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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 October 2020 (01.10.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/198509 A3

(51) International Patent Classification:
A61K 31/7084 (2006.01) *A61K 31/711* (2006.01)
A61K 31/7088 (2006.01) *A61K 31/7125* (2006.01)
A61K 31/7105 (2006.01)

(21) International Application Number:
PCT/US2020/025017

(22) International Filing Date:
26 March 2020 (26.03.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/824,136	26 March 2019 (26.03.2019)	US
62/826,454	29 March 2019 (29.03.2019)	US
62/864,792	21 June 2019 (21.06.2019)	US

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(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, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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(54) Title: MODIFIED OLIGONUCLEOTIDES WITH INCREASED STABILITY

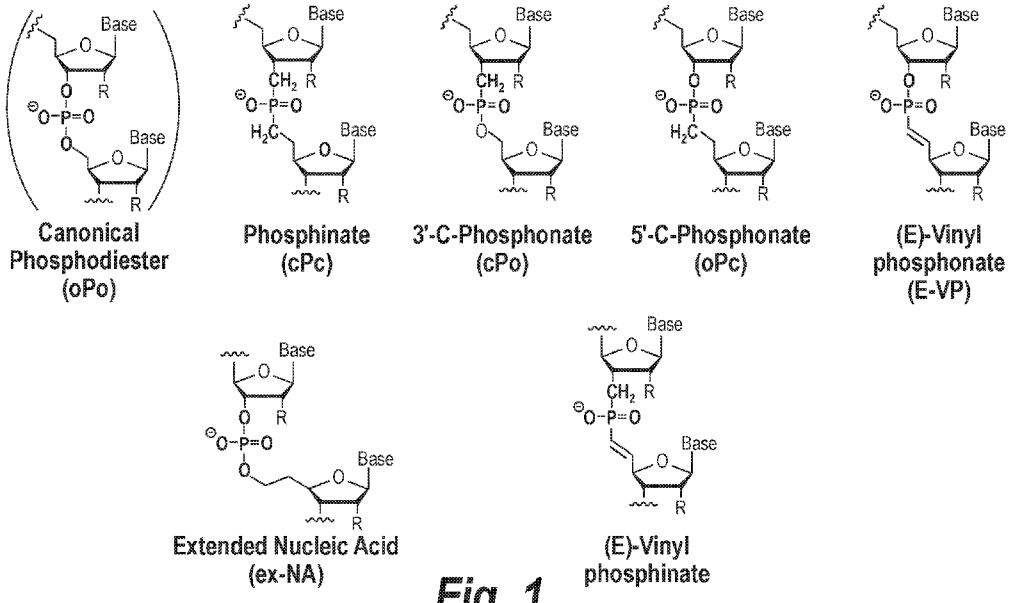

Fig. 1

(57) Abstract: This disclosure relates to novel modified oligonucleotides. Novel modified siRNA are also provided. In an aspect of the invention, the oligonucleotides and siRNA provided herein can be incorporated into a CRISPR/Cas system. In one embodiment, the present invention provides novel RNA silencing agents (e.g., siRNA and shRNAs), methods of making said RNA silencing agents, and methods (e.g., research and/or therapeutic methods) for using said improved RNA silencing agents (or portions thereof) for RNA silencing of for example, an ApoE, C9ORF72, or Htt protein.

[Continued on next page]

(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).

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))*

(88) Date of publication of the international search report:
05 November 2020 (05.11.2020)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025017

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-40 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025017

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 202, 256
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, and 257 to the extent that they read on Formula I, where W is O, X is OH, Y is S-, and Z is O; and Formula (1-1), where L is L1.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025017

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7084; A61K 31/7088; A61K 31/7105; A61K 31/711; A61K 31/7125 (2020.01)

CPC - A61K 31/7084; A61K 47/26; A61K 47/549; A61K 47/55; C07H 3/08; C07H 21/04; C12N 15/111; C12N 15/113; C12N 2310/11; C12N 2310/14; C12N 2310/351; C12N 2310/52 (2020.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0312367 A1 (UNIVERSITY OF MASSACHUSETTS) 02 November 2017 (02.11.2017) entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257
A	US 2010/0240730 A1 (BEIGELMAN et al) 23 September 2010 (23.09.2010) entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2020	18 SEP 2020

