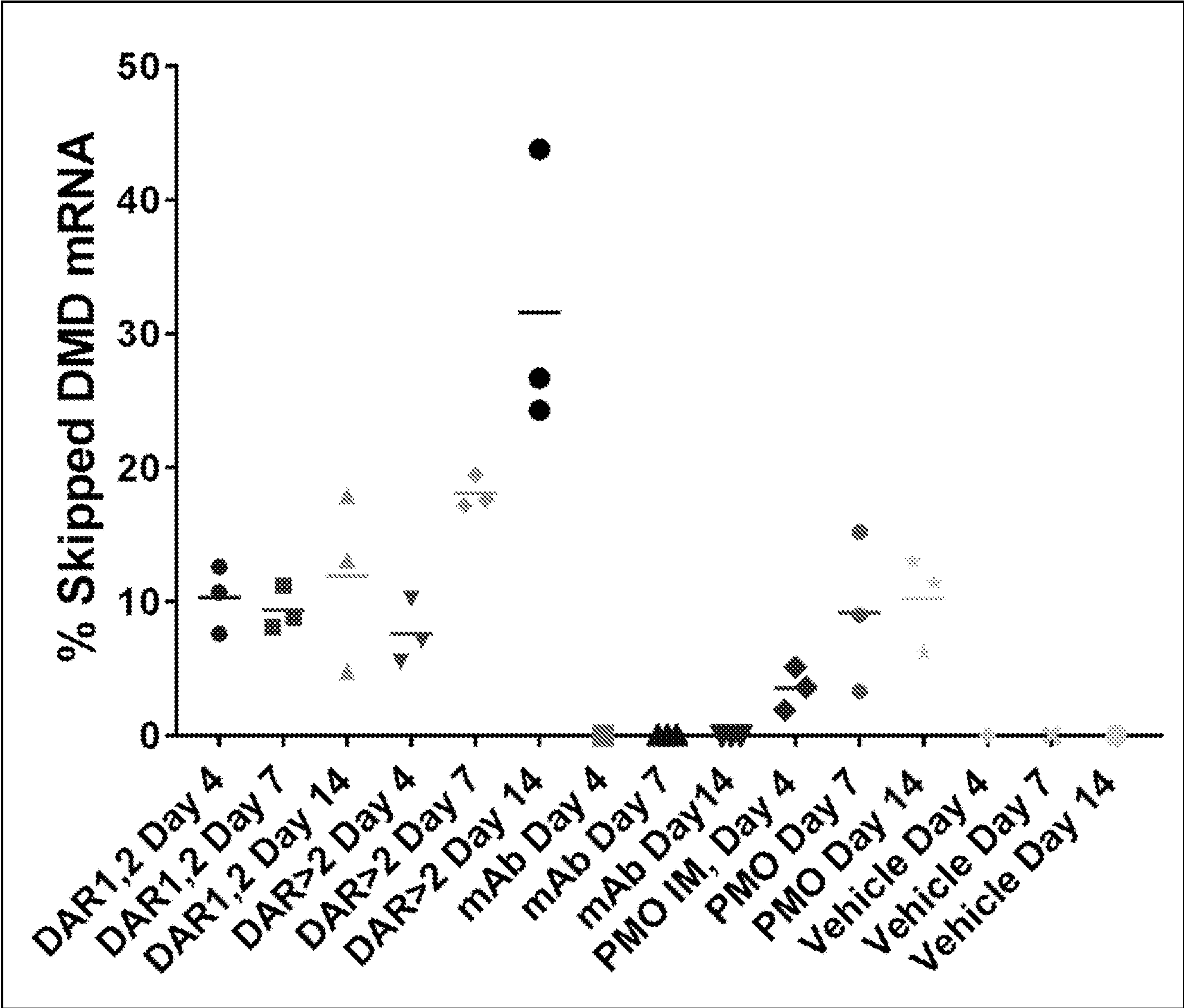


Fig. 12C

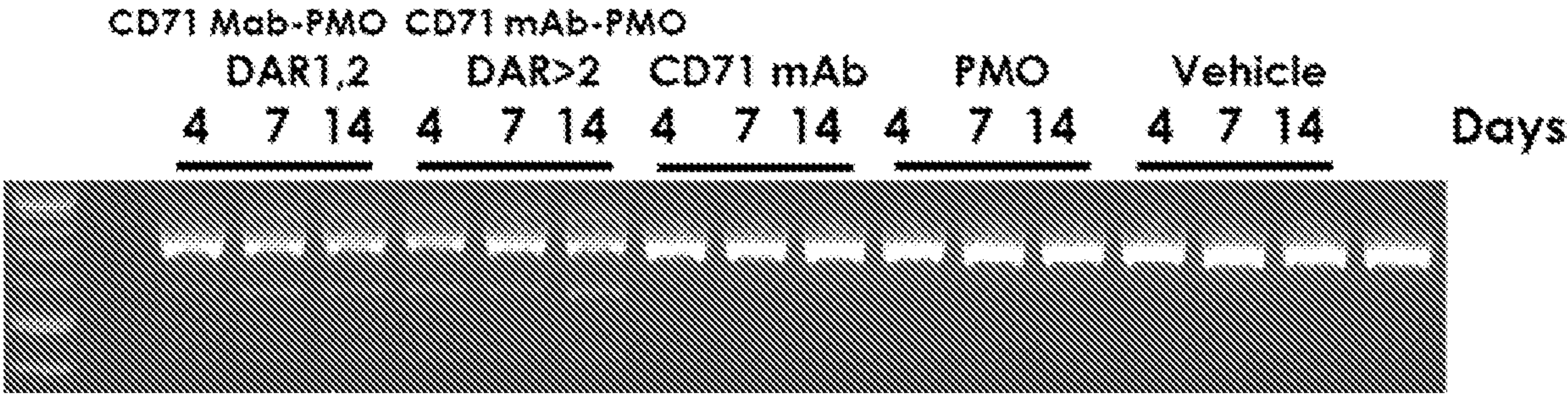


