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(54) HIGH AFFINITY NUCLEIC ACID LIGANDS OF COMPLEMENT SYSTEM PROTEINS

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Continuation-in-part of application No. 10/037,986, filed on Oct. 18, 2001, which is a continuation of application No. 09/502,344, filed on Feb. 10, 2000, now Pat. No. 6,331,398, which is a continuation of application No. 09/143,190, filed on Aug. 27, 1998, now Pat. No. 6,110,900, which is a continuation of application No. 08/469,609, filed on Jun. 6, 1995, now Pat. No. 5,843,653, which is a continuation of application No. 08/428,964, filed on Apr. 25, 1995, now abandoned, and which is a continuation of application No. 08/409,442, filed on Mar. 24, 1995, now Pat. No. 5,696,249, and which is a continuation of application No. 08/412,110, filed on Mar. 27, 1995, now Pat. No. 5,670,637.

Said application No. 08/428,964 is a continuation of application No. 07/714,131, filed on Jun. 10, 1991, now Pat. No. 5,475,096.

Said application No. 08/409,442 is a continuation of application No. 07/714,131, filed on Jun. 10, 1991, now Pat. No. 5,475,096.

Said application No. 08/412,110 is a continuation of application No. 07/714,131, filed on Jun. 10, 1991, now Pat. No. 5,475,096, which is a continuation of application No. 07/536,428, filed on Jun. 11, 1990, now abandoned.

Publication Classification

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(52) **U.S. Cl.** **514/44**; 536/23.2

(57) ABSTRACT

Methods are described for the identification and preparation of high-affinity Nucleic Acid Ligands to Complement System Proteins. Methods are described for the identification and preparation of high affinity Nucleic Acid Ligands to Complement System Proteins C1q, C3 and C5. Included in the invention are specific RNA ligands to C1q, C3 and C5 identified by the SELEX method.

