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(54) Title: COMPOSITIONS AND METHODS FOR MODULATION OF MCL-1 EXPRESSION

(57) Abstract: Disclosed herein are compounds, compositions and methods for modulating the expression of Mcl-1 in a cell, tissue or animal. Also provided are methods of target validation. Also provided are uses of disclosed compounds and compositions in the manufacture of a medicament for treatment of diseases and disorders.

COMPOSITIONS AND METHODS FOR MODULATION OF MCL-1 EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is an non-provisional application of U.S. Provisional Patent Application No. 60/783,505, entitled COMPOSITIONS AND METHODS FOR MODULATION OF MCL-1 EXPRESSION, filed March 16, 2006, the disclosure of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0079-SEQ.TXT, created March 16, 2007, which is 84.3 Kb in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] Disclosed herein are compounds, compositions and methods for modulating the expression of Mcl-1 in a cell, tissue or animal.

BACKGROUND

[0004] The Bcl-2 family of proteins, which plays an important role in regulating cell survival and cell death, can be divided into two groups, proapoptotic proteins (Bak and Bak) and anti-apoptotic proteins (Bcl-2, Bcl-x_L, A1, Mcl-1, and Bcl-w) (Adams *et al.* (1998) *Science 281*:1322-1326; Henson *et al.* (2006) *Clin. Cancer Res. 12(3)*:845-853). Antiapoptotic family member Mcl-1 was first characterized from a myeloid leukemia cell line (ML-1) induced to differentiate along the monocytic lineage (Kozopas *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*:3516-3520; Leuenroth *et al.* (2000) *J. Leukoc. Biol. 68*:158-166) and has since been shown to be expressed in a wide variety of tissues and neoplastic cells and to influence the development of numerous malignancies (Thallinger *et al.* (2004) *Clin. Cancer Res. 10*:4185-4191).

[0005] The role of Mcl-1 in regulating cell fate has made it a target of interest in many studies of apoptosis and hyperproliferative diseases. Many reports have demonstrated the importance of inhibiting Mcl-1 expression to increase apoptosis and regulate neoplastic disease.

[0006] U.S. Patent No. 6,001,992 discloses antisense oligonucleotides for inhibition of expression of anti-apoptotic bcl-2 related proteins, including Mcl-1.

[0007] U.S. Patent No. 6,800,750 discusses Mcl-1 regulatory elements, oligonucleotide primers which hybridize to Mcl-1 and methods of modulating apoptosis of a cell by modulating expression of Mcl-1.

[0008] WO 94/29330 discloses Mcl-1 polypeptide and polynucleotide sequences and discusses methods of treating a subject having a cell proliferative disorder.

[0009] Several studies have reported use of Mcl-1 antisense oligonucleotides or siRNA to inhibit Mcl-1 expression, increase apoptosis, decrease cell viability and/or decrease tumor weight of normal cells, cancer cell lines or xenograft tumors. Henson *et al.* ((2006) *Clin. Cancer Res. 12(3)*:845-853) disclose a Mcl-1 antisense oligonucleotide which sensitizes breast cancer cell lines to apoptosis. Selzer *et al.* ((2002) *Mol. Med. 8(12)*:877-884) disclose inhibition of Mcl-1 expression in melanoma cells using an antisense oligonucleotide targeting Mcl-1 and Skvara *et al.* ((2005) *Anticancer Res. 25(4)*:2697-2703) show a Mcl-1 antisense oligonucleotide which sensitizes human melanoma cells to ionizing radiation-induced apoptosis. Sieghart *et al.* ((2006) *J. Hepatol. 44(1)*:151-157) discuss the finding the Mcl-1 is overexpressed in hepatocellular carcinoma cells lines and disclose a Mcl-1 antisense oligonucleotide which decreases protein expression, increases apoptosis, decreases cell survival and sensitizes HCC cells to chemotherapy. Similarly, Song *et al.* ((2005) *Cancer Biol. Ther. 4(3)*:267-276) show that Mcl-1 is overexpressed in human lung cancer cells and treatment with a Mcl-1 antisense oligonucleotide resulted in an increase in apoptosis. Mcl-1 inhibition also caused sensitization of the lung cancer cells to apoptosis induced by chemotherapeutic agents and radiation.

[0010] Aichberger *et al.* ((2005) *Blood 105(8)*:3303-3011) discuss a Mcl-1 antisense oligonucleotide and siRNA which inhibit expression of Mcl-1 in chronic myeloid leukemia cells and decreased cell viability. Mcl-1 antisense oligonucleotide synergized with the BCR/ABL inhibitor Imatinib to produce growth arrest. Derenne *et al.* ((2002) *Blood 100(1)*:194-199) and Zhang *et al.* ((2002) *Blood 99(6)*:1885-1893) discuss use of Mcl-1 antisense oligonucleotide to decrease expression of Mcl-1, decrease cell viability and increase apoptosis of multiple myeloma cells lines and primary cells.

[0011] Mcl-1 has also been shown to exhibit increased expression in a variety of hematopoietic cells lines, including B cells, monocytes, macrophages and polymorphonuclear cells, and treatment of these cells with Mcl-1 antisense oligonucleotide reduces target expression and increases apoptosis (Michels *et al.* (2004) *Oncogene 23(28)*:4818-4827; Sly *et al.* (2003) *J. Immunol. 170(1)*:430-437; Liu *et al.* (2001) *J. Exp. Med. 194(2)*:113-126; Moulding *et al.* (2000) *Blood 96(5)*:1756-1763; and Leuenroth *et al.* (2000) *J. Leukoc. Biol. 68*:158-166).

[0012] Thallinger *et al.* ((2004) *Clin. Cancer Res. 10*:4185-4191) disclose an antisense oligonucleotide targeted to Mcl-1 which decreases expression of Mcl-1 in sarcoma xenotransplants and when used in combination with cyclophosphamide, reduces tumor weight and increases tumor cell apoptosis. Thallinger et al. ((2003) *J. Invest. Dermatol. 120(6)*:1081-1086) show a Mcl-1 antisense oligonucleotide administered systemically with or without dacarbazine in a human melanoma SCID mouse xenotransplantation model decreased target protein expression, increased apoptosis and decreased tumor weight.

[0013] Given the role of Mcl-1 in a variety of disorders, including hyperproliferative disorders, an efficient method of modulating expression of Mcl-1 is desirable. Antisense technology is an effective means for reducing the expression of one or more specific gene products and is uniquely useful in a number of therapeutic, diagnostic, and research applications. Provided herein are antisense compounds for use in modulation of Mcl-1 expression.

SUMMARY

[0014] Provided herein are oligomeric compounds targeting Mcl-1. Also provided are methods of modulating expression of Mcl-1 in cells, tissue or animals using oligomeric compounds targeting Mcl-1. Further provided are methods of increasing apoptosis and methods of decreasing cell proliferation using said compounds.

[0015] In certain jurisdictions, there may not be any generally accepted definition of the term "comprising." As used herein, the term "comprising" is intended to represent "open" language which permits the inclusion of any additional elements. With this in mind, additional embodiments of the present inventions are described with reference to the numbered paragraphs below:

[0016] 1. An oligomeric compound 12 to 50 nucleobases in length comprising at least an 8-nucleobase portion of a sequence selected from the group consisting of SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120.

[0017] 2. The compound of paragraph 1 which is 20 nucleobases in length and has a sequence selected from the group consisting of SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120.

[0018] 3. The compound of paragraph 1 comprising at least an 8-nucleobase portion of a sequence selected from the group consisting of SEQ ID NO: 64, 86, 87, 92, 95 and 96.

[0019] 4. The compound of paragraph 3 having a sequence selected from the group consisting of SEQ ID NO: 64, 86, 87, 92, 95 and 96.

[0020] 5. The compound of paragraph 1 which is at least 80% complementary to a nucleic acid molecule encoding human Mcl-1.

[0021] 6. The compound of paragraph 5 which is at least 90% complementary to a nucleic acid molecule encoding human Mcl-1.

[0022] 7. The compound of paragraph 6 which is at least 95% complementary to a nucleic acid molecule encoding human Mcl-1.

[0023] 8. The compound of paragraph 7 which is 100% complementary to a nucleic acid molecule encoding human Mcl-1.

[0024] 9. The compound of any one of paragraphs 5-8 wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 and 5.

[0025] 10. An oligomeric compound 16 to 50 nucleobases in length having at least 80% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

[0026] 11. The compound of paragraph 10 which is 17 to 50 nucleobases in length and has at least 85% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

[0027] 12. The compound of paragraph 10 which is 18 to 50 nucleobases in length and has at least 90% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

[0028] 13. The compound of paragraph 10 which is 19 to 50 nucleobases in length and has at least 95% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

[0029] 14. An oligomeric compound 12 to 50 nucleobases in length targeted to a nucleic acid molecule encoding human Mcl-1 (SEQ ID NO: 2), wherein said compound specifically hybridizes to at least a portion of a target region defined by nucleobases 123-150; 719-748; 808-839; 902-930; 902-1007; 938-1007; 1039-1074; 1083-1150; 1104-1128; 1252-1297; 1396-1423; 1651-1693; 1809-1851; 2062-2103; 2551-2585; 3118-3161; 3176-3202; or 3214-3254 of said nucleic acid molecule encoding human Mcl-1.

[0030] 15. The compound of any one of paragraphs 1, 3, 5, 6, 7 or 14 which is 15 to 30 nucleobases in length.

[0031] 16. The compound of any of paragraphs 1-15 comprising at least one modified sugar moiety, internucleoside linkage or nucleobase.

[0032] 17. The compound of paragraph 16, wherein the modified sugar moiety is 2'-O-methoxyethyl.

[0033] 18. The compound of paragraph 16, wherein the modified nucleobase is phosphorothioate.

[0034] 19. The compound of paragraph 16, wherein the modified nucleobase is 5-methylcytosine.

[0035] 20. A method of modulating expression of human Mcl-1 in a cell, tissue or animal, comprising administering to said cell, tissue or animal the oligomeric compound of any one of paragraphs 1-19.

[0036] 21. A method of inducing apoptosis of a cell, comprising administering to said cell the oligomeric compound of any one of paragraphs 1-19.

[0037] 22. A method of inhibiting proliferation of a cell, comprising administering to said cell the oligomeric compound of any one of paragraphs 1-19.

[0038] 23. A compound according to any one of paragraphs 1-19 for use in therapy.

[0039] 24. Use of a compound according to any one of paragraphs 1-19 for the preparation of a medicament for modulating expression of human Mcl-1.

[0040] 25. Use of a compound according to any one of paragraphs 1-19 for the preparation of a medicament for inducing apoptosis.

[0041] 26. Use of a compound according to any one of paragraphs 1-19 for the preparation of a medicament for inhibiting cellular proliferation.

[0042] 27. The use of any one of paragraphs 24-26, wherein the modulating, inducing or inhibiting occurs in a human cell.

DETAILED DESCRIPTION

[0043] Antisense technology is an effective means for reducing the expression of one or more specific gene products and is uniquely useful in a number of therapeutic, diagnostic, and research applications. Provided herein are antisense compounds useful for modulating gene expression and associated pathways via antisense mechanisms of action based on target degradation or target occupancy.

[0044] The principle behind antisense technology is that an antisense compound, which hybridizes to a target nucleic acid, modulates gene expression activities such as transcription, splicing or translation through one of a number of antisense mechanisms. The sequence specificity of antisense compounds makes them extremely attractive as tools for target validation and gene functionalization, as well as therapeutics to selectively modulate the expression of genes involved in disease.

[0045] Mcl-1 is known to play a significant role in cell viability. Overexpression of Mcl-1 has been associated with a variety of hyperproliferative disorders, including cancer (such as, for example, sarcomas, myelomas, melanomas, lymphomas, leukemias and carcinomas) and autoimmune disease (such as, for example, rheumatoid arthritis). Identification of compounds to modulate of Mcl-1 expression is critical. Thus, provided herein are antisense compounds targeting Mcl-1 which modulate expression of Mcl-1.

[0046] As used herein, the terms "target nucleic acid" and "nucleic acid molecule encoding Mcl-1" have been used for convenience to encompass DNA encoding Mcl-1, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA.

[0047] As used herein, "targeting" or "targeted to" refer to the process of designing an oligomeric compound such that the compound hybridizes with a selected nucleic acid molecule.

[0048] As used herein, "hybridization" means the pairing of complementary strands of oligomeric compounds. In the context of the present invention, an oligomeric compound is specifically hybridizable when there is a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target nucleic acid sequences.