Name and mailing address of the ISA/US	Authorized officer
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Facsimile No. 571-273-8300	Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025017

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2001/0027251 A1 (COOK et al) 04 October 2001 (04.10.2001) entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257
A	SHUKLA et al. "Exploring Chemical Modifications for siRNA Therapeutics: A Structural and Functional Outlook," ChemMedChem, 19 February 2010 (19.02.2010), Vol. 5, Iss. 3, Pgs. 328-349. entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257
A	US 2011/0086905 A1 (GLAZER) 14 April 2011 (14.04.2011) entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257
P, A	ALTERMAN et al. "A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system," Nature Biotechnology, 02 August 2019 (02.08.2019), Vol. 37, Pgs. 884-894. entire document	1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, 257

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-201, 203-255, and 257-271 are drawn to modified oligonucleotides comprising modified internucleoside linkages of Formula (I), and compounds of Formula (1) comprising the modified oligonucleotides.

The first invention of Group I+ is restricted to a modified oligonucleotide, and compounds of Formula (1) comprising the same, wherein the modified oligonucleotide comprises a sense and antisense strand, and at least one modified intersubunit linkage of Formula (I); wherein the modified intersubunit linkage is selected to be the modified intersubunit linkage of Formula I, where W is O, X is OH, Y is S-, and Z is O; wherein the linkage is inserted on position 1-2 of the antisense strand, wherein the modified nucleoside in position 1 of the antisense strand is uridine and the modified nucleoside in position 2 of the antisense strand is uridine; and the antisense strand comprises a 5' terminal group selected to be R1; and the compound of Formula (1) is selected to be N-L-N (Formula 1-1), where L is an ethylene glycol chain of Formula L1; and N is the modified oligonucleotide (above) comprising the modified intersubunit linkage of Formula (I). It is believed that claims 1, 15, 19, 20, 23, 32, 34, 36, 41, 55, 59, 60, 63, 72, 74, 76, 81, 95, 99, 100, 103, 112, 114, 120, 125, 129, 138, 139, 143, 150, 152, 154, 159, 163, 173, 177, 184, 186, 192, 197-201, 216, 217, 219-221, 226, 244-251, 253, 254, and 257 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional modified internucleoside linkages of Formula (I) and/or compounds of Formula (1) to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a modified oligonucleotide, and compounds of Formula (1) comprising the same, wherein the modified oligonucleotide comprises a sense and antisense strand, and at least one modified intersubunit linkage of Formula (I); wherein the modified intersubunit linkage is selected to be the modified intersubunit linkage of Formula III, where W is CH2, X is OH, Y is S-, and Z is CH2; wherein the linkage is inserted on position 6-7 of the antisense strand, wherein the modified nucleoside in position 6 of the antisense strand is adenosine, and the modified nucleoside in position 7 of the antisense strand is adenosine; and the antisense strand comprises a 5' terminal group selected to be R2; and the compound of Formula (1) is selected to be Formula (1-3), where L is an ethylene glycol chain of Formula L2; and N is the modified oligonucleotide (above) comprising the modified intersubunit linkage of Formula (III). Additional modified internucleoside linkages of Formula (I) and/or compounds of Formula (1) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element for treating or managing a neurodegenerative disease, requiring the selection of alternatives for the modified internucleoside linkages of Formula (I) and/or compounds of Formula (1), where "[the] modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is selected from the group consisting of O, OCH2, OCH, CH2, and CH; each X is, independently, selected from the group consisting of halo, hydroxy, and C1-6 alkoxy; Y is selected from the group consisting of O-, OH, OR, NH-, NH2, S-, and SH; Z is selected from the group consisting of O and CH2; R is a protecting group; and ---- is an optional double bond" and "[the] compound of Formula (1): L-(N)n (1); wherein: L is selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof, wherein Formula (1) optionally further comprises one or more branch point Bp, and one or more spacer S, wherein Bp is independently for each occurrence a polyvalent organic species or derivative thereof; S is independently for each occurrence selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof; N is an RNA duplex comprising a sense strand and an antisense strand, wherein the sense strand and antisense strand each independently comprise one or more chemical modifications; and n is 2, 3, 4, 5, 6, 7 or 8, wherein at least one N includes a modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is selected from the group consisting of O, OCH2, OCH, CH2, and CH; each X is, independently, selected from the group consisting of halo, hydroxy, and C1-6 alkoxy; Y is selected from the group consisting of O-, OH, OR, NH-, NH2, S-, and SH; Z is selected from the group consisting of O and CH2; R is a protecting group; and ---- is an optional double bond".