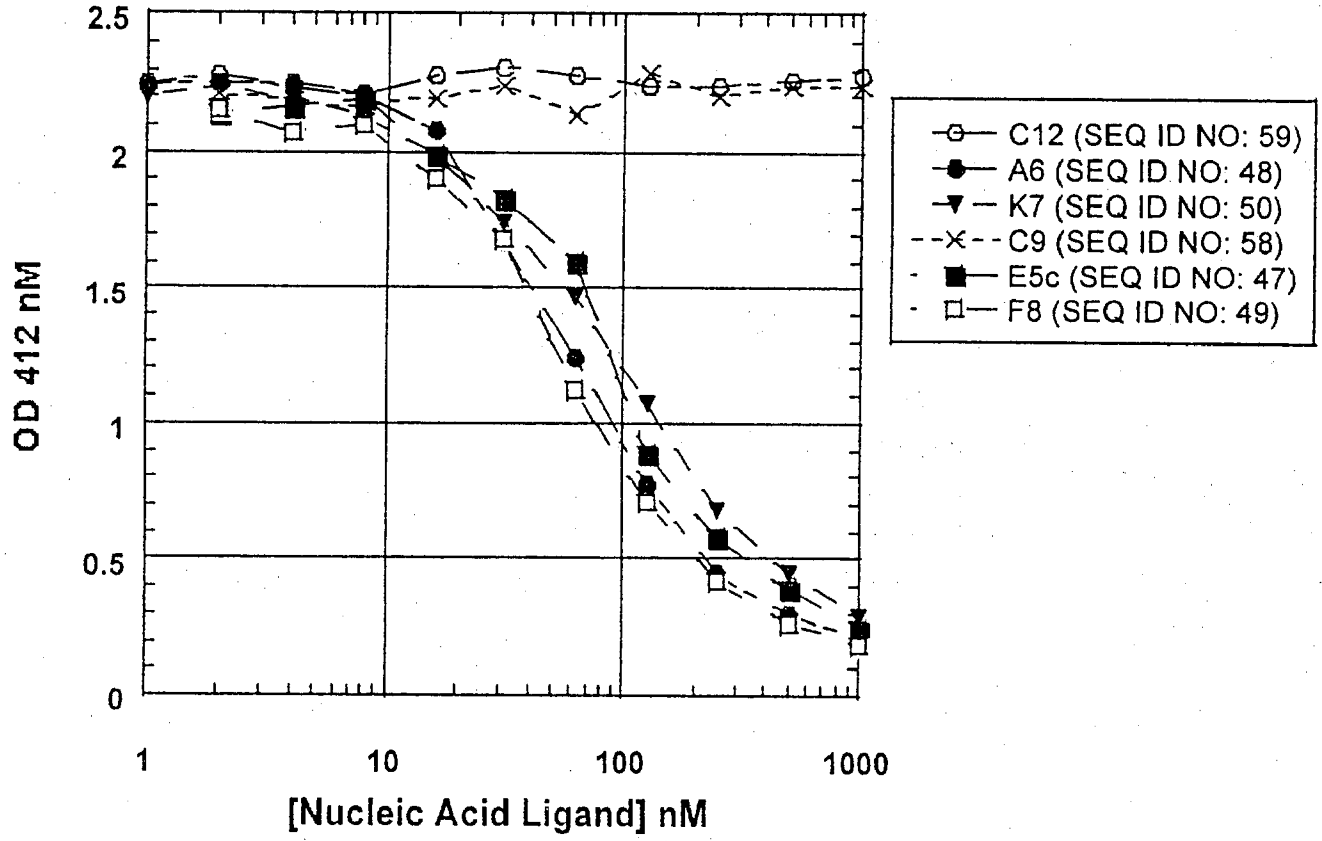

2.5
2
1.5
1
0.5
0
OD 412 nM
1
10
100
1000
[Nucleic Acid Ligand] nM
C12 (SEQ ID NO: 59)
A6 (SEQ ID NO: 48)
K7 (SEQ ID NO: 50)
C9 (SEQ ID NO: 58)
E5c (SEQ ID NO: 47)
F8 (SEQ ID NO: 49)