[0049] As used herein, "antisense mechanisms" are all those involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

[0050] In accordance with the present invention are compositions and methods for modulating the expression of Mcl-1 (also known as M yeloid cell differentiation protein-1). Listed in Table 1 are GENBANK® accession numbers of sequences used to design oligomeric compounds targeted to Mcl-1. Oligomeric compounds of the invention include oligomeric compounds which hybridize with one or more target nucleic acid molecules shown in Table 1, as well as oligomeric compounds which hybridize to other nucleic acid molecules encoding Mcl-1. The oligomeric compounds may target any region, segment, or site of nucleic acid molecules which encode Mcl-1. Suitable target regions, segments, and sites include, but are not limited to, the 5'UTR, the start codon, the stop codon, the coding region, the 3'UTR, the 5'cap region, introns, exons, intron-exon junctions, exon-intron junctions, and exon-exon junctions.

Table 1
Gene Target Names and Sequences

Target Name	Species	Genbank® #	SEQ ID NO
Mcl-1	Human	L08246.1	1
Mcl-1	Human	NM_021960.3	2
Mcl-1	Human	NM_182763.1	3
Mcl-1	Human	Complement of AI439011.1	4
Mcl-1	Human	Complement of NT_004487.17 (nucleotides 1036000 to 1044000)	5
Mcl-1	Mouse	BC005427.1	6
Mcl-1	Mouse	NM_008562.2	7
Mcl-1	Mouse	NT_039238.1 (nucleotides 1444531 to 1448899)	8
Mcl-1	Mouse	NT_039238.3 (nucleotides 3538000 to 3546000)	9
Mcl-1	Mouse	U35623.1	10

[0051] The term "oligomeric compound" refers to a polymeric structure capable of hybridizing to a region of a nucleic acid molecule. This term includes oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics and chimeric combinations of these. Oligomeric compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular. Moreover, branched structures are known in the art. An "antisense compound" or "antisense oligomeric compound" refers to an oligomeric compound that is at least partially complementary to the region of a nucleic acid molecule to which it hybridizes and which modulates (increases or decreases) its expression. Consequently, while all antisense compounds can be said to be oligomeric compounds, not all oligomeric compounds are antisense compounds. An "antisense oligonucleotide" is an antisense compound that is a nucleic acid-based oligomer. An antisense oligonucleotide can be chemically modified. Nonlimiting examples of oligomeric compounds include primers, probes, antisense compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides and alternate splicers. In one embodiment, the oligomeric compound comprises an antisense strand hybridized to a sense strand. Oligomeric compounds can be introduced in the form of single-stranded, double-stranded, circular, branched or hairpins and can contain structural elements such as internal or terminal bulges or loops. Oligomeric double-stranded compounds can be two strands hybridized to form double-stranded compounds or a single strand with sufficient self complementarity to allow for hybridization and formation of a fully or partially double-stranded compound.

[0052] The oligomeric compounds in accordance with this invention comprise compounds from about 8 to about 80 nucleobases (i.e. from about 8 to about 80 linked nucleosides). One of ordinary skill in the art will appreciate that this comprehends antisense compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 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, 79, or 80 nucleobases.

[0053] In one embodiment, the antisense compounds of the invention comprise 13 to 80 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 13, 14, 15, 16, 17, 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, 79, or 80 nucleobases.

[0054] In one embodiment, the antisense compounds of the invention comprise 12 to 50 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 12, 13, 14, 15, 16, 17, 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, or 50 nucleobases.

[0055] In one embodiment, the antisense compounds of the invention comprise 12 to 30 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleobases.

[0056] In some embodiments, the antisense compounds of the invention comprise 15 to 30 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleobases.

[0057] In one embodiment, the antisense compounds of the invention comprise 20 to 30 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases.

[0058] In one embodiment, the antisense compounds of the invention comprise 20 to 24 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 20, 21, 22, 23, or 24 nucleobases.

[0059] In one embodiment, the antisense compounds of the invention comprise 16 to 20 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 16, 17, 18, 19 or 20 nucleobases.

[0060] In one embodiment, the antisense compounds of the invention comprise 20 nucleobases.

[0061] In one embodiment, the antisense compounds of the invention comprise 19 nucleobases.

[0062] In one embodiment, the antisense compounds of the invention comprise 18 nucleobases.

[0063] In one embodiment, the antisense compounds of the invention comprise 17 nucleobases.

[0064] In one embodiment, the antisense compounds of the invention comprise 16 nucleobases.

[0065] In one embodiment, the antisense compounds of the invention comprise 15 nucleobases.

[0066] In one embodiment, the antisense compounds of the invention comprise 14 nucleobases.

[0067] In one embodiment, the antisense compounds of the invention comprise 13 nucleobases.

[0068] Antisense compounds 8-80 nucleobases in length, and any length within the range, comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative antisense compounds are considered to be suitable antisense compounds.

[0069] Compounds of the invention include oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of one of the illustrative antisense compounds (the remaining nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately upstream of the 5'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases). Other compounds are represented by oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative antisense compounds (the remaining nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately downstream of the 3'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases). It is also understood that compounds may be represented by oligonucleotide sequences that comprise at least 8 consecutive nucleobases from an internal portion of the sequence of an illustrative compound, and may extend in either or both directions until the oligonucleotide contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases.

[0070] One having skill in the art armed with the antisense compounds illustrated herein will be able, without undue experimentation, to identify further antisense compounds.

[0071] "Hybridization" means the pairing of complementary strands of oligomeric compounds. While not limited to a particular mechanism, the most common mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

[0072] An oligomeric compound is specifically hybridizable when there is a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

[0073] "Stringent hybridization conditions" or "stringent conditions" refer to conditions under which an oligomeric compound will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances, and "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated.

[0074] "Complementarity," as used herein, refers to the capacity for precise pairing between two nucleobases on one or two oligomeric compound strands. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA or RNA are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

[0075] It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure). The oligomeric compounds of the present invention comprise at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an oligomeric compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an oligomeric compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an oligomeric compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, *Genome Res.*, 1997, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482-489).

[0076] Oligomeric compounds, or a portion thereof, may have a defined percent identity to a SEQ ID NO, or a compound having a specific ISIS number. The oligomeric compounds of the present invention may have, for example, 80%, 85%, 90%, 95% or 100% identity with a specified compound. This identity may be over the entire length of the oligomeric compound, or in a portion of the oligomeric compound (e.g., nucleobases 1-20 of a 27-mer may be compared to a 20-mer to determine percent identity of the oligomeric compound to the SEQ ID NO). It is understood by those skilled in the art that an oligonucleotide need not have an identical sequence to those described herein to function similarly to the oligonucleotides described herein. Shortened (i.e., deleted, and therefore non-identical) versions of oligonucleotides taught herein, or non-identical (i.e., one base replaced with another) versions of the oligonucleotides taught herein fall within the scope of the invention. Percent identity is calculated according to the number of bases that are identical to the SEQ ID NO or compound to which it is being compared. The non-identical bases may be adjacent to each other, dispersed through out the oligonucleotide, or both.

[0077] For example, a 16-mer having the same sequence as nucleobases 2-17 of a 20-mer is 80% identical to the 20-mer. Alternatively, a 20-mer containing four nucleobases not identical to the 20-mer is also 80% identical to the 20-mer. A 14-mer having the same sequence as nucleobases 1-14 of an 18-mer is 78% identical to the 18-mer. Such calculations are well within the ability of those skilled in the art.

[0078] The percent identity is based on the percent of nucleobases in the original sequence present in a portion of the modified sequence. Therefore, a 30 nucleobase oligonucleotide comprising the full sequence of a 20 nucleobase SEQ ID NO would have a portion of 100% identity with the 20 nucleobase SEQ ID NO while further comprising an additional 10 nucleobase portion. In the context of the invention, the full length of the modified sequence may constitute a single portion.

[0079] The oligomeric compounds of the invention also include compounds in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, compounds may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the oligomeric compound. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of Mcl-1 mRNA.

[0080] As used herein, "targeting" or "targeted to" refer to the process of designing an oligomeric compound such that the compound hybridizes with a selected nucleic acid molecule. Targeting an oligomeric compound to a particular target nucleic acid molecule can be a multistep process. The process usually begins with the identification of a target nucleic acid whose expression is to be modulated. As used herein, the terms "target nucleic acid" and "nucleic acid encoding Mcl-1" encompass DNA encoding Mcl-1, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. For example, the target nucleic acid can be a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. As disclosed herein, the target nucleic acid encodes Mcl-1.

[0081] The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. "Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Target regions may include, for example, a particular exon or intron, or may include only selected nucleobases within an exon or intron which are identified as appropriate target regions. In some instances, an appropriate target region is defined by the range of nucleobases of a target nucleic acid to which one or more overlapping oligomeric compounds hybridize. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as unique nucleobase positions within a target nucleic acid. As used herein, the "target site" of an oligomeric compound is the 5'-most nucleotide of the target nucleic acid to which the compound binds.

[0082] Since, as is known in the art, the translation initiation codon is typically 5' AUG (in transcribed mRNA molecules; 5' ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon." A minority of genes have a translation initiation codon having the RNA sequence 5' GUG, 5' UUG or 5' CUG, and 5' AUA, 5' ACG and 5' CUG have been shown to function *in vivo*. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. "Start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA transcribed from a gene encoding a protein, regardless of the sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5' UAA, 5' UAG and 5' UGA (the corresponding DNA sequences are 5' TAA, 5' TAG and 5' TGA, respectively).

[0083] The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions which may be targeted effectively with oligomeric compounds of the invention.

[0084] The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, one region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

[0085] Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. The 5' cap region is also a target.

[0086] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence, resulting in exon-exon junctions at the site where exons are joined. Targeting exon-exon junctions can be useful in situations where aberrant levels of a normal splice product are implicated in disease, or where aberrant levels of an aberrant splice product are implicated in disease. Targeting splice sites, i.e., intron-exon junctions or exon-intron junctions can also be particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also suitable targets. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts" and are also suitable targets. It is also known that introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA. Single-stranded antisense compounds such as oligonucleotide compounds that work via an RNase H mechanism are effective for targeting pre-mRNA. Antisense compounds that function via an occupancy-based mechanism are effective for redirecting splicing as they do not, for example, elicit RNase H cleavage of the mRNA, but rather leave the mRNA intact and promote the yield of desired splice product(s).

[0087] It is also known in the art that alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants." More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence. Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants." Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants." If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

[0088] It is also known in the art that variants can be produced through the use of alternative signals to start or stop transcription and that pre-mRNAs and mRNAs can possess more that one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Consequently, the types of variants described herein are also suitable target nucleic acids.

[0089] As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base (sometimes referred to as a "nucleobase" or simply a "base"). The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[0090] Specific examples of oligomeric compounds useful of the present invention include oligonucleotides containing modified e.g. non-naturally occurring internucleoside linkages. As defined in this specification, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[0091] Oligomeric compounds can have one or more modified internucleoside linkages. Modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkyl-phosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkyl-phosphotriesters, phosphonoacetate and thiophosphonoacetate (see Sheehan *et al.*, *Nucleic Acids Research*, 2003, *31(14)*, 4109-4118 and Dellinger *et al.*, *J. Am. Chem. Soc.*, 2003, *125*, 940-950), selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage, i.e., a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

[0092] N3'-P5'-phosphoramidates have been reported to exhibit both a high affinity towards a complementary RNA strand and nuclease resistance (Gryaznov *et al.*, *J. Am. Chem. Soc.*, **1994**, *116*, 3143-3144). N3'-P5'-phosphoramidates have been studied with some success *in vivo* to specifically down regulate the expression of the *c-myc* gene (Skorski *et al., Proc. Natl. Acad. Sci.,* 1997, 94, 3966-3971; and Faira *et al., Nat. Biotechnol.,* **2001,** *19,* 40-44).

[0093] Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050.

[0094] In some embodiments of the invention, oligomeric compounds may have one or more phosphorothioate and/or heteroatom internucleoside linkages, in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ (known as a methylene (methylimino) or MMI backbone), $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ (wherein the native phosphodiester internucleotide linkage is represented as $-O-P(=O)(OH)-O-CH_2-$). The MMI type internucleoside linkages are disclosed in the above referenced U.S. patent 5,489,677. Amide internucleoside linkages are disclosed in the above referenced U.S. patent 5,602,240.

[0095] Some oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0096] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439.

[0097] Oligomeric compounds may also contain one or more substituted sugar moieties. Suitable compounds can comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Also suitable are $O((CH_2)_nO)_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON((CH_2)_nCH_3)_2$, where n and m are from 1 to about 10. Other oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. One modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, **1995**, *78*, 486-504), i.e., an alkoxyalkoxy group. A further modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-$(CH_2)_2$-O-$(CH_2)_2$-$N(CH_3)_2$, also described in examples hereinbelow.

[0098] Other modifications include 2'-methoxy (2'-O-CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$), 2'-allyl (2'-CH_2-$CH=CH_2$), 2'-O-allyl (2'-O-CH_2-$CH=CH_2$) and 2'-fluoro (2'-F). The 2'-modification may be in the arabino (up) position or ribo (down) position. One 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Antisense compounds may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; 5,700,920; and, 6,147,200.