Additionally, even if Groups I+ were considered to share the technical features of a modified oligonucleotide, said oligonucleotide having a 5' end and a 3' end, that is complementary to a target, wherein the oligonucleotide comprises a sense and antisense strand, and at least one modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is selected from the group consisting of O, OCH2, OCH, CH2, and CH; each X is, independently, selected from the group consisting of halo, hydroxy, and C1-6 alkoxy; Y is selected from the group consisting of O, OH, OR, NH-, NH2, S-, and SH; Z is selected from the group consisting of O and CH2; R is a protecting group; and ---- is an optional double bond; and a compound of Formula (1): L-(N)n (1); wherein: L is selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof, wherein Formula (1) optionally further comprises one or more branch point Bp, and one or more spacer S, wherein Bp is independently for each occurrence a polyvalent organic species or derivative thereof; S is independently for each occurrence selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof; N is an RNA duplex comprising a sense strand and an antisense strand, wherein the sense strand and antisense strand each independently comprise one or more chemical modifications; and n is 2, 3, 4, 5, 6, 7 or 8, wherein at least one N includes a modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is selected from the group consisting of O, OCH2, OCH, CH2, and CH; each X is, independently, selected from the group consisting of halo, hydroxy, and C1-6 alkoxy; Y is selected from the group consisting of O-, OH, OR, NH-, NH2, S-, and SH; Z is selected from the group consisting of O and CH2; R is a protecting group; and ---- is an optional double bond; these shared technical features do not represent a contribution over the prior art.

Specifically, US 2017/0312367 A1 to University of Massachusetts discloses a modified oligonucleotide (modified oligonucleotides, Para. [0074]), said oligonucleotide having a 5' end and a 3' end, that is complementary to a target, wherein the oligonucleotide comprises a sense and antisense strand (oligonucleotide ...is double-stranded and comprises a sense strand and an antisense strand, wherein the sense strand and the antisense strand each have a 5′ end and a 3′ end, Para. [0097]; the nucleic acid ...has complementarity to a target, Para. [0096]), and at least one modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is O; each X is hydroxy; Y is S-; and Z is O (the sense strand and the antisense strand each comprise one or more chemically-modified nucleotides. ...the nucleotides at positions 1 and 2 from the 5′ end of the sense and antisense strands are connected to adjacent nucleotides via phosphorothioate linkages, Para. [0100]; see also "phosphorothioates," Fig. 19, right-hand box); and a compound of Formula (1): L-(N)n (1) (provided herein is a compound of formula (I): L-(N)n (I), Para. [0102]); wherein: L is selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof, wherein Formula (1) optionally further comprises one or more branch point Bp, and one or more spacer S, wherein Bp is independently for each occurrence a polyvalent organic species or derivative thereof; S is independently for each occurrence selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof; N is an RNA duplex comprising a sense strand and an antisense strand, wherein the sense strand and antisense strand each independently comprise one or more chemical modifications; and n is 2, 3, 4, 5, 6, 7 or 8 (wherein L is selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof, wherein formula (I) optionally further comprises one or more branch point B, and one or more spacer S; wherein B is independently for each occurrence a polyvalent organic species or derivative thereof; S is independently for each occurrence selected from an ethylene glycol chain, an alkyl chain, a peptide, RNA, DNA, a phosphate, a phosphonate, a phosphoramidate, an ester, an amide, a triazole, and combinations thereof; N is an RNA duplex comprising a sense strand and an antisense strand, wherein the sense strand and antisense strand each independently comprise one or more chemical modifications; and n is 2, 3, 4, 5, 6, 7 or 8, Para. [0102]), wherein at least one N includes at least one modified intersubunit linkage of Formula (I); wherein: each B is, independently, a base pairing moiety; W is O; each X is hydroxy; Y is S-; and Z is O (the sense strand and the antisense strand each comprise one or more chemically-modified nucleotides. ...the nucleotides at positions 1 and 2 from the 5′ end of the sense and antisense strands are connected to adjacent nucleotides via phosphorothioate linkages, Para. [0100]; see also "phosphorothioates," Fig. 19, right-hand box).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.