Figure 1

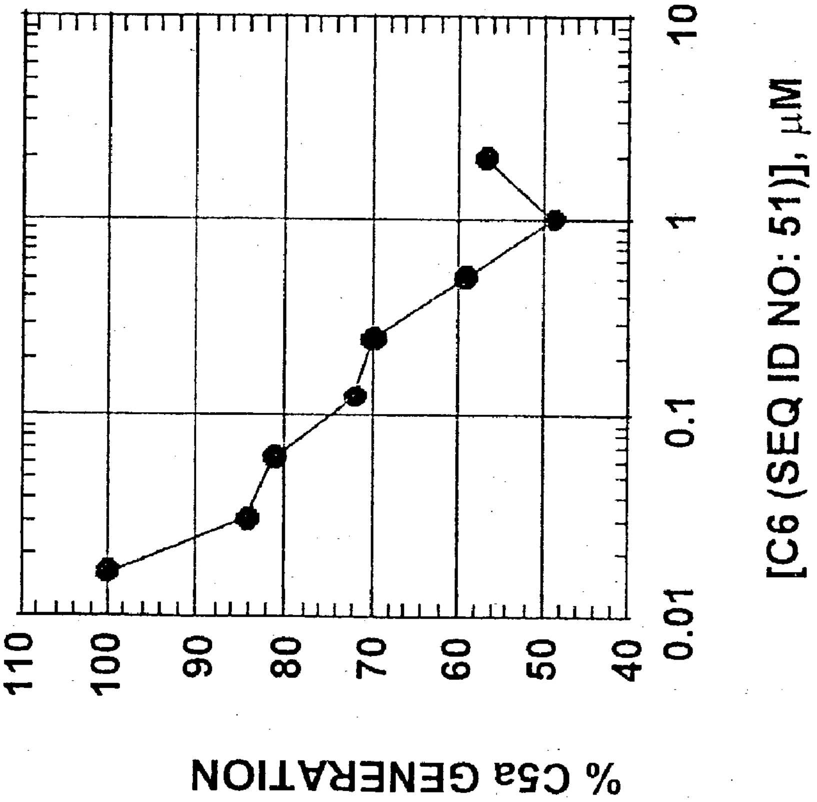

% C5a GENERATION
110
100
90
80
70
60
50
40
0.01
0.1
1
10
[C6 (SEQ ID NO: 51)], μM