[0099] The terms used to describe the conformational geometry of homoduplex nucleic acids are "A Form" for RNA and "B Form" for DNA. In general, RNA:RNA duplexes are more stable and have higher melting temperatures (Tm's) than DNA:DNA duplexes (Sanger et al., Principles of Nucleic Acid Structure, 1984, Springer-Verlag; New York, NY.; Lesnik et al., Biochemistry, 1995, 34, 10807-10815; Conte et al., Nucleic Acids Res., 1997, 25, 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved base stacking interactions that result from an A-form geometry (Searle et al., Nucleic Acids Res., 1993, 21, 2051-2056). The presence of the 2' hydroxyl in RNA biases the sugar toward a C3' endo pucker, i.e., also designated as Northern pucker, which causes the duplex to favor the A-form geometry. In addition, the 2' hydroxyl groups of RNA can form a network of water mediated hydrogen bonds that help stabilize the RNA duplex (Egli et al., Biochemistry, 1996, 35, 8489-8494). On the other hand, deoxy nucleic acids prefer a C2' endo sugar pucker, i.e., also known as Southern pucker, which is thought to impart a less stable B-form geometry (Sanger et al., Principles of Nucleic Acid Structure, 1984, Springer-Verlag; New York, NY). As used herein, B-form geometry is inclusive of both C2'-endo pucker and O4'-endo pucker.

[0100] The structure of a hybrid duplex is intermediate between A- and B-form geometries, which may result in poor stacking interactions (Lane et al., Eur. J. Biochem., 1993, 215, 297-306; Fedoroff et al., J. Mol. Biol., 1993, 233, 509-523; Gonzalez et al., Biochemistry, 1995, 34, 4969-4982; Horton et al., J. Mol. Biol., 1996, 264, 521-533). Consequently, compounds that favor an A-form geometry can enhance stacking interactions, thereby increasing the relative Tm and potentially enhancing a compound's antisense effect.

[0101] In one aspect of the present invention oligomeric compounds include nucleosides synthetically modified to induce a 3'-endo sugar conformation. A nucleoside can incorporate synthetic modifications of the heterocyclic base, the sugar moiety or both to induce a desired 3'-endo sugar conformation. These modified nucleosides are used to mimic RNA-like nucleosides so that particular properties of an oligomeric compound can be enhanced while maintaining the desirable 3'-endo conformational geometry.

[0102] There is an apparent preference for an RNA type duplex (A form helix, predominantly 3'-endo) as a requirement (e.g. trigger) of RNA interference which is supported in part by the fact that duplexes composed of 2'-deoxy-2'-F-nucleosides appears efficient in triggering RNAi response in the C. elegans system. Properties that are enhanced by using more stable 3'-endo nucleosides include but are not limited to: modulation of pharmacokinetic properties through modification of protein binding, protein off-rate, absorption and clearance; modulation of nuclease stability as well as chemical stability; modulation of the binding affinity and specificity of the oligomer (affinity and specificity for enzymes as well as for complementary sequences); and increasing efficacy of RNA cleavage. Also provided herein are oligomeric triggers of RNAi having one or more nucleosides modified in such a way as to favor a C3'-endo type conformation.

[0103] Nucleoside conformation is influenced by various factors including substitution at the 2', 3' or 4'-positions of the pentofuranosyl sugar. Electronegative substituents generally prefer the axial positions, while sterically demanding substituents generally prefer the equatorial positions (Principles of Nucleic Acid Structure, Wolfgang Sanger, 1984, Springer-Verlag.) Modification of the 2' position to favor the 3'-endo conformation can be achieved while maintaining the 2'-OH as a recognition element (Gallo et al., Tetrahedron (2001), 57, 5707-5713. Harry-O'kuru et al., J. Org. Chem., (1997), 62(6), 1754-1759 and Tang et al., J. Org. Chem. (1999), 64, 747-754.) Alternatively, preference for the 3'-endo conformation can be achieved by deletion of the 2'-OH as exemplified by 2'deoxy-2'F-nucleosides (Kawasaki et al., J. Med. Chem. (1993), 36, 831-841), which adopts the 3'-endo conformation positioning the electronegative fluorine atom in the axial position. Representative 2'-substituent groups amenable to the present invention that give A-form conformational properties (3'-endo) to the resultant duplexes include 2'-O-alkyl, 2'-O-substituted alkyl and 2'-fluoro substituent groups. Other suitable substituent groups are various alkyl and aryl ethers and thioethers, amines and monoalkyl and dialkyl substituted amines.

[0104] Other modifications of the ribose ring, for example substitution at the 4'-position to give 4'-F modified nucleosides (Guillerm et al., Bioorganic and Medicinal Chemistry Letters (1995), 5, 1455-1460 and Owen et al., J. Org. Chem. (1976), 41, 3010-3017), or for example modification to yield methanocarba nucleoside analogs (Jacobson et al., J. Med. Chem. Lett. (2000), 43, 2196-2203 and Lee et al., Bioorganic and Medicinal Chemistry Letters (2001), 11, 1333-1337) also induce preference for the 3'-endo conformation. Along similar lines, triggers of RNAi response might be composed of one or more nucleosides modified in such a way that conformation is locked into a C3'-

endo type conformation, i.e. Locked Nucleic Acid (LNA, Singh et al, Chem. Commun. (1998), 4, 455-456), and ethylene bridged Nucleic Acids (ENA™, Morita et al, Bioorganic & Medicinal Chemistry Letters (2002), 12, 73-76.)

[0105] It is further intended that multiple modifications can be made to one or more of the oligomeric compounds of the invention at multiple sites of one or more monomeric subunits (nucleosides are suitable) and or internucleoside linkages to enhance properties such as but not limited to activity in a selected application.

[0106] The synthesis of numerous of the modified nucleosides amenable to the present invention are known in the art (see for example, Chemistry of Nucleosides and Nucleotides Vol 1-3, ed. Leroy B. Townsend, 1988, Plenum press). The conformation of modified nucleosides and their oligomers can be estimated by various methods routine to those skilled in the art such as molecular dynamics calculations, nuclear magnetic resonance spectroscopy and CD measurements.

[0107] Another group of oligomeric compounds includes oligonucleotide mimetics. The term "mimetic" as it is applied to oligonucleotides includes oligomeric compounds wherein the furanose ring or the furanose ring and the internucleotide linkage are replaced with novel groups, replacement of only the furanose ring is also referred to in the art as being a sugar surrogate. The heterocyclic base moiety or a modified heterocyclic base moiety is maintained for hybridization with an appropriate target nucleic acid.

[0108] One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA) (Nielsen *et al.*, *Science*, 1991, 254, 1497-1500). PNAs have favorable hybridization properties, high biological stability and are electrostatically neutral molecules. PNA compounds have been used to correct aberrant splicing in a transgenic mouse model (Sazani *et al., Nat. Biotechnol.,* **2002**, *20*, 1228-1233). In PNA oligomeric compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA oligomeric compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262. PNA compounds can be obtained commercially from Applied Biosystems (Foster City, CA, USA). Numerous modifications to the basic PNA backbone are known in the art; particularly useful are PNA compounds with one or more amino acids conjugated to one or both termini. For example, 1-8 lysine or arginine residues are useful when conjugated to the end of a PNA molecule.

[0109] Another class of oligonucleotide mimetic that has been studied is based on linked morpholino units (morpholino nucleic acid) having heterocyclic bases attached to the morpholino ring. A number of linking groups have been reported that link the morpholino monomeric units in a morpholino nucleic acid. One class of linking groups have been selected to give a non-ionic oligomeric compound. Morpholino-based oligomeric compounds are non-ionic mimetics of oligonucleotides which are less likely to form undesired interactions with cellular proteins (Dwaine A. Braasch and David R. Corey, *Biochemistry*, 2002, *41(14)*, 4503-4510). Morpholino-based oligomeric compounds have been studied in zebrafish embryos (see: *Genesis*, volume 30, issue 3, 2001 and Heasman, J., *Dev. Biol.*, **2002**, *243*, 209-214). Further studies of morpholino-based oligomeric compounds have also been reported (Nasevicius *et al.*, *Nat. Genet.*, **2000**, *26*, 216-220; and Lacerra *et al.*, *Proc. Natl. Acad. Sci.*, **2000**, *97*, 9591-9596). Morpholino-based oligomeric compounds are disclosed in United States Patent 5,034,506. The morpholino class of oligomeric compounds have been prepared having a variety of different linking groups joining the monomeric subunits. Linking groups can be varied from chiral to achiral, and from charged to neutral. US Patent 5,166,315 discloses linkages including $-O-P(=O)(N(CH_3)_2)-O-$; US Patent 5,034,506 discloses achiral intermorpholino linkages; and US Patent 5,185,444 discloses phosphorus containing chiral intermorpholino linkages.

[0110] A further class of oligonucleotide mimetic is referred to as cyclohexene nucleic acids (CeNA). In CeNA oligonucleotides, the furanose ring normally present in a DNA or RNA molecule is replaced with a cyclohexenyl ring. CeNA DMT protected phosphoramidite monomers have been prepared and used for oligomeric compound synthesis following classical phosphoramidite chemistry. Fully modified CeNA oligomeric compounds and oligonucleotides having specific positions modified with CeNA have been prepared and studied (Wang *et al.*, *J. Am. Chem. Soc.*, 2000, *122*, 8595-8602). In general the incorporation of CeNA monomers into a DNA chain increases its stability of a DNA/RNA hybrid. CeNA oligoadenylates formed complexes with RNA and DNA complements with similar stability to the native complexes. The study of incorporating CeNA structures into natural nucleic acid structures was shown by NMR and circular dichroism to proceed with easy conformational adaptation. Furthermore the incorporation of CeNA into a sequence targeting RNA was stable to serum and able to activate *E. coli* RNase H resulting in cleavage of the target RNA strand.

[0111] A further modification includes bicyclic sugar moieties such as "Locked Nucleic Acids" (LNAs) in which the 2'-hydroxyl group of the ribosyl sugar ring is linked to the 4' carbon atom of the sugar ring thereby forming a 2'-C,4'-C-oxymethylene linkage to form the bicyclic sugar moiety (reviewed in Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, 2, 558-561; Braasch *et al., Chem. Biol.,* **2001**, *8* 1-7; and Orum *et al., Curr. Opinion Mol. Ther.,* **2001**, *3,* 239-243; see also U.S. Patents: 6,268,490 and 6,670,461). The linkage can be a methylene ($-CH_2-$) group bridging the 2' oxygen atom and the 4' carbon atom, for which the term LNA is used for the bicyclic moiety; in the case of an ethylene group in this position, the term ENA™ is used (Singh et al., Chem. Commun., 1998, 4, 455-456; ENA™: Morita *et al., Bioorganic Medicinal Chemistry,* 2003, *11,* 2211-2226). LNA and other bicyclic sugar analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm = +3 to +10° C), stability towards 3'-exonucleolytic degradation and good solubility properties. LNAs are commercially available from ProLigo (Paris, France and Boulder, CO, USA).

[0112] An isomer of LNA that has also been studied is alpha-L-LNA which has been shown to have superior stability against a 3'-exonuclease. The alpha-L-LNA's were incorporated into antisense gapmers and chimeras that showed potent antisense activity (Frieden *et al., Nucleic Acids Research,* **2003**, *21,* 6365-6372).

[0113] Another similar bicyclic sugar moiety that has been prepared and studied has the bridge going from the 3'-hydroxyl group via a single methylene group to the 4' carbon atom of the sugar ring thereby forming a 3'-C,4'-C-oxymethylene linkage (see U.S. Patent 6,043,060).

[0114] LNA has been shown to form exceedingly stable LNA:LNA duplexes (Koshkin et al., J. Am. Chem. Soc., 1998, 120, 13252-13253). LNA:LNA hybridization was shown to be the most thermally stable nucleic acid type duplex system, and the RNA-mimicking character of LNA was established at the duplex level. Introduction of 3 LNA monomers (T or A) significantly increased melting points (Tm = +15/+11° C) toward DNA complements. The universality of LNA-mediated hybridization has been stressed by the formation of exceedingly stable LNA:LNA duplexes. The RNA-mimicking of LNA was reflected with regard to the N-type conformational restriction of the monomers and to the secondary structure of the LNA:RNA duplex.

[0115] LNAs also form duplexes with complementary DNA, RNA or LNA with high thermal affinities. Circular dichroism (CD) spectra show that duplexes involving fully modified LNA (esp. LNA:RNA) structurally resemble an A-form RNA:RNA duplex. Nuclear magnetic resonance (NMR) examination of an LNA:DNA duplex confirmed the 3'-endo conformation of an LNA monomer. Recognition of double-stranded DNA has also been demonstrated suggesting strand invasion by LNA. Studies of mismatched sequences show that LNAs obey the Watson-Crick base pairing rules with generally improved selectivity compared to the corresponding unmodified reference strands. DNA·LNA chimeras have been shown to efficiently inhibit gene expression when targeted to a variety of regions (5'-untranslated region, region of the start codon or coding region) within the luciferase mRNA (Braasch *et al., Nucleic Acids Research,* **2002,** *30,* 5160-5167).