Fig. 13A

32/40

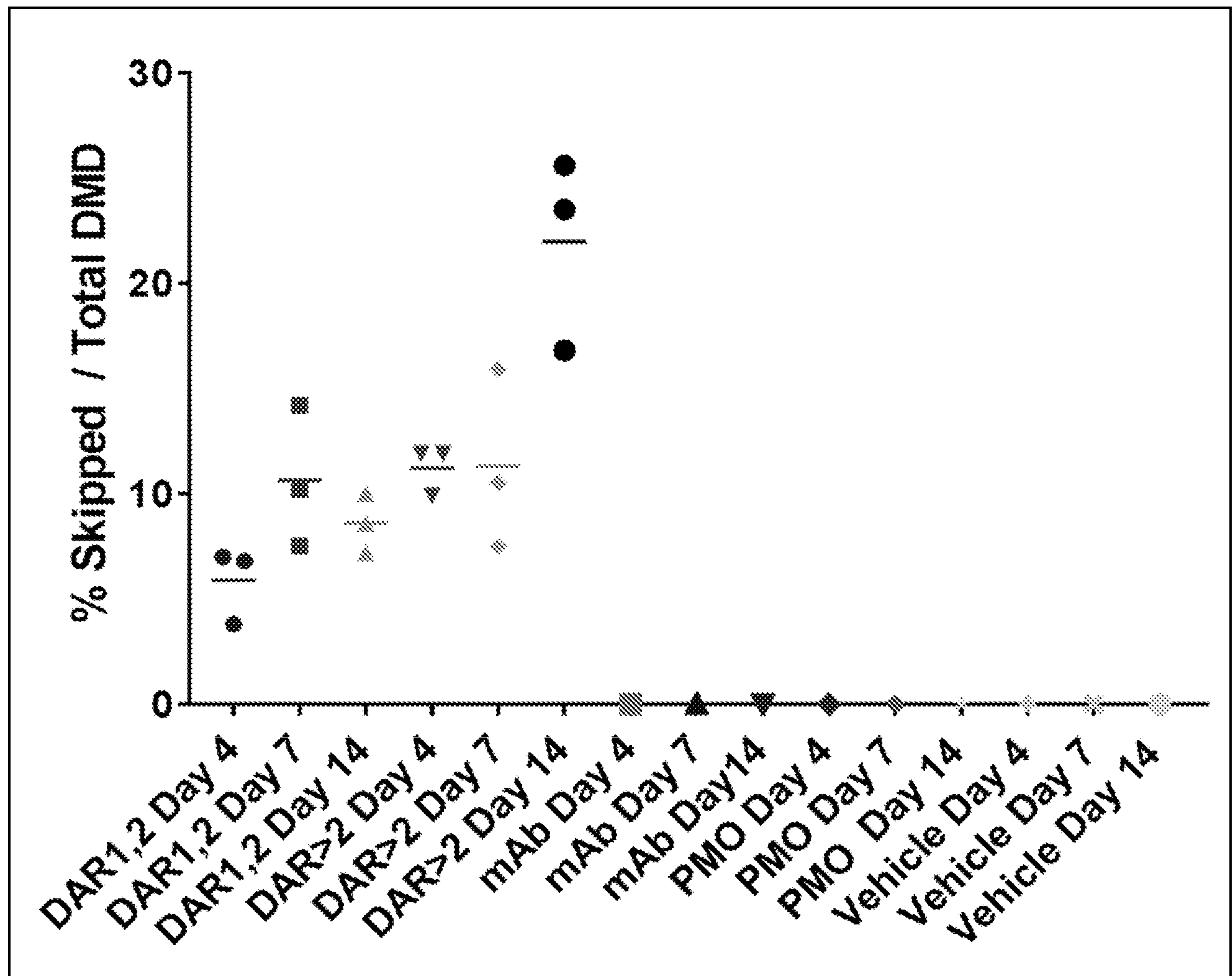
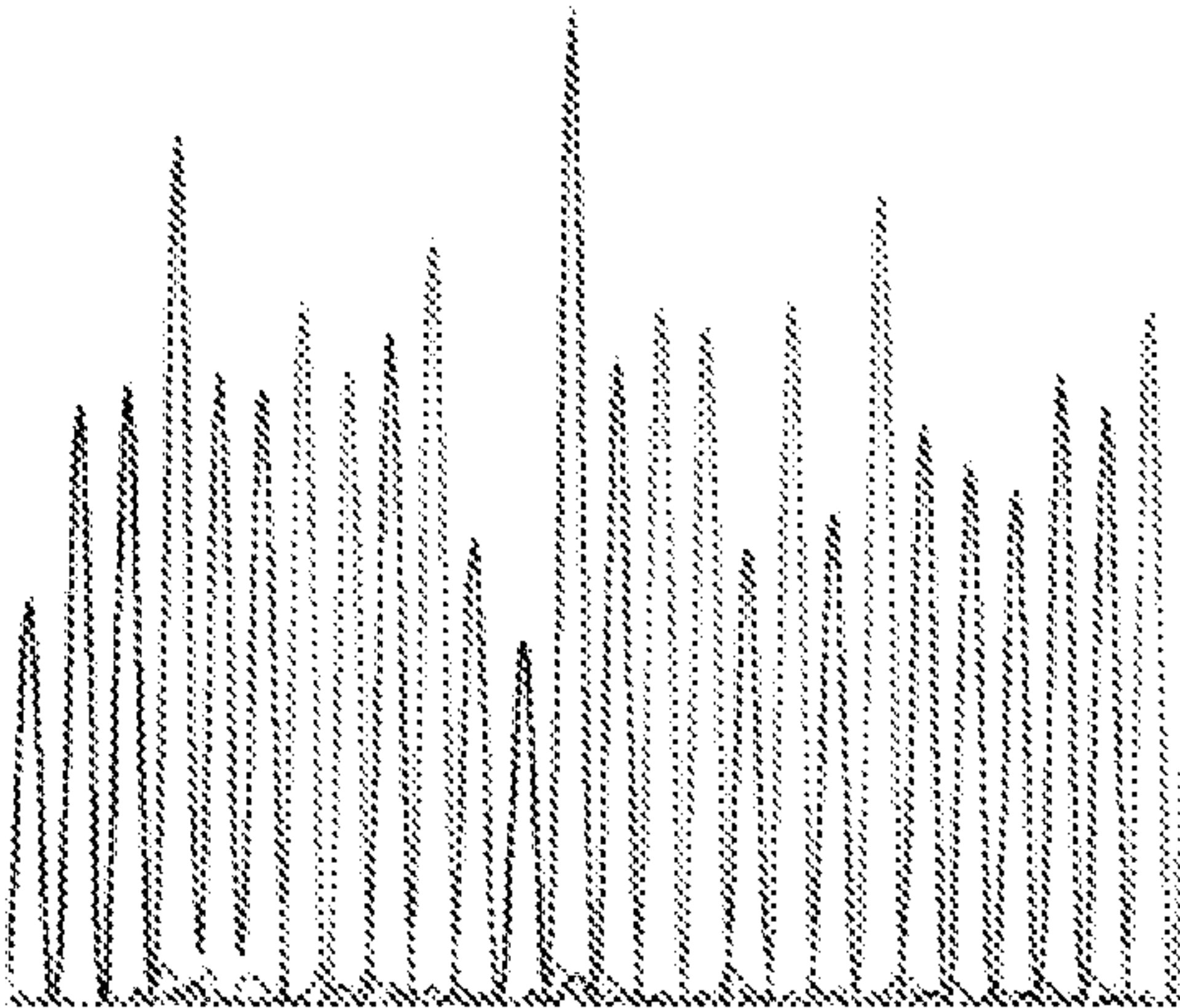