Figure 2

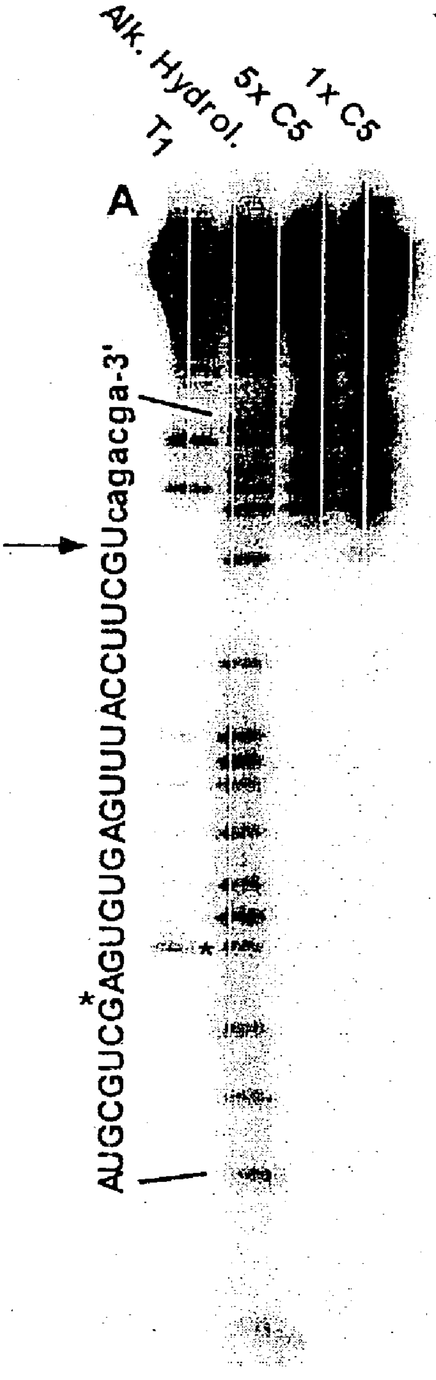


FIGURE 3A

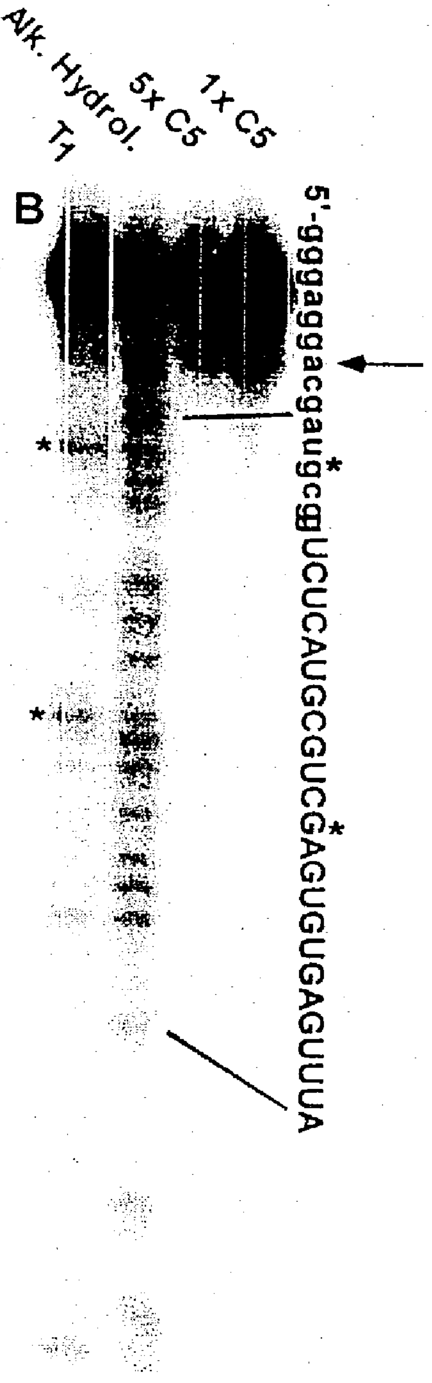


FIGURE 3B