[0116] Potent and nontoxic antisense oligonucleotides containing LNAs have been described (Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638). The authors have demonstrated that LNAs confer several desired properties. LNA/DNA copolymers were not degraded readily in blood serum and cell extracts. LNA/DNA copolymers exhibited potent antisense activity in assay systems as disparate as G-protein-coupled receptor signaling in living rat brain and detection of reporter genes in *Escherichia coli*. Lipofectin-mediated efficient delivery of LNA into living human breast cancer cells has also been accomplished. Further successful *in vivo* studies involving LNA's have shown knock-down of the rat delta opioid receptor without toxicity (Wahlestedt *et al., Proc. Natl. Acad. Sci.,* **2000,** *97,* 5633-5638) and in another study showed a blockage of the translation of the large subunit of RNA polymerase II (Fluiter *et al., Nucleic Acids Res.,* **2003,** *31,* 953-962).

[0117] The synthesis and preparation of the LNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). LNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

[0118] Analogs of LNA, phosphorothioate-LNA and 2'-thio-LNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs containing oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., WO 99/14226).

Furthermore, synthesis of 2'-amino-LNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-Amino- and 2'-methylamino-LNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

[0119] Another oligonucleotide mimetic that has been prepared and studied is threose nucleic acid. This oligonucleotide mimetic is based on threose nucleosides instead of ribose nucleosides. Initial interest in (3',2')-alpha-L-threose nucleic acid (TNA) was directed to the question of whether a DNA polymerase existed that would copy the TNA. It was found that certain DNA polymerases are able to copy limited stretches of a TNA template (reported in *Chemical and Engineering News*, **2003**, 81, 9). In another study it was determined that TNA is capable of antiparallel Watson-Crick base pairing with complementary DNA, RNA and TNA oligonucleotides (Chaput *et al., J. Am. Chem. Soc.*, **2003**, *125*, 856-857).

[0120] In one study (3',2')-alpha-L-threose nucleic acid was prepared and compared to the 2' and 3' amidate analogs (Wu *et al., Organic Letters*, **2002**, *4(8)*, 1279-1282). The amidate analogs were shown to bind to RNA and DNA with comparable strength to that of RNA/DNA.

[0121] Further oligonucleotide mimetics have been prepared to include bicyclic and tricyclic nucleoside analogs (see Steffens *et al., Helv. Chim. Acta*, 1997, *80*, 2426-2439; Steffens *et al., J. Am. Chem. Soc.*, 1999, *121*, 3249-3255; Renneberg *et al., J. Am. Chem. Soc.*, **2002**, *124*, 5993-6002; and Renneberg *et al., Nucleic acids res., 2002, 30*, 2751-2757). These modified nucleoside analogs have been oligomerized using the phosphoramidite approach and the resulting oligomeric compounds containing tricyclic nucleoside analogs have shown increased thermal stabilities (Tm's) when hybridized to DNA, RNA and itself. Oligomeric compounds containing bicyclic nucleoside analogs have shown thermal stabilities approaching that of DNA duplexes.

[0122] Another class of oligonucleotide mimetic is referred to as phosphonomonoester nucleic acids which incorporate a phosphorus group in the backbone. This class of oligonucleotide mimetic is reported to have useful physical and biological and pharmacological properties in the areas of inhibiting gene expression (antisense oligonucleotides, sense oligonucleotides and triplex-forming oligonucleotides), as probes for the detection of nucleic acids and as auxiliaries for use in molecular biology.

Further oligonucleotide mimetics amenable to the present invention have been prepared wherein a cyclobutyl ring replaces the naturally occurring furanosyl ring.

[0123] Oligomeric compounds can also include nucleobase (often referred to in the art as heterocyclic base or simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). A "substitution" is the replacement of an unmodified or natural base with another unmodified or natural base. "Modified" nucleobases mean other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl ($-C{\equiv}C-CH_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido(5,4-b)(1,4)benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido(5,4-b)(1,4)benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido(5,4-b)(1,4)benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido(4,5-b)indol-2-one), pyridoindole cytidine (H-pyrido(3',2':4,5)pyrrolo(2,3-d)pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, **1990**, those disclosed by Englisch *et al.*, *Angewandte Chemie*, International Edition, **1991**, *30*, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S.T. and Lebleu, B. , ed., CRC Press, **1993**. Certain of these nucleobases are known to those skilled in the art as suitable for increasing the binding affinity of the compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C and are presently suitable base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications. It is understood in the art that modification of the base does not entail such chemical modifications as to produce substitutions in a nucleic acid sequence.

[0124] Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,096; 5,681,941; and 5,750,692.

[0125] Oligomeric compounds of the present invention can also include polycyclic heterocyclic compounds in place of one or more of the naturally-occurring heterocyclic base moieties. A number of tricyclic heterocyclic compounds have been previously reported. These compounds are routinely used in antisense applications to increase the binding properties of the modified strand to a target strand. The most studied modifications are targeted to guanosines hence they have been termed G-clamps or cytidine analogs. Representative cytosine analogs that make 3 hydrogen bonds with a guanosine in a second strand include 1,3-diazaphenoxazine-2-one (Kurchavov, *et al., Nucleosides and Nucleotides*, 1997, 16, 1837-1846), 1,3-diazaphenothiazine-2-one , (Lin, K.-Y.; Jones, R. J.; Matteucci, M. J. Am. Chem. Soc. 1995, 117, 3873-3874) and 6,7,8,9-tetrafluoro-1,3-diazaphenoxazine-2-one (Wang, J.; Lin, K.-Y., Matteucci, M. Tetrahedron Lett. 1998, 39, 8385-8388). Incorporated into oligonucleotides these base modifications were shown to hybridize with complementary guanine and the latter was also shown to hybridize with adenine and to enhance helical thermal stability by extended stacking interactions (also see U.S. Pre-Grant Publications 20030207804 and 20030175906).

[0126] Further helix-stabilizing properties have been observed when a cytosine analog/substitute has an aminoethoxy moiety attached to the rigid 1,3-diaza-phenoxazine-2-one scaffold (Lin, K.-Y.; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532). Binding studies demonstrated that a single incorporation could enhance the binding affinity of a model oligonucleotide to its complementary target DNA or RNA with a ΔT_m of up to 18°C relative to 5-methyl cytosine ($dC5^{me}$), which is a high affinity enhancement for a single modification. On the other hand, the gain in helical stability does not compromise the specificity of the oligonucleotides.

[0127] Further tricyclic heterocyclic compounds and methods of using them that are amenable to use in the present invention are disclosed in United States Patents 6,028,183, and 6,007,992.

[0128] The enhanced binding affinity of the phenoxazine derivatives together with their uncompromised sequence specificity makes them valuable nucleobase analogs for the development of more potent antisense-based drugs. In fact, promising data have been derived from *in vitro* experiments demonstrating that heptanucleotides containing phenoxazine substitutions are capable to activate RNase H, enhance cellular uptake and exhibit an increased antisense activity (Lin, K-Y; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532). The activity enhancement was even more pronounced in case of G-clamp, as a single substitution was shown to significantly improve the *in vitro* potency of a 20mer 2'-deoxyphosphorothioate oligonucleotides (Flanagan, W. M.; Wolf, J.J.; Olson, P.; Grant, D.; Lin, K.-Y.; Wagner, R. W.; Matteucci, M. Proc. Natl. Acad. Sci. USA, 1999, 96, 3513-3518).

[0129] Further modified polycyclic heterocyclic compounds useful as heterocyclic bases are disclosed in but not limited to, the above noted U.S. Patent 3,687,808, as well as U.S. Patents: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,434,257; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,646,269; 5,750,692; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, and U.S. Pre-Grant Publication 20030158403.

[0130] Another modification of the oligomeric compounds of the invention involves chemically linking to the oligomeric compound one or more moieties or conjugates which enhance the properties of the oligomeric compound, such as to enhance the activity, cellular distribution or cellular uptake of the oligomeric compound. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmaco-kinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed October 23, 1992, and U.S. Patents 6,287,860 and 6,762,169.

[0131] Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligomeric compounds of the invention may also be conjugated to drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in U.S. Patent 6,656,730.

[0132] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

[0133] Oligomeric compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of an oligomeric compound to enhance properties such as for example nuclease stability. Included in stabilizing groups are cap structures. By "cap structure or terminal cap moiety" is meant chemical modifications, which have been incorporated at either terminus of oligonucleotides (see for example Wincott et al., WO 97/26270). These terminal modifications protect the oligomeric compounds having terminal nucleic acid molecules from exonuclease degradation, and can improve delivery and/or localization within a cell. The cap can be present at either the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both termini of a single strand, or one or more termini of both strands of a double-stranded compound. This cap structure is not to be confused with the inverted methylguanosine "5'cap" present at the 5' end of native mRNA molecules. In non-limiting examples, the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl riucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott et al., International PCT publication No. WO 97/26270).

[0134] Particularly suitable 3'-cap structures include, for example 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Tyer, 1993, Tetrahedron 49, 1925).

[0135] Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an oligomeric compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

[0136] It is not necessary for all positions in a given oligomeric compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even within a single nucleoside within an oligomeric compound.

[0137] The present invention also includes oligomeric compounds which are chimeric compounds. "Chimeric" oligomeric compounds or "chimeras," in the context of this invention, are single-or double-stranded oligomeric compounds, such as oligonucleotides, which contain two or more chemically distinct regions, each comprising at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. Chimeric antisense oligonucleotides are one form of oligomeric compound. These oligonucleotides typically contain at least one region which is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, alteration of charge, increased stability and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for RNAses or other enzymes. By way of example, RNAse H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target when bound by a DNA-like oligomeric compound, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. The cleavage of RNA:RNA hybrids can, in like fashion, be accomplished through the actions of endoribonucleases, such as RNase III or RNAseL which cleaves both cellular and viral RNA. Cleavage products of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0138] Chimeric oligomeric compounds of the invention can be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides, oligonucleotide mimetics, or regions or portions thereof. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922.

[0139] A "gapmer" is defined as an oligomeric compound, generally an oligonucleotide, having a 2'-deoxyoligonucleotide region flanked by non-deoxyoligonucleotide segments. The central region is referred to as the "gap." The flanking segments are referred to as "wings." While not wishing to be bound by theory, the gap of the gapmer presents a substrate recognizable by RNase H when bound to the RNA target whereas the wings do not provide such a substrate but can confer other properties such as contributing to duplex stability or advantageous pharmacokinetic effects. Each wing can be one or more non-deoxyoligonucleotide monomers (if one of the wings has zero non-deoxyoligonucleotide monomers, a "hemimer" is described). In one embodiment, the gapmer is a ten deoxynucleotide gap flanked by five non-deoxynucleotide wings. This is referred to as a 5-10-5 gapmer. Other configurations are readily recognized by those skilled in the art. In one embodiment the wings comprise 2'-MOE modified nucleotides. In another embodiment the gapmer has a phosphorothioate backbone. In another embodiment the gapmer has 2'-MOE wings and a phosphorothioate backbone. Other suitable modifications are readily recognizable by those skilled in the art.

[0140] Oligomerization of modified and unmodified nucleosides can be routinely performed according to literature procedures for DNA (Protocols for Oligonucleotides and Analogs, Ed. Agrawal (1993), Humana Press) and/or RNA (Scaringe, Methods (2001), 23, 206-217. Gait et al., Applications of Chemically synthesized RNA in RNA: Protein Interactions, Ed. Smith (1998), 1-36. Gallo et al., Tetrahedron (2001), 57, 5707-5713).

