CHIRAL DESIGN

Cross-Reference to Related Applications

[0001] This application claims priority to United States Provisional Application Serial No. 61/928,405, filed January 16, 2014, and 62/063,359, filed October 13, 2014, the entirety of each of which is incorporated herein by reference.

Background of the Invention

[0002] Oligonucleotides are useful in therapeutic, diagnostic, research and nanomaterials applications. The use of naturally occurring nucleic acids (e.g., unmodified DNA or RNA) for therapeutics can be limited, for example, because of their instability against extra- and intracellular nucleases and/or their poor cell penetration and distribution. Additionally, *in vitro* studies have shown that properties of antisense oligonucleotides such as binding affinity, sequence specific binding to the complementary RNA (Cosstick and Eckstein, 1985; LaPlanche *et al.,* 1986; Latimer *et al.,* 1989; Hacia *et al.,* 1994; Mesmaeker *et al.,* 1995), and stability to nucleases can be affected by the absolute stereochemical configurations of the phosphorus atoms (Cook, *et al.* US005599797A). Therefore, there is a need for new and improved oligonucleotides and oligonucleotide compositions, such as, e.g., new antisense and siRNA oligonucleotides and oligonucleotide compositions.

Summary

[0002a] In one aspect of the present disclosure, there is provided a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers,

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type; wherein:

the oligonucleotides of the particular oligonucleotide each comprise three or more chiral, modified phosphate linkages;

the common pattern of backbone chiral centers comprises from 5' to 3' $Rp(Sp)_2$;

the common base sequence has at least 17 bases; andthe common pattern of backbone chiral centers comprises at least 50% of backbone chiral centers in the *S*p conformation.

[0003] Among other things, the present disclosure encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Moreover, the present disclosure encompasses the insight that it is typically unlikely that a stereorandom oligonucleotide preparation will include every possible stereoisomer of the relevant oligonucleotide. Thus, among other things, the present disclosure provides new chemical entities that are particular stereoisomers of oligonucleotides of interest. That is, the present disclosure provides substantially pure preparations of single oligonucleotide compounds, where a particular oligonucleotide compound may be defined by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers.

[0004] The present disclosure demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity from each other. Moreover, the present disclosure demonstrates that stability improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of certain modified backbone linkages, bases, and/or sugars (e.g., through use of certain types of modified phophates, 2'-modifications, base modifications, etc.).

[0005] Among other things, the present disclosure recognizes that properties and activities of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers. In some embodiments, the present disclosure provides compositions of oligonucleotides, wherein the oligonucleotides have a common pattern of backbone chiral centers which, unexpectedly, greatly enhances the stability and/or biological activity of the oligonucleotides. In some embodiments, a pattern of backbone chiral centers provides increased stability. In some embodiments, a pattern of backbone chiral centers provides surprisingly increased activity. In some embodiments, a pattern of backbone chiral centers provides increased stability and activity. In some embodiments, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers of the oligonucleotide, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity between two target sequences differing only by a single nucleotide polymorphism (SNP).

Claims

1. A chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers,

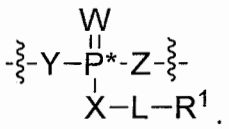
which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type; wherein:

the oligonucleotides of the particular oligonucleotide each comprise three or more chiral, modified phosphate linkages;

the common pattern of backbone chiral centers comprises from 5' to 3' *R*p(*S*p)$_2$;

the common base sequence has at least 17 bases; andthe common pattern of backbone chiral centers comprises at least 50% of backbone chiral centers in the *S*p conformation.

2. The composition of claim 1, wherein each chiral, modified phosphate linkage of the oligonucleotides of the particular oligonucleotide type independently has the structure of formula **I**:



(I)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

W is O, S or Se;

each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene,

C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each $-\overset{}{\underset{}{\lesssim}}-$ independently represents a connection to a nucleoside.

3. The composition of claim 2, wherein W is O, X is –S–, and Y and Z are –O–..

4. The composition of claim 2, wherein X is –S– and –L–R^1 is not hydrogen.

5. The composition of claim 2, wherein each chiral, modified phosphate linkage of the oligonucleotides of the particular oligonucleotide type is a phosphorothioate diester linkage.