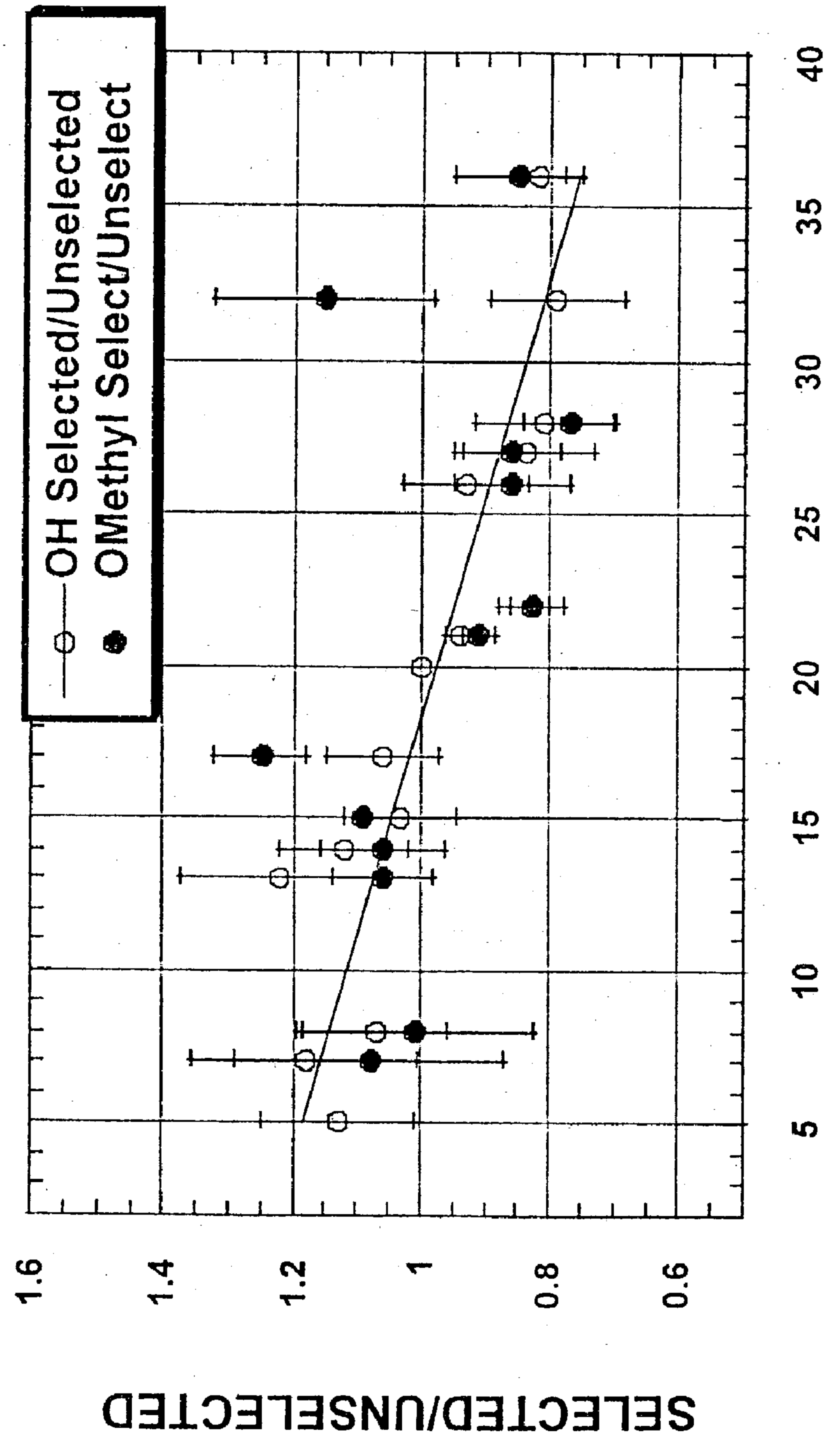
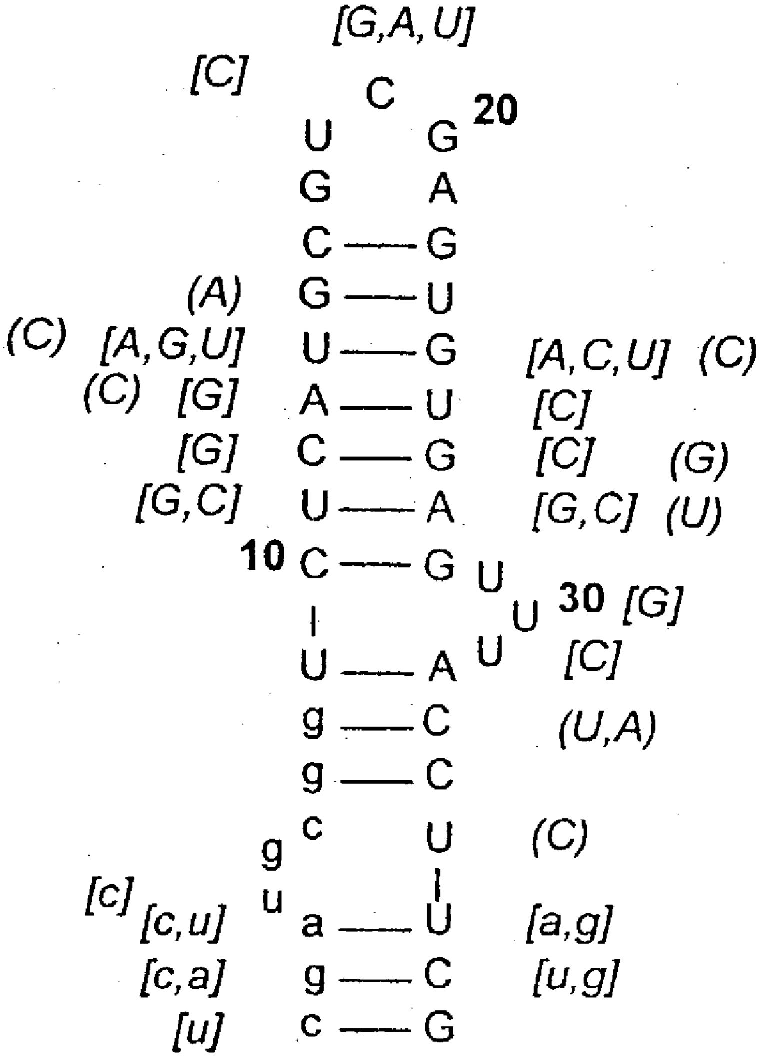
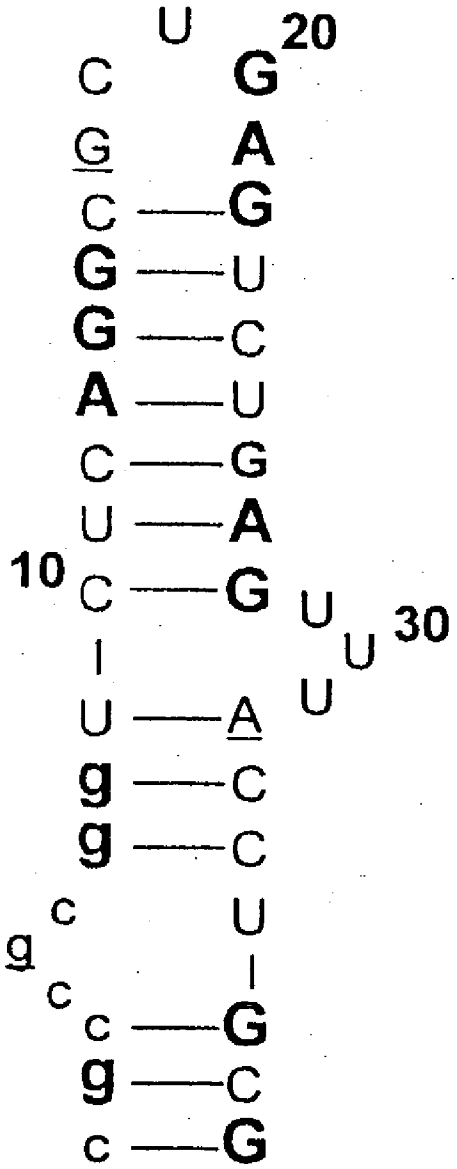