[0141] Oligomeric compounds of the present invention can be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0142] The following precursor compounds, including amidites and their intermediates can be prepared by methods routine to those skilled in the art; 5'-O-Dimethoxytrityl-thymidine intermediate for 5-methyl dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-5-methylcytidine intermediate for 5-methyl-dC amidite, 5'-O-Dimethoxytrityl-2'-

deoxy-N4-benzoyl-5-methylcytidine penultimate intermediate for 5-methyl dC amidite, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-deoxy-N^4-benzoyl-5-methylcytidin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (5-methyl dC amidite), 2'-Fluorodeoxyadenosine, 2'-Fluorodeoxyguanosine, 2'-Fluorouridine, 2'-Fluorodeoxycytidine, 2'-O-(2-Methoxyethyl) modified amidites, 2'-O-(2-methoxyethyl)-5-methyluridine intermediate, 5'-O-DMT-2'-O-(2-methoxyethyl)-5-methyluridine penultimate intermediate, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-5-methyluridin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE T amidite), 5'-O-Dimethoxytrityl-2'-O-(2-methoxyethyl)-5-methylcytidine intermediate, 5'-O-dimethoxytrityl-2'-*O*-(2-methoxyethyl)-N^4-benzoyl-5-methyl-cytidine penultimate intermediate, (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^4-benzoyl-5-methylcytidin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE 5-Me-C amidite), (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^6-benzoyladenosin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE A amdite), (5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(2-methoxyethyl)-N^4-isobutyrylguanosin-3'-*O*-yl)-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (MOE G amidite), 2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites, 2'-(Dimethylaminooxyethoxy) nucleoside amidites, 5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine , 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine, 2'-O-((2-phthalimidoxy)ethyl)-5'-*t*-butyldiphenylsilyl-5-methyluridine , 5'-O-*tert*-butyldiphenylsilyl-2'-O-((2-formadoximinooxy)ethyl)-5-methyluridine, 5'-O-*tert*-Butyldiphenylsilyl-2'-O-(N,N dimethylaminooxyethyl)-5-methyluridine, 2'-O-(dimethylaminooxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-((2-cyanoethyl)-N,N-diisopropylphosphoramidite), 2'-(Aminooxyethoxy) nucleoside amidites, N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-((2-cyanoethyl)-N,N-diisopropylphosphoramidite), 2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites, 2'-O-(2(2-N,N-dimethylaminoethoxy)ethyl)-5-methyl uridine, 5'-O-dimethoxytrityl-2'-O-(2(2-N,N-dimethylaminoethoxy)-ethyl))-5-methyl uridine and 5'-O-Dimethoxytrityl-2'-O-(2(2-N,N-dimethylaminoethoxy)-ethyl))-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite.

[0143] The preparation of such precursor compounds for oligonucleotide synthesis are routine in the art and disclosed in US Patent 6,426,220 and published PCT WO 02/36743.

[0144] 2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites can be purchased from commercial sources (e.g. Chemgenes, Needham, MA or Glen Research, Inc. Sterling, VA). Other 2'-O-alkoxy substituted nucleoside amidites can be prepared as described in U.S. Patent 5,506,351.

[0145] Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides can be synthesized routinely according to published methods (Sanghvi, et. al., *Nucleic Acids Research*, **1993**, *21*, 3197-3203) using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham, MA).

[0146] 2'-fluoro oligonucleotides can be synthesized routinely as described (Kawasaki, et. al., *J. Med. Chem.*, **1993**, *36*, 831-841) and U. S. Patent 5,670,633.

[0147] 2'-O-Methoxyethyl-substituted nucleoside amidites can be prepared routinely as per the methods of Martin, P., *Helvetica Chimica Acta*, **1995**, *78*, 486-504.

[0148] Aminooxyethyl and dimethylaminooxyethyl amidites can be prepared routinely as per the methods of U.S. Patent 6,127,533.

[0149] Phosphorothioate-containing oligonucleotides (P=S) can be synthesized by methods routine to those skilled in the art (see, for example, Protocols for Oligonucleotides and Analogs, Ed. Agrawal (1993), Humana Press). Phosphinate oligonucleotides can be prepared as described in U.S. Patent 5,508,270.

[0150] Alkyl phosphonate oligonucleotides can be prepared as described in U.S. Patent 4,469,863.

[0151] 3'-Deoxy-3'-methylene phosphonate oligonucleotides can be prepared as described in U.S. Patents 5,610,289 or 5,625,050.

[0152] Phosphoramidite oligonucleotides can be prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878.

[0153] Alkylphosphonothioate oligonucleotides can be prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively).

[0154] 3'-Deoxy-3'-amino phosphoramidate oligonucleotides can be prepared as described in U.S. Patent 5,476,925.

[0155] Phosphotriester oligonucleotides can be prepared as described in U.S. Patent 5,023,243.

[0156] Borano phosphate oligonucleotides can be prepared as described in U.S. Patents 5,130,302 and 5,177,198.

[0157] 4'-thio-containing oligonucleotides can be synthesized as described in U.S. Patent 5,639,873.

[0158] Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages can be prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289.

[0159] Formacetal and thioformacetal linked oligonucleosides can be prepared as described in U.S. Patents 5,264,562 and 5,264,564.

[0160] Ethylene oxide linked oligonucleosides can be prepared as described in U.S. Patent 5,223,618.

[0161] Peptide nucleic acids (PNAs) can be prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, **1996**, *4*, 5-23. They may also be prepared in accordance with U.S. Patents 5,539,082, 5,700,922, 5,719,262, 6,559,279 and 6,762,281.

[0162] Oligomeric compounds incorporating at least one 2'-O-protected nucleoside by methods routine in the art. After incorporation and appropriate deprotection the 2'-O-protected nucleoside will be converted to a ribonucleoside at the position of incorporation. The number and position of the 2-ribonucleoside units in the final oligomeric compound can vary from one at any site or the strategy can be used to prepare up to a full 2'-OH modified oligomeric compound.

[0163] A large number of 2'-O-protecting groups have been used for the synthesis of oligoribonucleotides and any can be used. Some of the protecting groups used initially for oligoribonucleotide synthesis included tetrahydropyran-1-yl and 4-methoxytetrahydropyran-4-yl. These two groups are not compatible with all 5'-O-protecting groups so modified versions were used with 5'-DMT groups such as 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp). Reese et al. have identified a number of piperidine derivatives (like Fpmp) that are useful in the synthesis of oligoribonucleotides including 1-[(chloro-4-methyl)phenyl]-4'-methoxypiperidin-4-yl (Reese et al., Tetrahedron Lett., 1986, (27), 2291). Another approach is to replace the standard 5'-DMT (dimethoxytrityl) group with protecting groups that were removed under non-acidic conditions such as levulinyl and 9-fluorenylmethoxycarbonyl. Such groups enable the use of acid labile 2'-protecting groups for oligoribonucleotide synthesis. Another more widely used protecting group, initially used for the synthesis of oligoribonucleotides, is the t-butyldimethylsilyl group (Ogilvie et al., Tetrahedron Lett., 1974, 2861; Hakimelahi et al., Tetrahedron Lett., 1981, (22), 2543; and Jones et al., J. Chem. Soc. Perkin I., 2762). The 2'-O-protecting groups can require special reagents for their removal. For example, the t-butyldimethylsilyl group is normally removed after all other cleaving/deprotecting steps by treatment of the oligomeric compound with tetrabutylammonium fluoride (TBAF).

[0164] One group of researchers examined a number of 2'-O-protecting groups (Pitsch, S., Chimia, 2001, (55), 320-324.) The group examined fluoride labile and photolabile protecting groups that are removed using moderate conditions. One photolabile group that was examined was the [2-(nitrobenzyl)oxy]methyl (nbm) protecting group (Schwartz et al., Bioorg. Med. Chem. Lett., 1992, (2), 1019.) Other groups examined included a number structurally related formaldehyde acetal-derived, 2'-O-protecting groups. Also prepared were a number of related protecting groups for preparing 2'-O-alkylated nucleoside phosphoramidites including 2'-O-[(triisopropylsilyl)oxy]methyl (2'-O-CH_2-O-Si(iPr)$_3$, TOM). One 2'-O-protecting group that was prepared to be used orthogonally to the TOM group was 2'-O-[(R)-1-(2-nitrophenyl)ethyloxy)methyl] ((R)-mnbm).

[0165] Another strategy using a fluoride labile 5'-O-protecting group (non-acid labile) and an acid labile 2'-O-protecting group has been reported (Scaringe, Stephen A., Methods, 2001, (23) 206-217). A number of possible silyl ethers were examined for 5'-O-protection and a number of acetals and orthoesters were examined for 2'-O-protection. The protection scheme that gave the best results was 5'-O-silyl ether-2'-ACE (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether (DOD)-2'-O-bis(2-acetoxyethoxy)methyl (ACE). This approach uses a modified phosphoramidite synthesis approach in that some different reagents are required that are not routinely used for RNA/DNA synthesis.

[0166] The main RNA synthesis strategies that are presently being used commercially include 5'-O-DMT-2'-O-t-butyldimethylsilyl (TBDMS), 5'-O-DMT-2'-O-[1(2-fluorophenyl)-4-methoxypiperidin-4-yl] (FPMP), 2'-O-[(triisopropylsilyl)oxy]methyl (2'-O-CH_2-O-Si(iPr)$_3$ (TOM), and the 5'-O-silyl ether-2'-ACE (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether (DOD)-2'-O-bis(2-acetoxyethoxy)methyl (ACE). Some companies currently offering RNA products include Pierce Nucleic Acid Technologies (Milwaukee, WI), Dharmacon Research Inc. (a subsidiary of Fisher Scientific, Lafayette, CO), and Integrated DNA Technologies, Inc. (Coralville, IA). One company, Princeton Separations, markets an RNA synthesis activator advertised to reduce coupling times especially with TOM and TBDMS chemistries. Such an activator would also be amenable to the oligomeric compounds of the present invention.

[0167] All of the aforementioned RNA synthesis strategies are amenable to the oligomeric compounds of the present invention. Strategies that would be a hybrid of the above e.g. using a 5'-protecting group from one strategy with a 2'-O-protecting from another strategy is also contemplated herein.

(2'-O-Me)--(2'-deoxy)--(2'-O-Me) Chimeric Phosphorothioate Oligonucleotides

[0168] Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments can be routinely synthesized by one skilled in the art, using, for example, an Applied Biosystems automated DNA synthesizer Model 394. Oligonucleotides can be synthesized using an automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxy-trityl-2'-O-methyl-3'-O-phosphoramidite for the 2'–O-alkyl portion. In one nonlimiting example, the standard synthesis cycle is modified by incorporating coupling steps with increased reaction times for the 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite. The fully protected oligonucleotide is cleaved from the support and deprotected in concentrated ammonia (NH_4OH) for 12-16 hr at 55°C. The deprotected oligonucleotide is then recovered by an appropriate method (precipitation, column chromatography, volume reduced *in vacuo*) and analyzed by methods routine in the art.

(2'-O-(2-Methoxyethyl))--(2'-deoxy)--(2'-O-(2-Methoxyethyl)) Chimeric Phosphorothioate Oligonucleotides

[0169] (2'-O-(2-methoxyethyl))--(2'-deoxy)--(-2'-O-(2-methoxyethyl)) chimeric phosphorothioate oligonucleotides can be prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

(2'-O-(2-Methoxyethyl)Phosphodiester)--(2'-deoxy Phosphorothioate)--(2'-O-(2-Methoxyethyl) Phosphodiester) Chimeric Oligonucleotides

[0170] (2'-O-(2-methoxyethyl phosphodiester)--(2'-deoxy phosphorothioate)--(2'-O-(methoxyethyl) phosphodiester) chimeric oligonucleotides can be prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidation with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

[0171] Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides can be synthesized according to United States patent 5,623,065.

[0172] Methods of oligonucleotide purification and analysis are known to those skilled in the art. Analysis methods include capillary electrophoresis (CE) and electrospray-mass spectroscopy. Such synthesis and analysis methods can be performed in multi-well plates.

[0173] Modulation of expression of a target nucleic acid can be achieved through alteration of any number of nucleic acid (DNA or RNA) functions. "Modulation" means a perturbation of function, for example, either an increase (stimulation or induction) or a decrease (inhibition or reduction) in expression. As another example, modulation of expression can include perturbing splice site selection of pre-mRNA processing. "Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. These structures include the products of transcription and translation. "Modulation of expression" means the perturbation of such functions. The functions of DNA to be modulated can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of

RNA to be modulated can include translocation functions, which include, but are not limited to, translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, and translation of protein from the RNA. RNA processing functions that can be modulated include, but are not limited to, splicing of the RNA to yield one or more RNA species, capping of the RNA, 3' maturation of the RNA and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA. Modulation of expression can result in the increased level of one or more nucleic acid species or the decreased level of one or more nucleic acid species, either temporally or by net steady state level. One result of such interference with target nucleic acid function is modulation of the expression of Mcl-1. Thus, in one embodiment modulation of expression can mean increase or decrease in target RNA or protein levels. In another embodiment modulation of expression can mean an increase or decrease of one or more RNA splice products, or a change in the ratio of two or more splice products.

[0174] Modulation of Mcl-1 expression can be assayed in a variety of ways known in the art. Mcl-1 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA by methods known in the art. Methods of RNA isolation are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993.

[0175] Northern blot analysis is routine in the art and is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., **1996**. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

[0176] Levels of a protein encoded by Mcl-1 can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Antibodies directed to a protein encoded by Mcl-1 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional antibody generation methods. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 11.4.1-11.11.5, John Wiley & Sons, Inc., 1997.

[0177] Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 10.16.1-10.16.11, John Wiley & Sons, Inc., 1998. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 10.8.1-10.8.21, John Wiley & Sons, Inc., 1997. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991.

[0178] Once one or more target regions, segments or sites have been identified, oligomeric compounds are designed which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect. The oligomeric compounds of the present invention can be targeted to features of a target nucleobase sequence.

[0179] The locations on the target nucleic acid to which active oligomeric compounds hybridize are hereinbelow referred to as "validated target segments." As used herein the term "validated target segment" is defined as at least an 8-nucleobase portion of a target region to which an active oligomeric compound is targeted. While not wishing to be bound by theory, it is presently believed that these target segments represent portions of the target nucleic acid which are accessible for hybridization.