Fig. 13B

Skipped DMD

Exon22  Exon24
GGGAAATTACAGGAATCACAATAAAAAAC



WT DMD

Exon22  Exon23
GGGAAATTACAGGCTCTGCAAAGTTCTTT

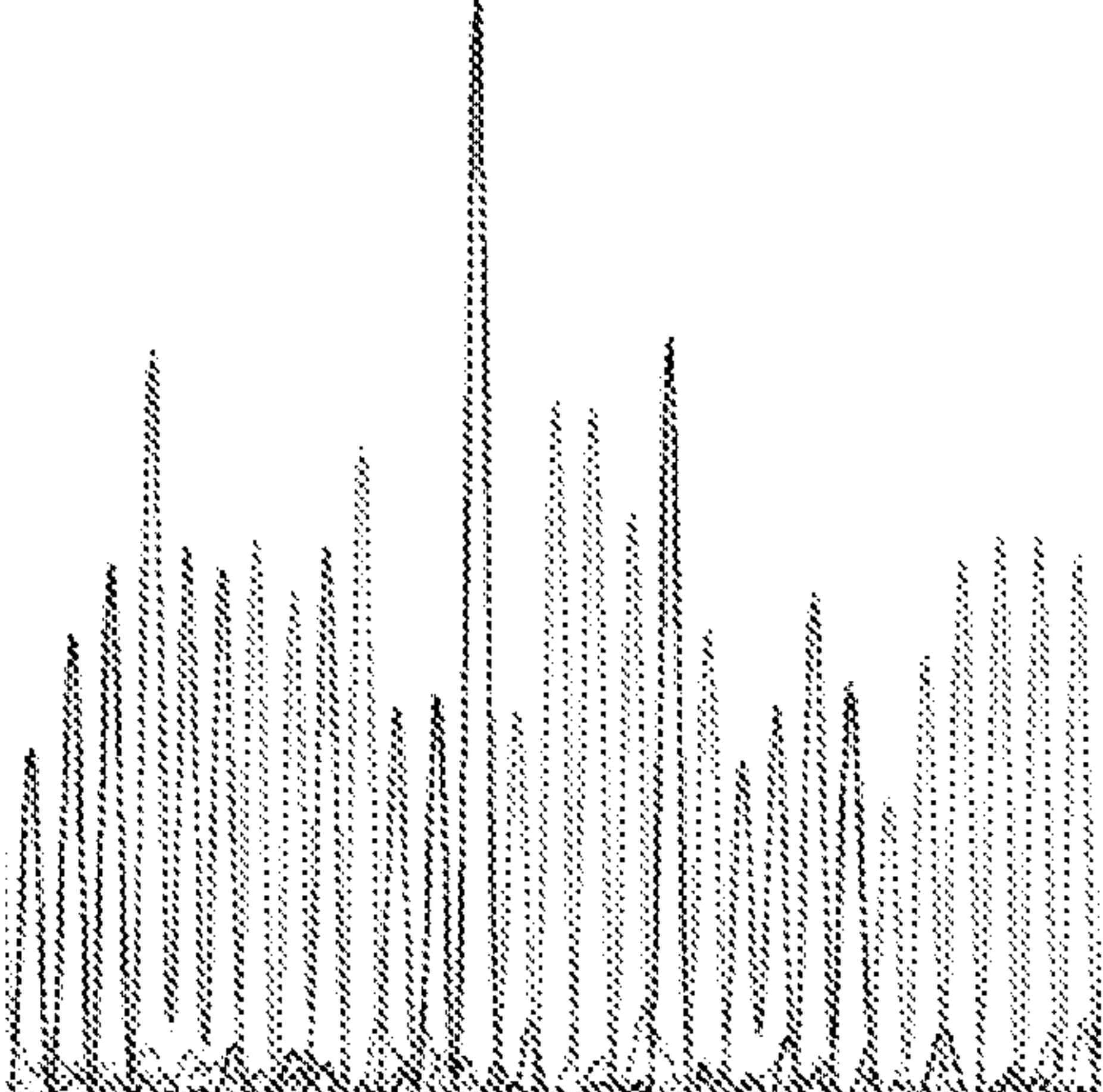


Fig. 14

34/40

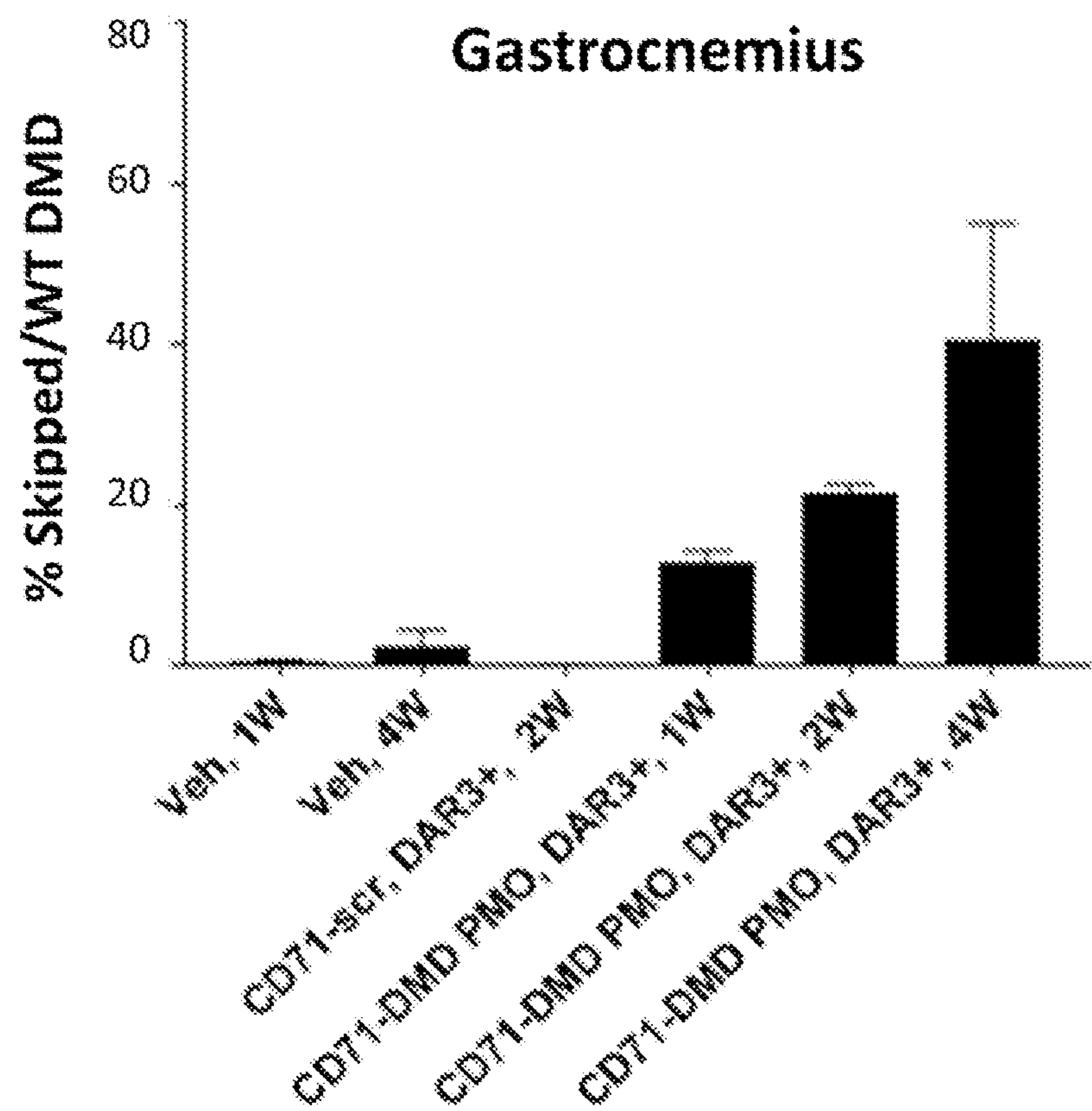


Fig. 15A

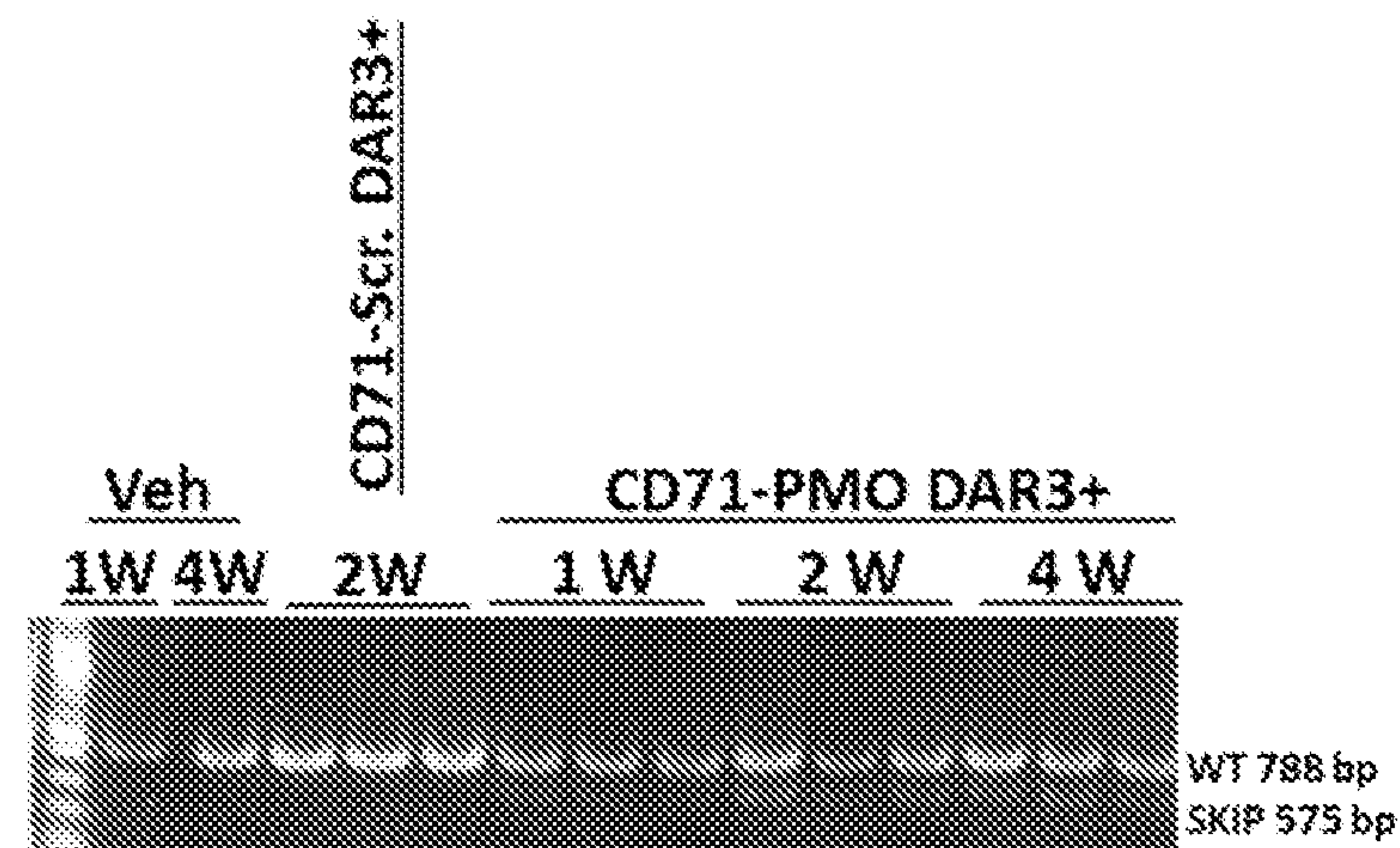