OH Selected/Unselected
OMethyl Select/Unselect
1.6
1.4
1.2
1
0.8
0.6
SELECTED/UNSELECTED
5
10
15
20
25
30
35
40
NUCLEOTIDE NUMBER

Figure 4



SEQ ID NO: 160

Figure 5A



SEQ ID NO: 193

Figure 5B

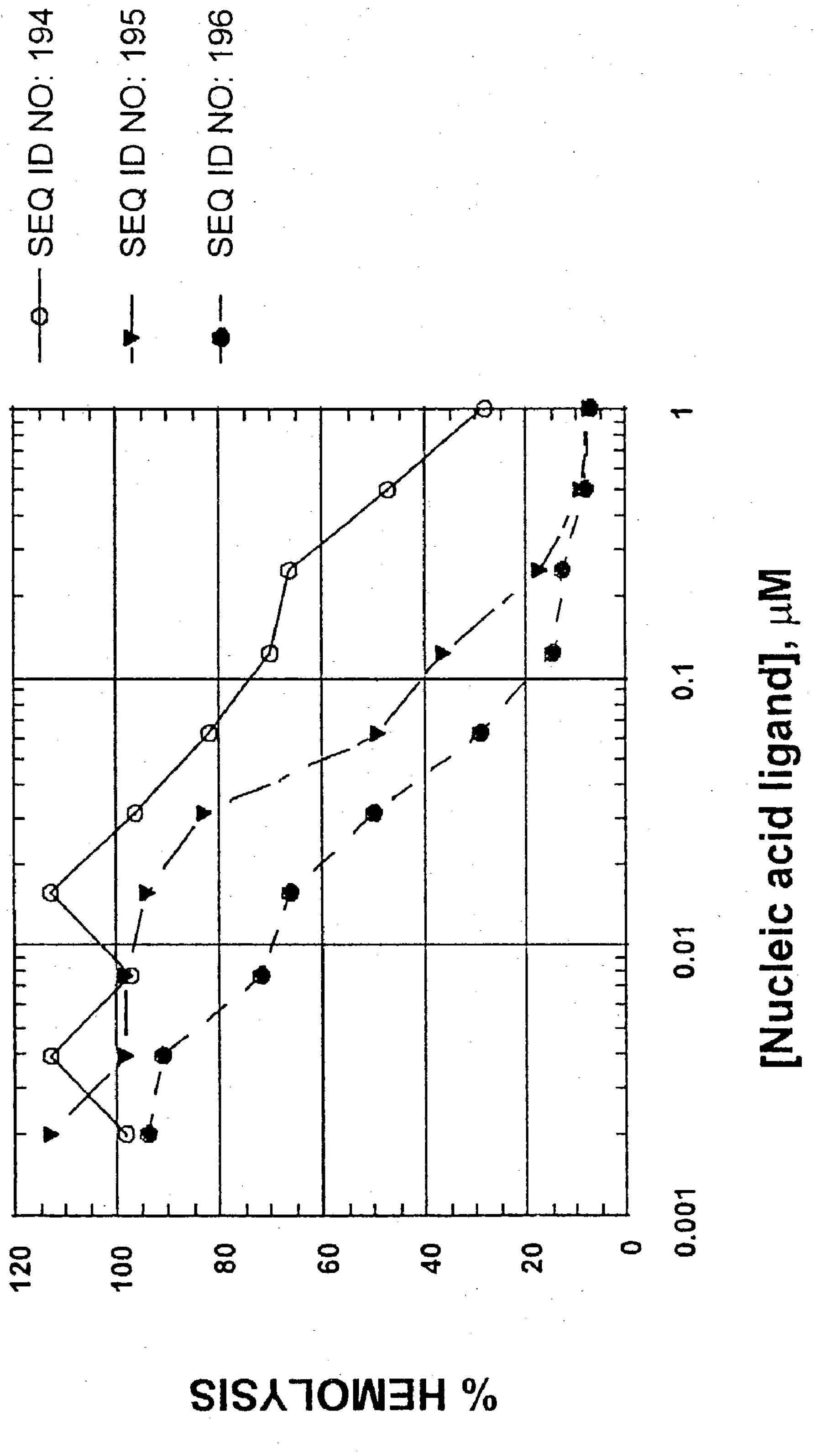

SEQ ID NO: 194
SEQ ID NO: 195
SEQ ID NO: 196
% HEMOLYSIS
120
100
80
60
40
20
0
0.001
0.01
0.1
1
[Nucleic acid ligand], μM