[0180] Target segments can include DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of a validated target segment (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases). Similarly validated target segments are represented by DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of a validated target segment (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases). It is also understood that a validated oligomeric target segment can be represented by DNA or RNA sequences that comprise at least 8 consecutive nucleobases from an internal portion of the sequence of a validated target segment, and can extend in either or both directions until the oligonucleotide contains about 8 to about 80, or about 13 to about 80, or about 12 to about 50, or about 12 to about 30, or about 15 to about 30, or about 20 to about 30, or about 20 to about 24, or about 16 to about 20 nucleobases.

[0181] In another embodiment, the validated target segments identified herein can be employed in a screen for additional compounds that modulate the expression of Mcl-1. "Modulators" are those compounds that modulate the expression of Mcl-1 and which comprise at least an 8-nucleobase portion which is complementary to a validated target segment. The screening method comprises the steps of contacting a validated target segment of a nucleic acid molecule encoding Mcl-1 with one or more candidate modulators, and selecting for one or more candidate modulators which perturb the expression of a nucleic acid molecule encoding Mcl-1. Once it is shown that the candidate modulator or modulators are capable of modulating the expression of a nucleic acid molecule encoding Mcl-1, the modulator can then be employed in further investigative studies of the function of Mcl-1, or for use as a research, diagnostic, or therapeutic agent. The validated target segments can also be combined with a second strand as disclosed herein to form stabilized double-stranded (duplexed) oligonucleotides for use as a research, diagnostic, or therapeutic agent.

[0182] The oligomeric compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. Furthermore, antisense compounds, which are able to inhibit gene expression with specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

[0183] For use in kits and diagnostics, the oligomeric compounds of the present invention, either alone or in combination with other compounds or therapeutics, can be used as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

[0184] As one nonlimiting example, expression patterns within cells or tissues treated with one or more compounds or compositions of the present invention are compared to control cells or tissues not treated with compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds which affect expression patterns.

[0185] Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett.*, **2000**, 480, 17-24; Celis, *et al.*, *FEBS Lett.*, **2000**, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, *et al.*, *Drug Discov. Today*, **2000**, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.*, **1999**, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, **2000**, 97, 1976-81), protein arrays and proteomics (Celis, *et al.*, *FEBS Lett.*, **2000**, 480, 2-16; Jungblut, *et al.*, *Electrophoresis*, **1999**, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, *et al.*, *FEBS Lett.*, **2000**, 480, 2-16; Larsson, *et al.*, *J. Biotechnol.*, **2000**, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, *et al.*, *Anal. Biochem.*, **2000**, 286, 91-98; Larson, *et al.*, *Cytometry*, **2000**, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol.*, **2000**, 3, 316-21), comparative genomic hybridization (Carulli, *et al.*, *J. Cell Biochem. Suppl.*, **1998**, 31, 286-96), FISH (fluorescent *in situ* hybridization) techniques (Going and Gusterson, *Eur. J. Cancer*, **1999**, 35, 1895-904) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen*, **2000**, 3, 235-41).

[0186] Compounds of the invention can be used to modulate the expression of Mcl-1 in an animal, such as a human. In one non-limiting embodiment, the methods comprise the step of administering to said animal an effective amount of an antisense compound that inhibits expression of Mcl-1. In one embodiment, the antisense compounds of the present invention effectively inhibit the levels or function of Mcl-1 RNA. Because reduction in Mcl-1 mRNA levels can lead to alteration in Mcl-1 protein products of expression as well, such resultant alterations can also be measured. Antisense compounds of the present invention that effectively inhibit the levels or function of Mcl-1 RNA or protein products of expression are considered active antisense compounds. In one embodiment, the antisense compounds of the invention inhibit the expression of Mcl-1 causing a reduction of RNA by at least 10%, by at least 20%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100%.

[0187] For example, the reduction of the expression of Mcl-1 can be measured in a bodily fluid, tissue or organ of the animal. Bodily fluids include, but are not limited to, blood (serum or plasma), lymphatic fluid, cerebrospinal fluid, semen, urine, synovial fluid and saliva and can be obtained by methods routine to those skilled in the art. Tissues or organs include, but are not limited to, blood (e.g., hematopoietic cells, such as human hematopoietic progenitor cells, human hematopoietic stem cells, CD34+ cells CD4+ cells), lymphocytes and other blood lineage cells, skin, bone marrow, spleen, thymus, lymph node, brain, spinal cord, heart, skeletal muscle, liver, pancreas, prostate, kidney, lung, oral mucosa, esophagus, stomach, ilium, small intestine, colon, bladder, cervix, ovary, testis, mammary gland, adrenal gland, and adipose (white and brown). Samples of tissues or organs can be routinely obtained by biopsy. In some alternative situations, samples of tissues or organs can be recovered from an animal after death.

[0188] The cells contained within said fluids, tissues or organs being analyzed can contain a nucleic acid molecule encoding Mcl-1 protein and/or the Mcl-1-encoded protein itself. For example, fluids, tissues or organs procured from an animal can be evaluated for expression levels of the target mRNA or protein. mRNA levels can be measured or evaluated by real-time PCR, Northern blot, in situ hybridization or DNA array analysis. Protein levels can be measured or evaluated by ELISA, immunoblotting, quantitative protein assays, protein activity assays (for example, caspase activity assays) immunohistochemistry or immunocytochemistry. Furthermore, the effects of treatment can be assessed by measuring biomarkers associated with the target gene expression in the aforementioned fluids, tissues or organs, collected from an animal contacted with one or more compounds of the invention, by routine clinical methods known in the art. These biomarkers include but are not limited to: glucose, cholesterol, lipoproteins, triglycerides, free fatty acids and other markers of glucose and lipid metabolism; liver transaminases, bilirubin, albumin, blood urea nitrogen, creatine and other markers of kidney and liver function; interleukins, tumor necrosis factors, intracellular adhesion molecules, C-reactive protein and other markers of inflammation; testosterone, estrogen and other hormones; tumor markers; vitamins, minerals and electrolytes.

[0189] The compounds of the present invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. In one aspect, the compounds of the present invention inhibit the expression of Mcl-1. The compounds of the invention can also be used in the manufacture of a medicament for the treatment of diseases and disorders related to Mcl-1 expression.

[0190] Methods whereby bodily fluids, organs or tissues are contacted with an effective amount of one or more of the antisense compounds or compositions of the invention are also contemplated. Bodily fluids, organs or tissues can be contacted with one or more of the compounds of the invention resulting in modulation of Mcl-1 expression in the cells of bodily fluids, organs or tissues. An effective amount can be determined by monitoring the modulatory effect of the antisense compound or compounds or compositions on target nucleic acids or their products by methods routine to the skilled artisan. Further contemplated are *ex vivo* methods of treatment whereby cells or tissues are isolated from a subject, contacted with an effective amount of the antisense compound or compounds or compositions and reintroduced into the subject by routine methods known to those skilled in the art.

[0191] The oligomeric compounds of the present invention comprise any pharmaceutically acceptable salts, esters, or salts of such esters, or any other functional chemical equivalent which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the oligomeric compounds of the present invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

[0192] The term "prodrug" indicates a therapeutic agent that is prepared in an inactive or less active form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE ((S-acetyl-2-thioethyl) phosphate) derivatives according to the methods disclosed in WO 93/24510 or WO 94/26764.

[0193] The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

[0194] The oligomeric compounds of the invention may also be formulated with active or inert ingredients, or a combination of both, for delivery via parenteral and non-parenteral routes of administration. Compositions and methods of preparing formulations are well known to those skilled in the art.

[0195] While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same. Each of the references, GENBANK® accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Example 1

Cell culture and treatment with oligomeric compounds

[0196] The effect of oligomeric compounds on target nucleic acid expression was tested in A549 and b.END cells. The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (Manassas, VA). A549 cells were routinely cultured in DMEM, high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 units per ml penicillin, and 100 micrograms per ml streptomycin (Invitrogen Life Technologies, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached approximately 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of approximately 5000 cells/well for use in oligomeric compound transfection experiments.

[0197] The mouse brain endothelial cell line b.END was obtained from Dr. Werner Risau at the Max Plank Institute (Bad Nauheim, Germany). b.END cells were routinely cultured in DMEM, high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached approximately 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria

#3872) at a density of approximately 3000 cells/well for use in oligomeric compound transfection experiments.

[0198] When cells reached appropriate confluency, they were treated with oligonucleotide using Lipofectin™ as described. When cells reached 65-75% confluency, they were treated with oligonucleotide. Oligonucleotide was mixed with LIPOFECTIN™ Invitrogen Life Technologies, Carlsbad, CA) in Opti-MEM™-1 reduced serum medium (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired concentration of oligonucleotide and a LIPOFECTIN™ concentration of 2.5 or 3 µg/mL per 100 nM oligonucleotide. This transfection mixture was incubated at room temperature for approximately 0.5 hours. For cells grown in 96-well plates, wells were washed once with 100 µL OPTI-MEM™-1 and then treated with 130 µL of the transfection mixture. Cells grown in 24-well plates or other standard tissue culture plates were treated similarly, using appropriate volumes of medium and oligonucleotide. Cells were treated and data were obtained in duplicate or triplicate. After approximately 4-7 hours of treatment at 37°C, the medium containing the transfection mixture was replaced with fresh culture medium. Cells were harvested 16-24 hours after oligonucleotide treatment.

[0199] Control oligonucleotides were used to determine the optimal oligomeric compound concentration for a particular cell line. Furthermore, when oligomeric compounds of the invention were tested in oligomeric compound screening experiments or phenotypic assays, control oligonucleotides were tested in parallel with compounds of the invention. In some embodiments, the control oligonucleotides were used as negative control oligonucleotides, i.e., as a means for measuring the absence of an effect on gene expression or phenotype. In alternative embodiments, control oligonucleotides were used as positive control oligonucleotides, i.e., as oligonucleotides known to affect gene expression or phenotype. Control oligonucleotides are shown in Table 2. "Target Name" indicates the gene to which the oligonucleotide is targeted. "Species of Target" indicates species in which the oligonucleotide is perfectly complementary to the target mRNA. "Motif" is indicative of chemically distinct regions comprising the oligonucleotide. The compounds in Table 2 are chimeric oligonucleotides, composed of a central "gap" region consisting of 2'-deoxynucleotides, which is flanked on both sides (5' and 3') by "wings". The wings are composed of 2'-O-(2-methoxyethyl) nucleotides, also known as 2'-MOE nucleotides. The "motif" of each gapmer oligonucleotide is illustrated in Table 2 and indicates the number of nucleotides in each gap region and wing, for example, "5-10-5" indicates a gapmer having a 10-nucleotide gap region flanked by 5-nucleotide wings. Similarly, the motif "5-9-6" indicates a 9-nucleotide gap region flanked by 5-nucleotide wing on the 5' side and a 6-nucleotide wing on the 3' side. ISIS 29848 is a mixture of randomized oligomeric compounds, where each nucleotide can be A, T, C or G. For each compound listed in Table 2, the internucleoside (backbone) linkages are phosphorothioate throughout the oligonucleotide. Unmodified cytosines are indicated by "^{u}C" in the nucleotide sequence; all other cytosines are 5-methylcytosines.

Table 2

Control oligonucleotides for cell line testing, oligomeric compound screening and phenotypic assays

ISIS #	Target Name	Species of Target	Sequence (5' to 3')	Motif	SEQ ID NO
113131	CD86	Human	CGTGTGTCTGTGCTAGTCCC	5-10-5	11
289865	forkhead box O1A (rhabdomyosarcoma)	Human	GGCAACGTGAACAGGTCCAA	5-10-5	12
25237	integrin beta 3	Human	GCCCATTGCTGGACATGC	4-10-4	13
196103	integrin beta 3	Human	AGCCCATTGCTGGACATGCA	5-10-5	14
148715	Jagged 2	Human; Mouse; Rat	TTGTCCCAGTCCCAGGCCTC	5-10-5	15
18076	Jun N-Terminal Kinase - 1	Human	CTTTC^{u}CGTTGGA^{u}C^{u}CCCTGGG	5-9-6	16
18078	Jun N-Terminal Kinase - 2	Human	GTGCG^{u}CG^{u}CGAG^{u}C^{u}C^{u}CGAAATC	5-9-6	17
183881	kinesin-like 1	Human	ATCCAAGTGCTACTGTAGTA	5-10-5	18
29848	none	none	NNNNNNNNNNNNNNNNNNNN	5-10-5	19
226844	Notch (Drosophila) homolog 1	Human; Mouse	GCCCTCCATGCTGGCACAGG	5-10-5	20
105990	Peroxisome proliferator-activated receptor gamma	Human	AGCAAAAGATCAATCCGTTA	5-10-5	21
336806	Raf kinase C	Human	TACAGAAGGCTGGGCCTTGA	5-10-5	22
15770	Raf kinase C	Mouse; Murine sarcoma virus; Rat	ATGCATT^{u}CTG^{u}C^{u}C^{u}C^{u}C^{u}CAAGGA	5-10-5	23

[0200] The concentration of oligonucleotide used varies from cell line to cell line. To determine the optimal oligonucleotide concentration for a particular cell line, the cells were treated with a positive control oligonucleotide at a range of concentrations. The concentration of positive control oligonucleotide that results in 80% inhibition of the target mRNA is then utilized as the screening concentration for new oligonucleotides in subsequent experiments for that cell line. If 80% inhibition is not achieved, the lowest concentration of positive control oligonucleotide that results in 60% inhibition of the target mRNA is then utilized as the oligonucleotide screening concentration in subsequent experiments for that cell line. If 60% inhibition is not achieved, that particular cell line is deemed as unsuitable for oligonucleotide transfection experiments. The concentrations of antisense oligonucleotides used herein are from 50 nM to 300 nM when the antisense oligonucleotide is transfected using a liposome reagent and 1 μM to 40 μM when the antisense oligonucleotide is transfected by electroporation.