Fig. 15B

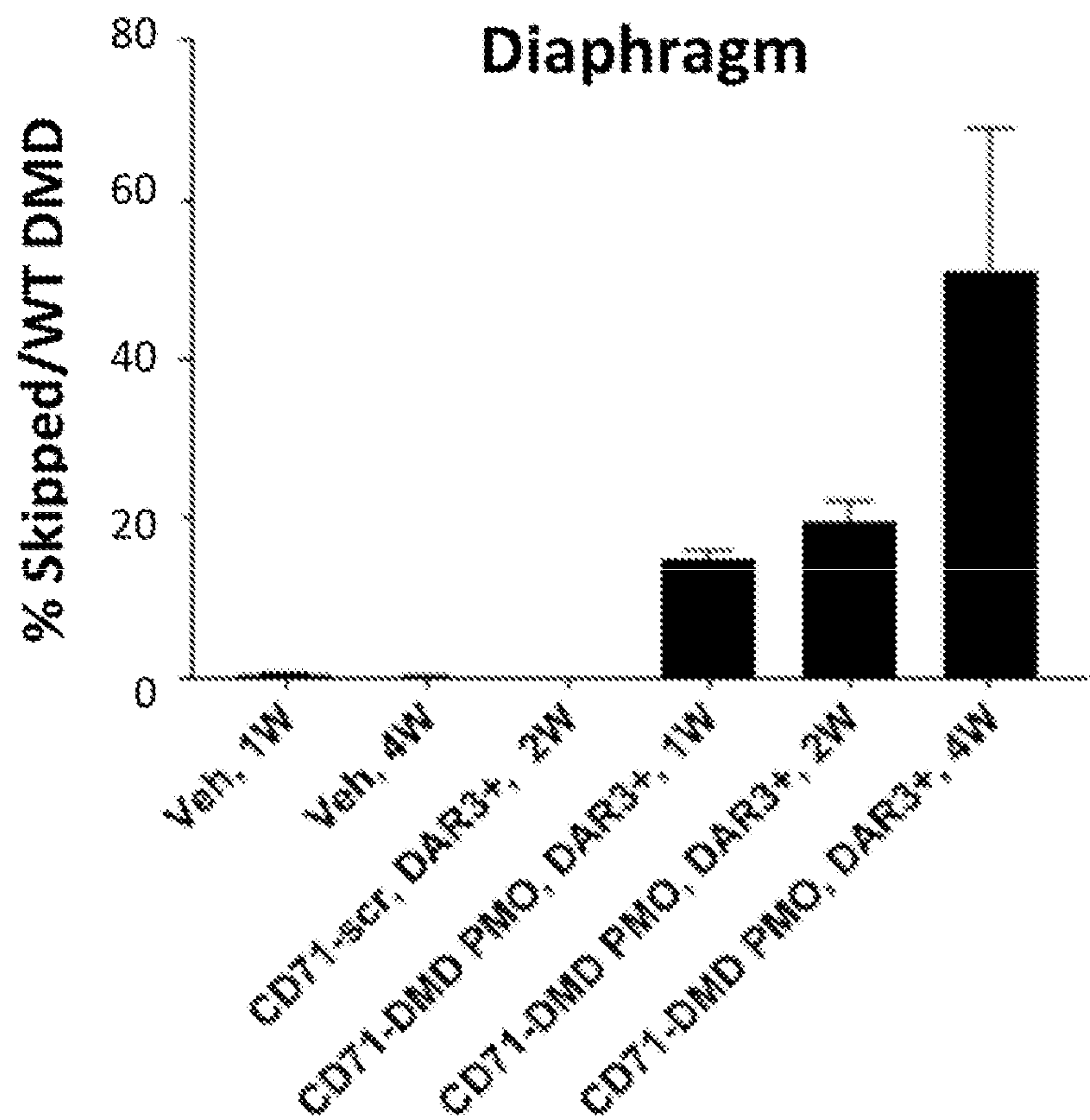


Fig. 15C

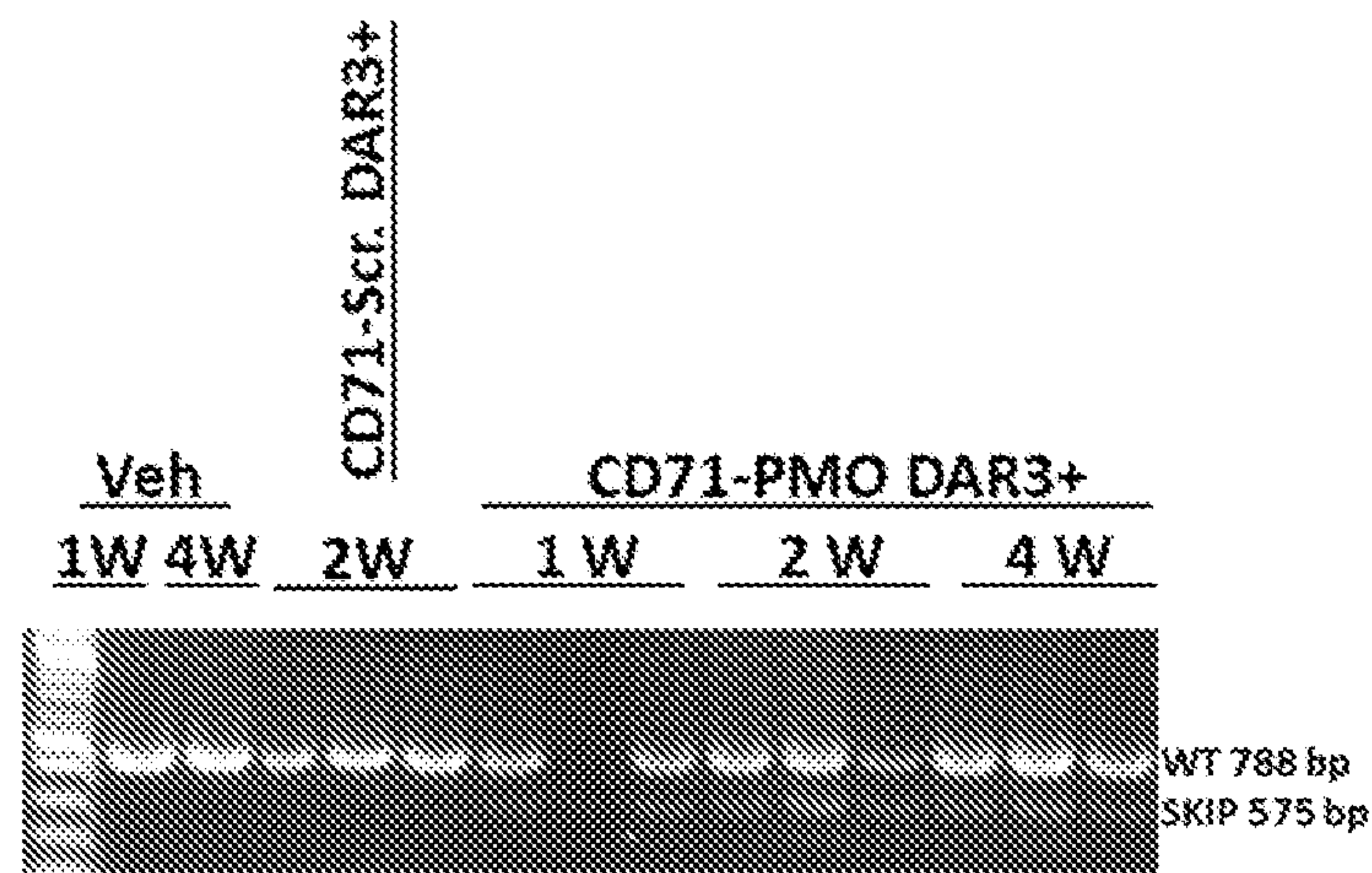


Fig. 15D

36/40