6. The composition of claim 5, wherein the common pattern of backbone chiral centers comprises from 5' to 3' *S*p*S*p*R*p*S*p*S*p.

7. The composition of claim 5, wherein the pattern of backbone chiral centers comprises from 5' to 3' (*N*p)t(*R*p)n(*S*p)m, wherein t is 1, 2, 3, 4, 5, 6, 7 or 8, m is 2, 3, 4, 5, 6, 7 or 8, n is 1, and each *N*p is independent *R*p or *S*p.

8. The composition of claim 7, wherein Np is Sp.

9. The composition of claim 7, wherein t is greater than 5.

10. The composition of claim 9, wherein the pattern of backbone chiral centers comprises $(Sp)_2Rp(Sp)_2$

11. The composition of claim 5, wherein the oligonucleotides of the particular oligonucleotide type each comprise one or more phosphate diester linkages.

12. The composition of claim 5, wherein the nucleobases of the oligonucleotides of the particular oligonucleotide type are independently selected from adenine, thymine, cytosine, guanine, uracil and 5-methylcytosine.

13. The composition of claim 5, wherein the oligonucleotides of the particular oligonucleotide type each comprise a modified sugar.

14. The composition of claim 13, wherein the modified sugar comprises a 2'-modification.

15. The composition of claim 14, wherein the 2'-modification is 2'-OR^1, wherein R^1 is optionally substituted C_{1-6} aliphatic.

16. The composition of claim 14, wherein the 2'-modification is 2'-OMe.

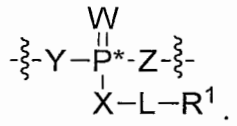
17. The composition of claim 14, wherein the 2'-modification is 2'-OCH_2CH_2OMe.

18. The composition of claim 5, wherein the oligonucleotides of the particular oligonucleotide type each comprise a locked nucleic acid sugar.

19. The composition of claim 13, wherein the modified sugar comprises a bivalent substituent –L–, wherein –L– is $-O-CH_2-$ between C_2 and C_4 of the sugar, wherein the $-CH_2-$ is optionally substituted.

20. The composition of claim 13, wherein the modified sugar comprises a bivalent substituent –L–, wherein –L– is $-O-CH_2(Et)-$ between C_2 and C_4 of the sugar.

21. The composition of claim 1, wherein the oligonucleotides of the particular oligonucleotide type each comprise at least one chiral, modified phosphate linkage having the structure of formula **I**:



(I)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

W is O, S or Se;

each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each  independently represents a connection to a nucleoside.

22. The composition of claim 21, wherein the chiral, modified phosphate linkage having the structure of formula **I** is a phosphorothioate diester linkage.

23. The composition of claim 22, wherein the oligonucleotides of the particular oligonucleotide type each comprise a modified sugar.

24. The composition of claim 23, wherein the modified sugar comprises a 2'-modification.

25. The composition of claim 24, wherein the 2'-modification is 2'-OR^1, wherein R^1 is optionally substituted C_{1-6} aliphatic.

26. The composition of claim 25, wherein the 2'-modification is 2'-OMe.

27. The composition of claim 25, wherein the 2'-modification is 2'-OCH_2CH_2OMe.

28. The composition of claim 23, wherein the modified sugar is a locked nucleic acid sugar.

29. The composition of claim 23, wherein the modified sugar comprises a bivalent substituent –L–, wherein –L– is $-O-CH_2-$ between C_2 and C_4 of the sugar, wherein the $-CH_2-$ is optionally substituted.

30. The composition of claim 23, wherein the modified sugar comprises a bivalent substituent –L–, wherein –L– is $-O-CH_2(Et)-$ between C_2 and C_4 of the sugar.

31. The composition of claim 5, wherein at least about 20% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

32. The composition of claim 5, wherein the common base sequence has at least 19 bases.

33. The composition of claim 5, wherein the common base sequence has at least 20 bases.

34. The composition of claim 8, wherein m is 3, 4, 5, 6, 7 or 8.

35. The composition of claim 8, wherein t is 6, 7 or 8.