Example 2

Real-time Quantitative PCR Analysis of Mcl-1 mRNA Levels

[0201] Quantitation of Mcl-1 mRNA levels was accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions.

[0202] Prior to quantitative PCR analysis, primer-probe sets specific to the target gene being measured were evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. After isolation the RNA is subjected to sequential reverse transcriptase (RT) reaction and real-time PCR, both of which are performed in the same well. RT and PCR reagents were obtained from Invitrogen Life Technologies (Carlsbad, CA). RT, real-time PCR was carried out in the same by adding 20 μL PCR cocktail (2.5x PCR buffer minus $MgCl_2$, 6.6 mM $MgCl_2$, 375 μM each of dATP, dCTP, dCTP and dGTP, 375 nM each of forward primer and reverse primer, 125 nM of probe, 4 Units RNAse inhibitor, 1.25 Units PLATINUM® Taq, 5 Units MuLV reverse transcriptase, and 2.5x ROX dye) to 96-well plates containing 30 μL total RNA solution (20-200 ng). The RT reaction was carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the PLATINUM® Taq, 40 cycles of a two-step PCR protocol were carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension).

[0203] Gene target quantities obtained by RT, real-time PCR were normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, OR). GAPDH expression was quantified by RT, real-time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA was quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR).

[0204] 170 μL of RiboGreen™ working reagent (RiboGreen™ reagent diluted 1:350 in 10mM Tris-HCl, 1 mM EDTA, pH 7.5) was pipetted into a 96-well plate containing 30 μL purified cellular RNA. The plate was read in a CytoFluor 4000 (PE Applied Biosystems) with excitation at 485nm and emission at 530nm.

[0205] Presented in Table 3 are primers and probes used to measure GAPDH expression in the cell types described herein. The GAPDH PCR probes have JOE covalently linked to the 5' end and TAMRA or MGB covalently linked to the 3' end, where JOE is the fluorescent reporter dye and TAMRA or MGB is the quencher dye. In some cell types, primers and probe designed to a GAPDH sequence from a different species are used to measure GAPDH expression. For example, a human GAPDH primer and probe set is used to measure GAPDH expression in monkey-derived cells and cell lines.

Table 3
GAPDH primers and probes for use in real-time PCR

Target Name	Species	Sequence Description	Sequence (5' to 3')	SEQ ID NO
GAPDH	Human	Forward Primer	CAACGGATTTGGTCGTATTGG	24
GAPDH	Human	Reverse Primer	GGCAACAATATCCACTTTACCAGAGT	25
GAPDH	Human	Probe	CGCCTGGTCACCAGGGCTGCT	26
GAPDH	Human	Forward Primer	GAAGGTGAAGGTCGGAGTC	27
GAPDH	Human	Reverse Primer	GAAGATGGTGATGGGATTTC	28
GAPDH	Human	Probe	CAAGCTTCCCGTTCTCAGCC	29
GAPDH	Human	Forward Primer	GAAGGTGAAGGTCGGAGTC	27
GAPDH	Human	Reverse Primer	GAAGATGGTGATGGGATTTC	28
GAPDH	Human	Probe	TGGAATCATATTGGAACATG	30
GAPDH	Mouse	Forward Primer	GGCAAATTCAACGGCACAGT	31
GAPDH	Mouse	Reverse Primer	GGGTCTCGCTCCTGGAAGAT	32
GAPDH	Mouse	Probe	AAGGCCGAGAATGGGAAGCTTGTCATC	33
GAPDH	Rat	Forward Primer	TGTTCTAGAGACAGCCGCATCTT	34
GAPDH	Rat	Reverse Primer	CACCGACCTTCACCATCTTGT	35
GAPDH	Rat	Probe	TTGTGCAGTGCCAGCCTCGTCTCA	36

[0206] Probes and primers for use in real-time PCR were designed to hybridize to target-specific sequences. The primers and probes and Mcl-1 target nucleic acid sequences to which they hybridize are presented in Table 4. The target-specific PCR probes have FAM covalently linked to the 5' end and TAMRA or MGB covalently linked to the 3' end, where FAM is the fluorescent dye and TAMRA or MGB is the quencher dye.

Table 4

Mcl-1-specific primers and probes for use in real-time PCR

Target Name	Species	Target SEQ ID NO	Sequence Description	Sequence (5' to 3')	SEQ ID NO
Mcl-1	Human	3	Forward Primer	AAGATCTGGTTACGGTAACTAAAAAAGC	37
Mcl-1	Human	3	Reverse Primer	GGGCCCCTAAAAACCAATTC	38
Mcl-1	Human	3	Probe	TGTCTGCCAAATCCAGTGGAAACAAGTG	39
Mcl-1	Mouse	7	Forward Primer	TCCAGGGTGTGCTTGACAAA	40
Mcl-1	Mouse	7	Reverse Primer	TCATCCAAACCAAGCCAAAGT	41
Mcl-1	Mouse	7	Probe	TCCCAAGTGCTCAGGACTTTTAGCCCTG	42

Example 3

Treatment of cultured cells with oligomeric compounds

[0207] Oligomeric compounds targeted to Mcl-1 nucleic acids presented in Table 1 were tested for their effects on gene target expression in cultured cells. Table 5 shows the experimental conditions, including cell type, transfection method, dose of oligonucleotide and control SEQ ID NO used to evaluate the inhibition of gene expression by the oligomeric compounds of the invention. The control oligonucleotide was chosen from the group presented in Table 2, and in these experiments was used as a negative control. Each cell type was treated with the indicated dose of oligonucleotide as described by other examples herein. The oligomeric compounds and the data describing the degree to which they inhibit gene expression are shown in Table 6.

Table 5
Treatment conditions of cultured cells with oligomeric compounds

Target Name	Cell Type	Transfection Method	Dose of Oligonucleotide (nM)	Control SEQ ID NO
Mcl-1	A549	Lipofectin	100	17
Mcl-1	b.END	Cytofectin	100	17

Example 4

Antisense inhibition of gene targets by oligomeric compounds

[0208] A series of oligomeric compounds was designed to target different regions of Mcl-1, using published sequences cited in Table 1. The compounds are shown in Table 6 (human) and Table 7 (mouse). All compounds in Table 6 and Table 7 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of 10 2'-deoxynucleotides, which is flanked on both sides (5' and 3') by five-nucleotide "wings". The wings are composed of 2'-O-(2-methoxyethyl) nucleotides, also known as 2'-MOE nucleotides. The internucleoside (backbone) linkages are phosphorothioate throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on gene target mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from experiments in which cultured cells were treated with the disclosed oligomeric compounds. Shown in Table 6 and Table 7 is the SEQ ID NO of the sequence to which each oligomeric compound is targeted. The inhibition data presented in Table 6 and Table 7 were obtained in human A549 cells and mouse b.END cells, respectively.

[0209] A reduction in expression is expressed as percent inhibition. If the target expression level of oligomeric compound-treated cell was higher than control, percent inhibition is expressed as zero inhibition. The target regions to which these oligomeric compounds are inhibitory are herein referred to as "validated target segments."

Table 6
Inhibition of human Mcl-1 mRNA levels by chimeric oligonucleotides having 2'-MOE wings and deoxy gap

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
387600	2	123	GAGGCCAAACATTGCCAGTC	54	43
387601	2	131	TTTCTTTTGAGGCCAAACAT	29	44
387602	2	573	GGTGTTATTACCAGATTCCC	32	45
387603	2	719	CCAGACCTGCCCATTGGCTT	67	46
387604	2	729	GCTGGTGGCCCCAGACCTGC	39	47
387605	2	808	GAAGCATGCCTTGGAAGGCC	29	48
387606	2	820	TGTCCAGTTTCCGAAGCATG	62	49
387607	2	873	GAAAACATGGATCATCACTC	33	50
385360	2	902	ATCCTGCCCCAGTTTGTTAC	48	51
385361	2	911	AGAGTCACAATCCTGCCCCA	47	52
387608	2	938	TTAGCCACAAAGGCACCAAA	47	53
387609	2	959	TGGTTTATGGTCTTCAAGTG	50	54
387610	2	972	GATGCAGCTTTCTTGGTTTA	24	55
387611	2	977	GGTTCGATGCAGCTTTCTTG	50	56
387612	2	988	TTTCTGCTAATGGTTCGATG	66	57
387613	2	1039	TTTGTTTAACTAGCCAGTCC	24	58
387614	2	1055	AACCCATCCCAGCCTCTTTG	36	59
387615	2	1083	TAGGTCCTCTACATGGAAGA	43	60
387616	2	1104	CACATTCCTGATGCCACCTT	56	61
387617	2	1109	AGCAGCACATTCCTGATGCC	43	62
387618	2	1131	TCCAGCAACACCTGCAAAAG	52	63
387619	2	1156	TTAGATATGCCAAACCAGCT	71	64
387620	2	1183	TATTGCACTTACAGTAAGGC	34	65
387621	2	1252	GAAGTTACAGCTTGGAGTCC	63	66
387622	2	1268	TAGGGTGCAACTCTAGGAAG	63	67
387623	2	1278	GCTAGGTTGCTAGGGTGCAA	67	68

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
387624	2	1319	TATTCTTGTTAGCCATAATC	72	69
387625	2	1341	GGGAGCACTCTTCCCATGTA	52	70
387626	2	1396	GTTTGTTGCTGAAACTGAAC	51	71
387627	2	1404	CAAAGTTTGTTTGTTGCTGA	68	72
387628	2	1471	GGAAATTAAGTCTTTCCACC	64	73
387629	2	1651	GAGGAAAAGCTTCCCTTGTA	47	74
387630	2	1669	GGGAAAGCTAATTAGAGAGA	5	75
387631	2	1674	ATACTGGGAAAGCTAATTAG	55	76
387632	2	1809	ATATTCATAACTAATTACTG	4	77
387633	2	1824	GAATTGAGGATATCCATATT	37	78
387634	2	1832	TGTCTTAAGAATTGAGGATA	52	79
387635	2	1890	TCAAAGAAATAGACTTTCTG	58	80
387636	2	2062	CTACAACCAGTCTGCATACA	70	81
387637	2	2075	CAGATTTGTTCCACTACAAC	62	82
387638	2	2084	CATAGTTATCAGATTTGTTC	66	83
387639	2	2178	GAATTTCCATTCATCAACCT	52	84
387640	2	2207	ATTGAAAACTTGCATATAAT	32	85
387641	2	2248	GTAAGTCATCAGTAACCTTA	77	86
387642	2	2270	GCCCAATCAGAGCCCATTAT	71	87
387643	2	2307	TAAATTAGGTCAAATGGAAG	30	88
387644	2	2515	AAGCCTAATAATAGCACCAT	70	89
387645	2	2551	TGACATACTAGGCTTAGACC	62	90
387646	2	2566	AAGTATTTGCTTTATTGACA	66	91
387647	2	2637	ACTGAAATCCAAAGATGCCA	71	92
387648	2	2766	AAAGAGTTCAGGGATGGCAG	65	93
387649	2	2823	GAGGGTCACTCAGGTTTCCA	50	94
387650	2	2903	ACAGCACCCATGGTATTACC	73	95
387651	2	3118	GGTTTAACACAGCTCACCTC	73	96
387652	2	3126	AACTCTGAGGTTTAACACAG	65	97
387653	2	3142	TTATCAGTAGCTTTTAAACT	28	98
387654	2	3176	TGACCCTAGTTCCAATATAG	65	99
387655	2	3183	TTTCAAATGACCCTAGTTCC	59	100
387656	2	3214	CAGACTAAAGGTCATGTTCC	68	101
387657	2	3235	CCTATTTTTAAATGGAGTCC	70	102
387658	2	3388	GGTCCTTAGAGATACATGAT	64	103
387659	2	3482	TATGCACTTGTTTCCACTGG	92	104
387660	2	3579	GCCCCAAGCCCAAAATATCA	20	105
387661	2	3829	CCCACAGAATGTACATGAAA	63	106
387662	2	3902	GTAGTTGGTCCTAACCCTTC	63	107
387663	2	3940	TAGGGAAACACACTACATTT	26	108
387664	3	809	CAAACCCATCCTTGGAAGGC	0	109
385368	3	870	GCAAAAGCCAGCAGCACATT	49	110
385372	3	920	AAGGCTATCTTATTAGATAT	9	111
385421	3	2941	TGAAGCTTTCAAATGACCCT	53	112
387665	3	3428	CAGTCAGCACTTAGACCACC	70	113
387666	5	2439	TGGCTTCAGGAATAGGATGA	27	114
387667	5	2668	GAAGCATGCCTGAGAAAGAA	14	115
387668	5	2919	AGGCAAACTTACCCAGCCTC	22	116
387669	5	2996	AAAAACCTTTAGATATCCCC	34	117