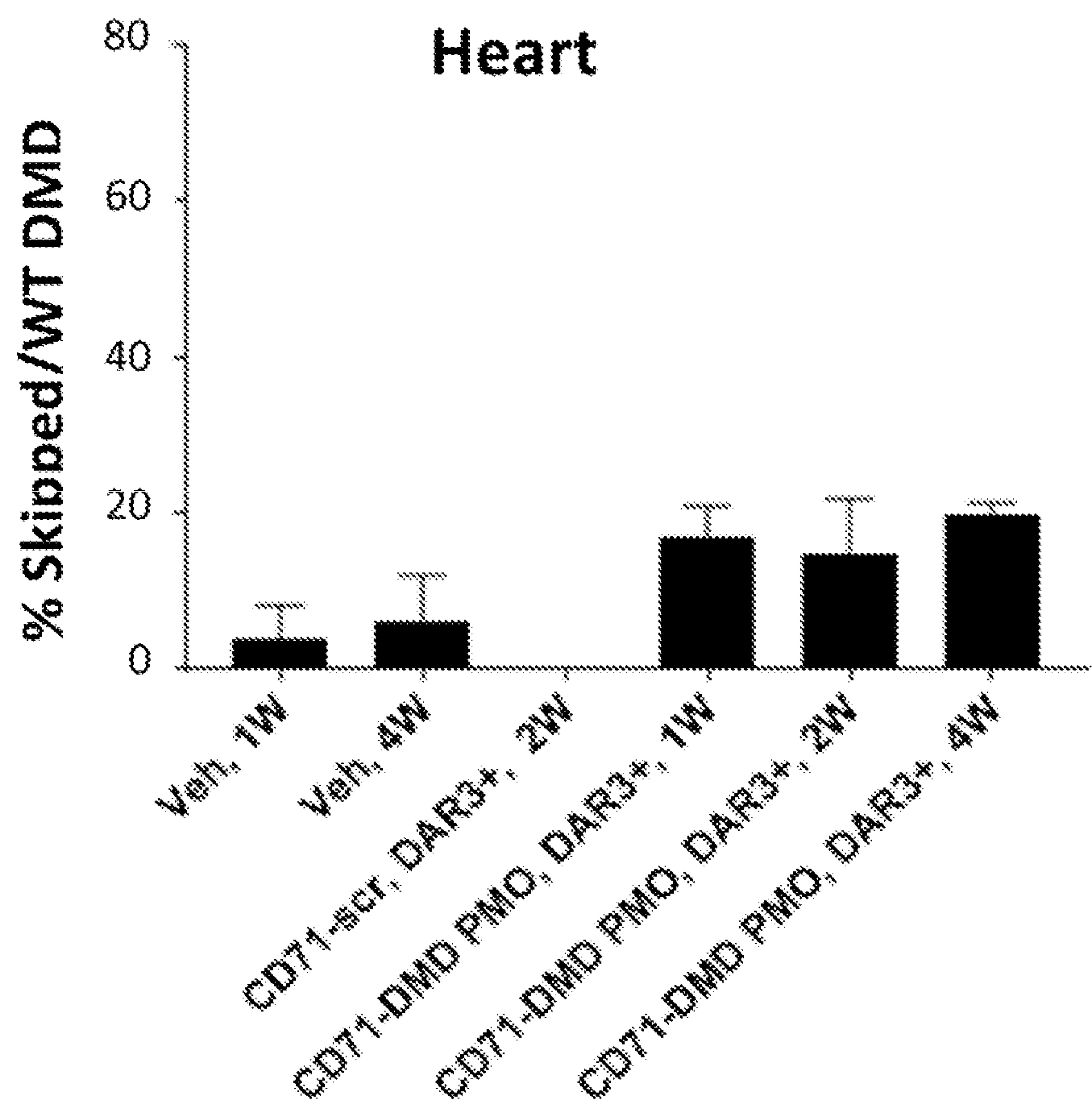


Fig. 15E

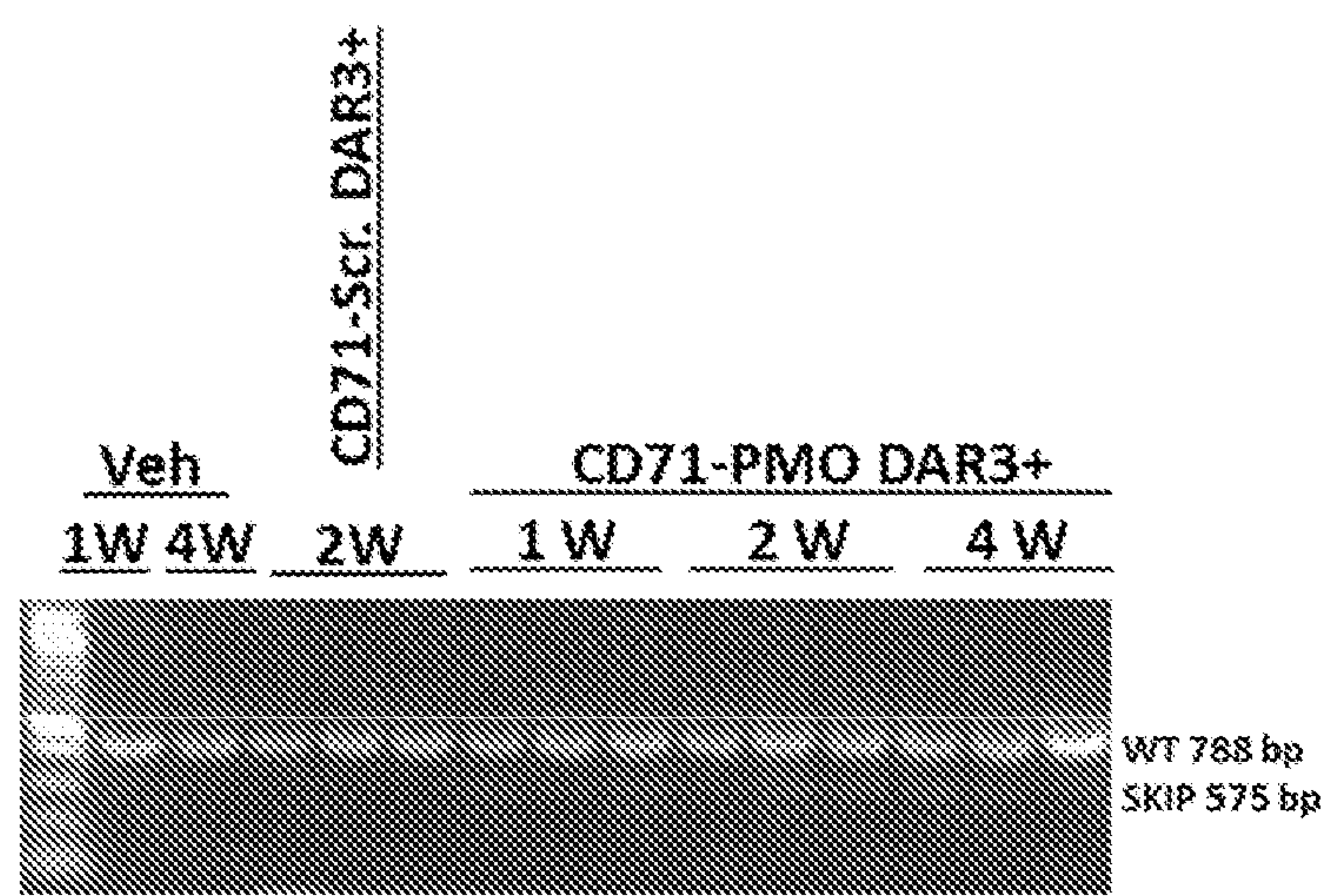


Fig. 15F

37/40

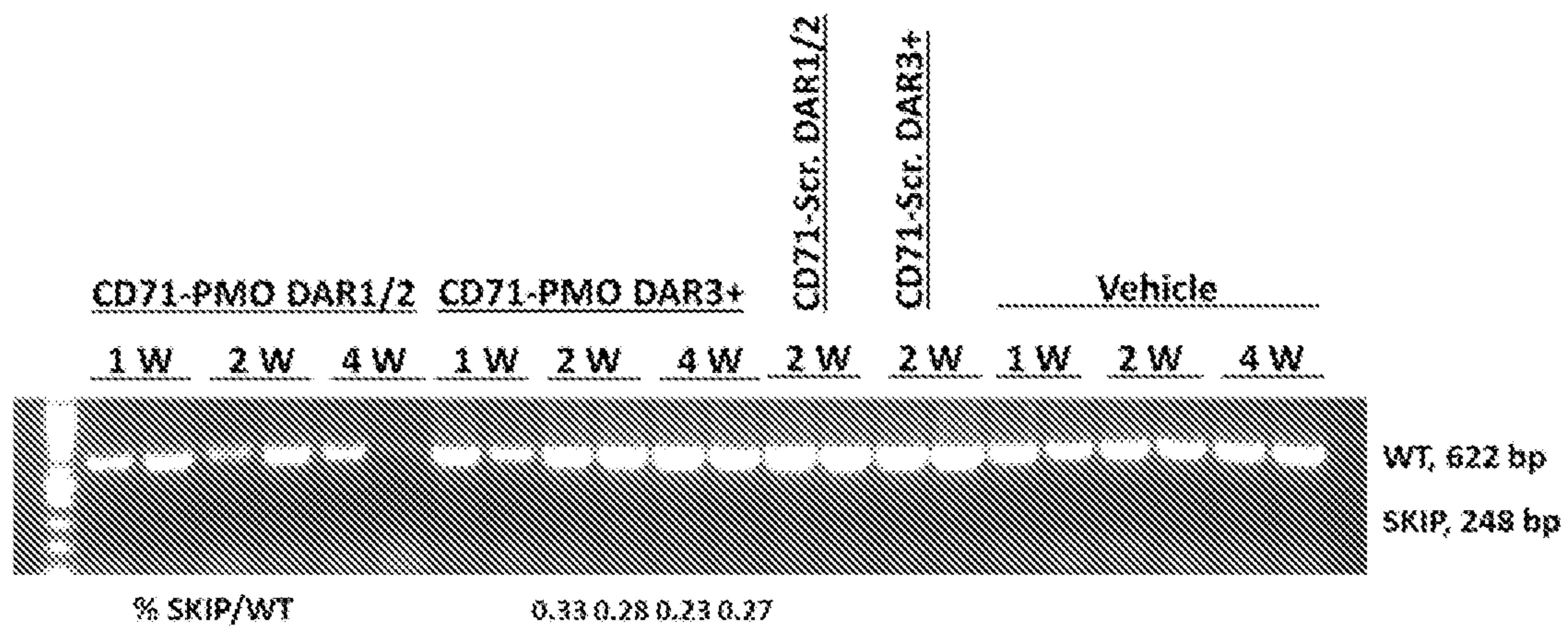