Figure 6

HIGH AFFINITY NUCLEIC ACID LIGANDS OF COMPLEMENT SYSTEM PROTEINS

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 10/037,282, filed Jan. 3, 2002, which is a continuation of U.S. patent application Ser. No. 09/163,025, filed Sep. 29, 1998, now U.S. Pat. No. 6,395,888, which is a continuation-in-part of U.S. patent application Ser. No. 09/023,228, filed Feb. 12, 1998, now U.S. Pat. No. 6,140,490, which is a continuation-in-part of PCT/US97/01739 (International Publication No. WO 97/28178), filed Jan. 30, 1997, which is a continuation-in-part of U.S. patent. application Ser. No. 08/595,335, filed Feb. 1, 1996, now abandoned. This application is also a continuation-in-part of U.S. patent application Ser. No. 10/037,986, filed Oct. 18, 2001, which is a continuation of U.S. patent application Ser. No. 09/502,344, filed Feb. 10, 2000, now U.S. Pat. No. 6,331,398, which is a continuation of U.S. Patent. application Ser. No. 09/143,190, filed Aug. 27, 1998, now U.S. Pat. No. 6,110,900, which is a continuation of U.S. patent application Ser. No. 08/469,609, filed Jun. 6, 1995, now U.S. Pat. No. 5,843,653, which is a continuation of U.S. patent application Ser. No. 08/428,964, filed Apr. 25, 1995, now abandoned. U.S. patent application Ser. No. 08/469,609 is also a continuation of U.S. patent application Ser. No. 08/409,442, filed Mar. 24, 1995, now U.S. Pat. No. 5,696,249 and a continuation of U.S. patent application Ser. No. 08/412,110, filed Mar. 27, 1995, now U.S. Pat. No. 5,670,637. Ser. Nos. 08/428,964, 08/409,442, and 08/412,110 are continuations of U.S. patent application Ser. No. 07/714,131, filed Jun. 10, 1991, now U.S. Pat. No. 5,475,096, which is a continuation-in-part application of U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, now abandoned.

FIELD OF THE INVENTION

[0002] Described herein are methods for identifying and preparing high-affinity Nucleic Acid Ligands to Complement System Proteins. The method utilized herein for identifying such Nucleic Acid Ligands is called SELEX™, an acronym for Systematic Evolution of Ligands by EXponential enrichment. Described herein are methods for identifying and preparing high-affinity Nucleic Acid Ligands to the Complement System Proteins C1q, C3 and C5. This invention includes high affinity Nucleic Acid Ligands of C1q, C3 and C5. Also disclosed are RNA ligands of C1q, C3 and C5. Also disclosed are Nucleic Acid Ligands that inhibit and/or activate the Complement System. The oligonucleotides of the present invention are useful as pharmaceuticals or diagnostic agents.

BACKGROUND OF THE INVENTION

[0003] The complement system comprises a set of at least 20 plasma and membrane proteins that act together in a regulated cascade system to attack extracellular forms of pathogens (Janeway et al. (1994) *Immunobiology: The Immune System in Health and Disease*. Current Biology Ltd, San Francisco, pp. 8:35-8:55; Morgan (1995) Crit. Rev. in Clin Lab. Sci. 32(3):265-298). There are two distinct enzymatic activation cascades, the classical and alternative pathways, and a non-enzymatic pathway known as the membrane attack pathway.

[0004] The classical pathway is usually triggered by an antibody bound to a foreign particle. It comprises several components, C1, C4, C2, C3 and C5 (listed by order in the pathway). Initiation of the classical pathway of the Complement System occurs following binding and activation of the first complement component (C1) by both immune and non-immune activators (Cooper (1985) Adv. Immunol. 37:151). C1 comprises a calcium-dependent complex of components C1q, C1r and C1s, and is activated through binding of the C1q component. C1 q contains six identical subunits and each subunit comprises three chains (the A, B and C chains). Each chain has a globular head region which is connected to a collagen-like tail. Binding and activation of C1q by antigen-antibody complexes occurs through the C1q head group region. Numerous non-antibody C1q activators, including proteins, lipids and nucleic acids (Reid et al. (1993) *The Natural Immune System: Humoral Factors*. E. Sim, ed. IRL Press, Oxford, p. 151) bind and activate through a distinct site on the collagen-like stalk region.