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
387670	5	3047	TCAAATAAACAATGGTCCTT	62	118
387671	5	3176	ATGGTTTGAATCCACTGAAG	49	119
387672	5	3412	GACTTCCAGAGTTCCCATGA	35	120

[0210] As shown in Table 6, SEQ ID NOs 43-74, 76, 78-108, 110, 112-114 and 116-120 inhibited expression of Mcl-1 mRNA by at least 20%; SEQ ID NOs 43, 46, 49, 54, 56, 57, 61, 63, 64, 66-73, 76, 52-84, 86, 87, 89-97, 99-104, 106, 107, 112, 113 and 118 inhibited expression of Mcl-1 mRNA by at least 50%; and SEQ ID NOs 64, 69, 81, 86, 87, 89, 92, 95, 96, 102, 104 and 113 inhibited expression of Mcl-1 mRNA by at least 70%. Preferred target regions of SEQ ID NO: 2 include but are not limited to nucleotides 123-150; 719-748; 808-839; 902-930; 902-1007; 938-1007; 1039-1074; 1083-1150; 1104-1128; 1252-1297; 1396-1423; 1651-1693; 1809-1851; 2062-2103; 2551-2585; 3118-3161; 3176-3202; and 3214-3254, and any range therewithin.

Table 7
Inhibition of mouse Mcl-1 mRNA levels by chimeric oligonucleotides having 2'-MOE wings and deoxy gap

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
385358	7	763	TTGAAAACATGGACCATTAC	75	121
385359	7	769	CCATCTTTGAAAACATGGAC	60	122
385360	7	790	ATCCTGCCCCAGTTTGTTAC	70	51
385361	7	799	AGAGTCACAATCCTGCCCCA	67	52
385362	7	805	GAAATAAGAGTCACAATCCT	28	123
385363	7	829	TGTTTGGCCACAAAGGCACC	45	124
385364	7	837	TCTTTAAGTGTTTGGCCACA	61	125
385365	7	881	GATAGTTTCTGCTAATGGTT	59	126
385366	7	927	TTTGTTTGACAAGCCAGTCC	55	127
385367	7	997	AGCAGCACATTTCTGATGCC	55	128
385368	7	1006	GCAAAAGCCAGCAGCACATT	60	110
385369	7	1038	ATGCCAGACCAGCCCCTACT	69	129
385370	7	1043	TAGATATGCCAGACCAGCCC	69	130
385371	7	1048	CTTATTAGATATGCCAGACC	57	131
385372	7	1056	AAGGCTATCTTATTAGATAT	38	111
385373	7	1060	TCACAAGGCTATCTTATTAG	65	132
385374	7	1099	TAGTTTGGTGGCTGGAGCTT	59	133
385375	7	1115	TTTTCACAGATGCATGTAGT	71	134
385376	7	1130	TCATAAATACACATGTTTTC	43	135
385377	7	1156	AATCCTGGGCAGCTTCAAGT	80	136
385378	7	1274	TCATTCAGACAGTGACTCTT	70	137
385379	7	1279	TTGCTTCATTCAGACAGTGA	71	138

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
385380	7	1284	GAACTTTGCTTCATTCAGAC	71	139
385381	7	1335	TCATTCATTCTAGAAGTCCT	65	140
385382	7	1415	TATTGAGCTTTGTGACTAGC	87	141
385383.	7	1434	GAGCAGAGTAATGGATATTT	77	142
385384	7	1443	CAACACTCTGAGCAGAGTAA	74	143
385385	7	1488	CCATTTTACACAAGTCACCA	45	144
385386	7	1500	TAGGTTACAAATCCATTTTA	51	145
385387	7	1506	GACTTGTAGGTTACAAATCC	36	146
385388	7	1598	GCACTTGGGACTTTGTCAAG	79	147
385389	7	1610	TAAAAGTCCTGAGCACTTGG	65	148
385390	7	1620	TAGACAGGGCTAAAAGTCCT	58	149
385391	7	1630	CAAGCCAAAGTAGACAGGGC	85	150
385392	7	1671	TTGGCCATCACTAGGCTAAT	85	151
385393	7	1700	TAATTAGTGAACCTTAAGTC	69	152
385394	7	1711	AGTTTTGTAACTAATTAGTG	45	153
385395	7	1758	TACAGACAAATACATTTCAT	53	154
385396	7	1763	ATTTTTACAGACAAATACAT	19	155
385397	7	1769	TATACAATTTTTACAGACAA	33	156
385398	7	1800	TGTTCAAAGAAATAGACTTT	53	157
385399	7	1850	CAAATTTCAAAAGGGTATGG	63	158
385400	7	1873	TACATTTCTAACTAGAGAAG	22	159
385401	7	1878	AGAAATACATTTCTAACTAG	21	160
385402	7	1935	CACACAACAGGCTCTGCATA	75	161
385403	7	1947	AACCAGTCCACACACACAAC	71	162
385404	7	1955	AAATCTATAACCAGTCCACA	65	163
385405	7	2035	GATCAAATGTCTTACATCTA	72	164
385406	7	2055	AATTTTGTAAGTCAACAGGG	75	165
385407	7	2095	TAGCACCATGGTTAAGACTC	77	166
385408	7	2124	ACTTGTGTTAAACAAGTAAA	23	167
385409	7	2146	GTTTTATTGACACCAGGTAT	69	168
385410	7	2149	TTTGTTTTATTGACACCAGG	67	169
385411	7	2161	AAGAAATACATATTTGTTTT	25	170
385412	7	2178	GGCAATCCTTAGTAGACAAG	84	171
385413	7	2234	GTAAAGGAAGTAAAGGCTAC	61	172
385414	7	2295	AGAGCACAGGGAGGAAGTGT	58	173
385415	7	2302	CCAGTGAAGAGCACAGGGAG	74	174
385416	7	2472	CCAAGAATGCCAATCCCTGG	76	175
385417	7	2552	CCAACCTTTGAAATTCCCAA	75	176
385418	7	2593	CATACTTGGAGCAAACTAAT	53	177
385419	7	2611	AACTTAAGTGAACACAGTCA	60	178
385420	7	2665	TCAGTTACCAGTGGCTTTTG	71	179
385421	7	2698	TGAAGCTTTCAAATGACCCT	69	112
385422	7	2724	CCTGACTAAAGGTCACATTC	46	180
385423	7	2799	CCAAGCTGGCAGGCAGGGCA	78	181
385424	7	3055	CCAAGTCTTCATGGCCCTGG	73	182
385425	7	3127	ATTCATCTAGTCAGCACTCA	66	183
385426	7	3283	TACCAGAATGAAGGTGTTCA	53	184
385427	7	3290	GGTGCTCTACCAGAATGAAG	66	185
385428	7	3345	CCACATTAACTTGCAGTTGG	71	186

ISIS #	Target SEQ ID NO	Target Site	Sequence (5' to 3')	% Inhibition	SEQ ID NO
385429	9	2764	TCTAGAGCAGTCAGGCAGAC	46	187
385430	9	2869	GGAGCATGCCTGAGAAGAAA	66	188
385431	9	3166	CAAAATCCTGCACCCCATTT	55	189
385432	9	3358	AGATTATCCAACTGAATTAG	49	190
385433	9	3491	GAGCTGGTGTATTTGGGTAT	56	191
385434	9	3587	TGAGCAGGGCCCATAACCAA	73	192
385435	9	3784	ACATTTTCAAGTATGGGTTT	43	193

[0211] As shown in Table 7, SEQ ID NOs 51, 52, 110, 112, 121, 122, 124-145, 147, 148, 150-154, 157, 158, 161-166, 168, 169 and 171-193 inhibited expression of Mcl-1 mRNA by at least 40%; SEQ ID NOs 51, 52, 110, 112, 121, 122, 125, 129, 130, 132, 134, 136-143, 147, 148, 150-152, 158, 161-166, 168, 169 and 171, 172, 174-176, 178, 179, 181-183, 185, 186, 188 and 192 inhibited expression of Mcl-1 mRNA by at least 60%; and SEQ ID NOs 136, 141, 150, 151 and 171 inhibited expression of Mcl-1 mRNA by at least 80%. Preferred target regions of SEQ ID NO: 7 include but are not limited to nucleobases 763-856; 763-824; 763-788; 790-856; 790-824; 829-856; 997-1025; 1038-1079; 1099-1149; 1274-1303; 1415-1462; 1488-1525; 1598-1649; 1700-1730; 1758-1788; 1873-1897; 1935-1974; 2146-2197; 2295-2321; 2593-2630; and 3283-3319, and any range therewithin.

WHAT IS CLAIMED IS:

1. An oligomeric compound 12 to 50 nucleobases in length comprising at least an 8-nucleobase portion of a sequence selected from the group consisting of SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120.

2. The compound of claim 1 which is 20 nucleobases in length and has a sequence selected from the group consisting of SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120.

3. The compound of claim 1 comprising at least an 8-nucleobase portion of a sequence selected from the group consisting of SEQ ID NO: 64, 86, 87, 92, 95 and 96.

4. The compound of claim 3 having a sequence selected from the group consisting of SEQ ID NO: 64, 86, 87, 92, 95 and 96.

5. The compound of claim 1 which is at least 80% complementary to a nucleic acid molecule encoding human Mcl-1.

6. The compound of claim 5 which is at least 90% complementary to a nucleic acid molecule encoding human Mcl-1.

7. The compound of claim 6 which is at least 95% complementary to a nucleic acid molecule encoding human Mcl-1.

8. The compound of claim 7 which is 100% complementary to a nucleic acid molecule encoding human Mcl-1.

9. The compound of any one of claims 5-8 wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 and 5.

10. An oligomeric compound 16 to 50 nucleobases in length having at least 80% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

11. The compound of claim 10 which is 17 to 50 nucleobases in length and has at least 85% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

12. The compound of claim 10 which is 18 to 50 nucleobases in length and has at least 90% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

13. The compound of claim 10 which is 19 to 50 nucleobases in length and has at least 95% identity with SEQ ID NO: 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120.

14. An oligomeric compound 12 to 50 nucleobases in length targeted to a nucleic acid molecule encoding human Mcl-1 (SEQ ID NO: 2), wherein said compound specifically hybridizes to at least a portion of a target region defined by nucleobases 123-150; 719-748; 808-839; 902-930; 902-1007; 938-1007; 1039-1074; 1083-1150; 1104-1128; 1252-1297; 1396-1423; 1651-1693; 1809-1851; 2062-2103; 2551-2585; 3118-3161; 3176-3202; or 3214-3254 of said nucleic acid molecule encoding human Mcl-1.

15. The compound of any one of claims 1, 3, 5, 6, 7 or 14 which is 15 to 30 nucleobases in length.

16. The compound of any of claims 1-15 comprising at least one modified sugar moiety, internucleoside linkage or nucleobase.

17. The compound of claim 16, wherein the modified sugar moiety is 2'-O-methoxyethyl.

18. The compound of claim 16, wherein the modified nucleobase is phosphorothioate.

19. The compound of claim 16, wherein the modified nucleobase is 5-methylcytosine.

20. A method of modulating expression of human Mcl-1 in a cell, tissue or animal, comprising administering to said cell, tissue or animal the oligomeric compound of any one of claims 1-19.

21. A method of inducing apoptosis of a cell, comprising administering to said cell the oligomeric compound of any one of claims 1-19.

22. A method of inhibiting proliferation of a cell, comprising administering to said cell the oligomeric compound of any one of claims 1-19.

23. A compound according to any one of claims 1-19 for use in therapy.

24. Use of a compound according to any one of claims 1-19 for the preparation of a medicament for modulating expression of human Mcl-1.

25. Use of a compound according to any one of claims 1-19 for the preparation of a medicament for inducing apoptosis.

26. Use of a compound according to any one of claims 1-19 for the preparation of a medicament for inhibiting cellular proliferation.

27. The use of any one of claims 24-26, wherein the modulating, inducing or inhibiting occurs in a human cell.