Fig. 16A

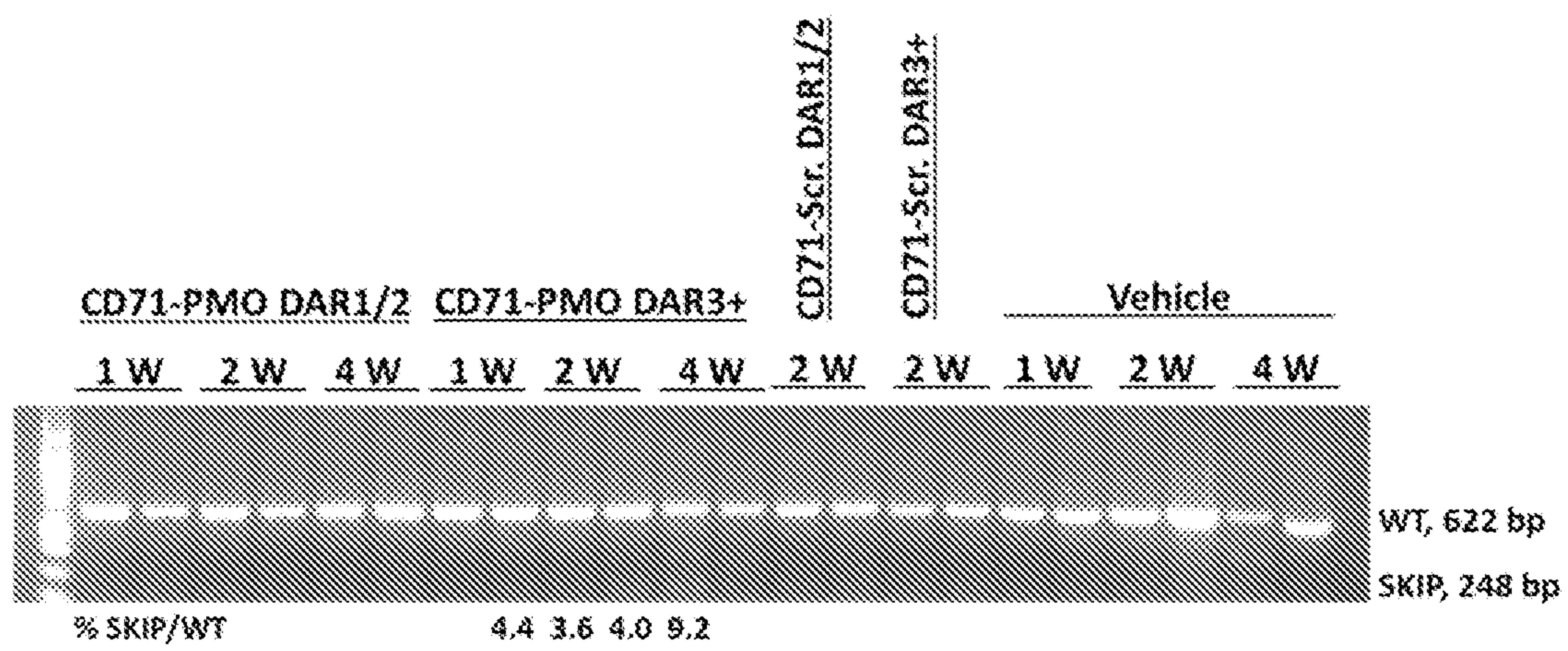


Fig. 16B

38/40

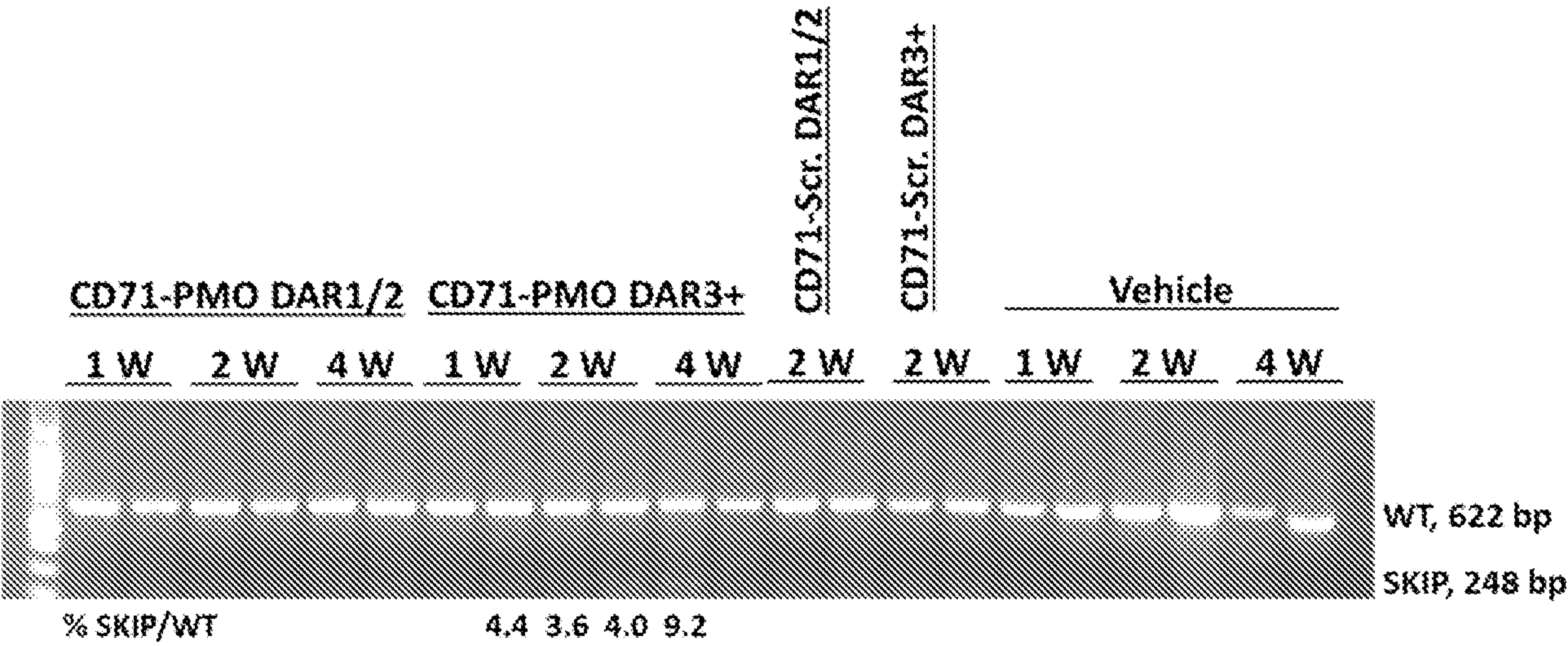


Fig. 16C

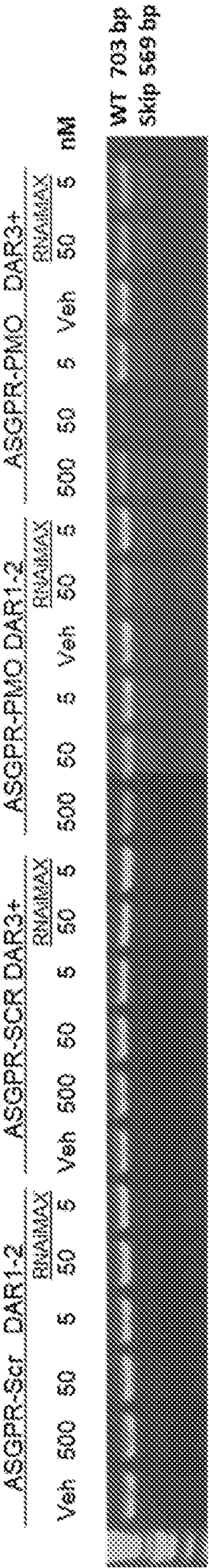


Fig. 17

40/40

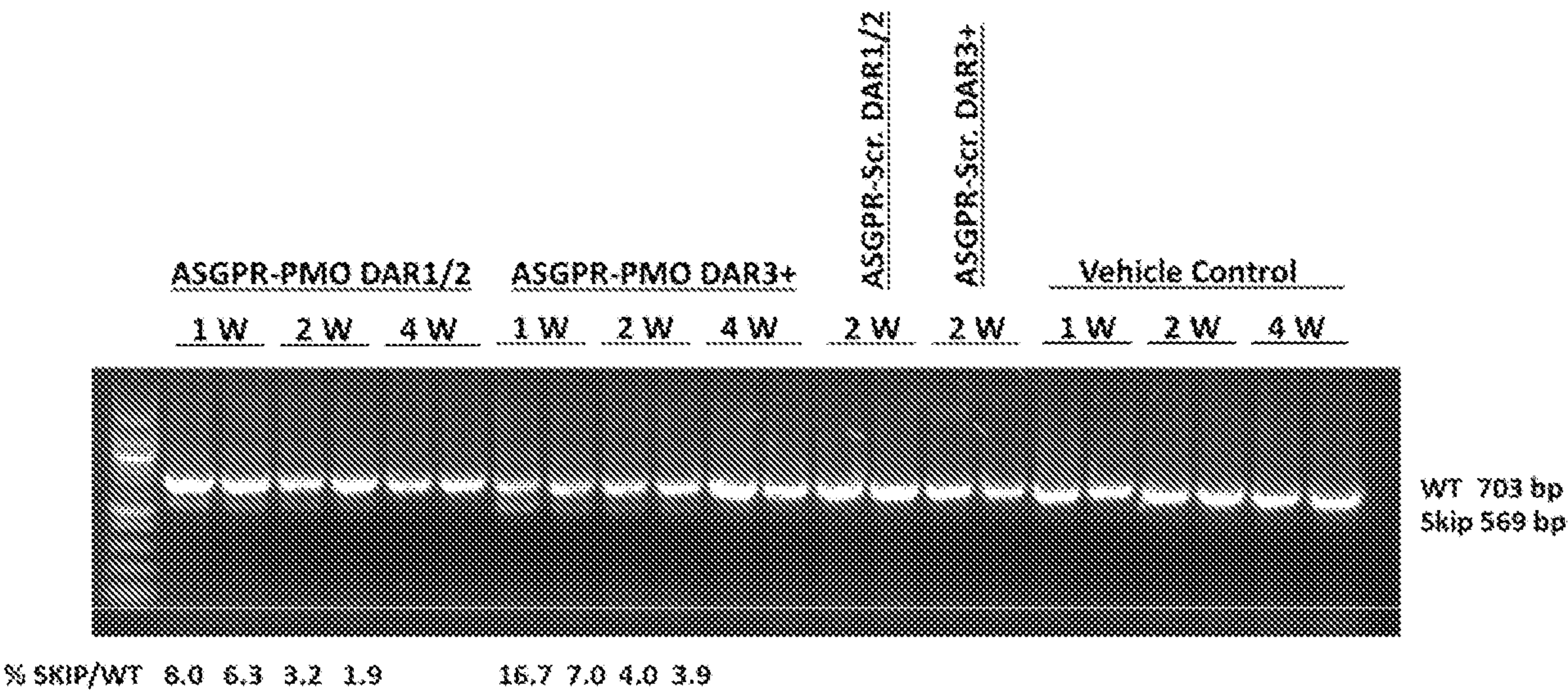


Fig. 18

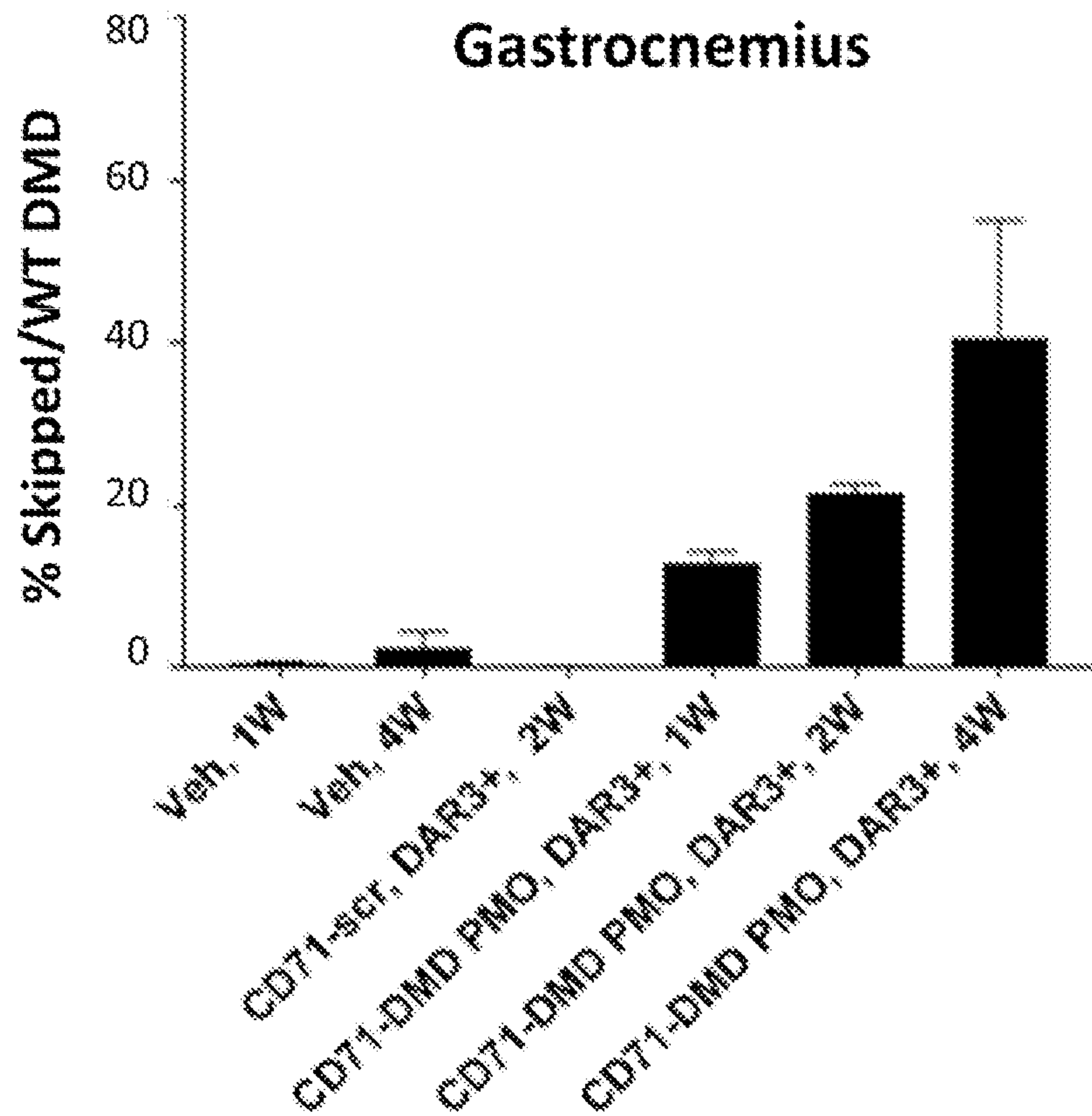


Fig. 15A