[0005] Non-antibody C1q protein activators include C-reactive protein (CRP) (Jiang et al. (1991) J. Immunol. 146:2324) and serum amyloid protein (SAP) (Bristow et al. (1986) Mol. Immunol. 23:1045); these will activate C1q when aggregated by binding to phospholipid or carbohydrate, respectively. Monomeric CRP or SAP do not activate C1q. C1q is also activated through binding to aggregated β-amyloid peptide (Schultz et al. (1994) Neurosci. Lett. 175:99; Snyder et al. (1994) Exp. Neurol. 128:136), a component of plaques seen in Alzheimer's disease (Jiang et al. (1994) J. Immunol. 152:5050; Eikelenboom and Stam (1982) Acta Neuropathol (Berl) 57:239; Eikelenboom et al. (1989) Virchows Arch. [B] 56:259; Rogers et al. (1992) Proc. Natl. Acad. Sci. USA 89:10016; Dietzschold et al. (1995) J. Neurol. Sci. 130:11). C1q activation might also exacerbate the tissue damage associated with Alzheimer's disease. These activators bind C1q on its collagen-like region, distant from the head-group region where immunoglobulin activators bind. Other proteins which bind the C1q collagen-like region include collagen (Menzel et al. (1981) Biochim. Biophys. Acta 670:265), fibronectin (Reid et al. (1984) Acta Pathol. Microbiol. Immunol. Scand. Sect. C 92 (Suppl. 284):11), laminin (Bohnsack et al. (1985) Proc. Natl. Acad. Sci. USA 82:3824), fibrinogen and fibrin (Entwistle et al. (1988) Biochem. 27:507), HIV rsgp41 (Stoiber et al. (1995) Mol. Immunol. 32:371), actin (Nishioka et al. (1982) Biochem. Biophys. Res. Commun. 108:1307) and tobacco glycoprotein (Koethe et al. (1995) J. Immunol. 155:826).

[0006] C1q also binds and can be activated by anionic carbohydrates (Hughes-Jones et al. (1978) Immunology 34:459) including mucopolysaccharides (Almeda et al. (1983) J. Biol. Chem. 258:785), fucans (Blondin et al. (1994) Mol. Immunol. 31 :247), proteoglycans (Silvestri et al. (1981) J. Biol. Chem. 256:7383), and by lipids including lipopolysaccharide (LPS) (Zohair et al. (1989) Biochem. J. 257:865; Stoiber et al. (1994) Eur. J. Immunol. 24:294). Both DNA (Schravendijk and Dwek (1982) Mol. Immunol. 19:1179; Rosenberg et al. (1988) J. Rheumatol 15:1091; Uwatoko et al. (1990) J. Immunol. 144:3484) and RNA (Acton et al. (1993) J. Biol. Chem. 268:3530) can also bind and potentially activate C1q. Intracellular components which activate C1q include cellular and subcellular membranes (Linder (1981) J. Immunol. 126:648; Pinckard et al. (1973) J. Immunol. 110: 1376; Storrs et al. (1981) J. Biol. Chem. 256:10924; Giclas et al. (1979) J. Immmunol. 122:146; Storrs et al. (1983) J. Immunol. 131:416), intermediate filaments (Linder et al. (1979) Nature 278:176) and actin (Nishioka et al. (1982) Biochem. Biophys. Res. Commun. 108:1307). All of these interactions would recruit the classical pathway for protection against bacterial (or viral) infection, or as a response to tissue injury (Li et al. (1994) J. Immunol. 152:2995) in the absence of antibody.

[0007] A binding site for non-antibody activators including CRP (Jiang et al. (1991) J. Immunol. 146:2324), SAP (Ying et al. (1993) J. Immunol. 150:169), β-amyloid peptide (Newman (1994) Curr. Biol. 4:462) and DNA (Jiang et al. (1992) J. Biol. Chem. 267:25597) has been localized to the amino terminus of C1q A chain at residues 14-26. A synthetic peptide comprising this sequence effectively inhibits both binding and activation. The peptide 14-26 contains several basic residues and matches one of the heparin binding motifs (Yabkowitz et al. (1989) J. Biol. Chem. 264:10888; Cardin et al. (1989) Arteriosclerosis 9:21). The peptide is also highly homologous with peptide 145-156 in collagen-tailed acetylcholinesterase; this site is associated with heparin-sulfate basement membrane binding (Deprez et al. (1995) J. Biol. Chem. 270:11043). A second C1q A chain site at residues 76-92 also might be involved in weaker binding; this site is at the junction of the globular head region and the collagen-like tail.

[0008] The second enzymatically activated cascade, known as the alternative pathway, is a rapid, antibody-independent route for the Complement System activation and amplification. The alternative pathway comprises several components, C3, Factor B, and Factor D. Activation of the alternative pathway occurs when C3b, a proteolytic cleavage form of C3, is bound to an activating surface such as a bacterium. Factor B is then bound to C3b, and cleaved by Factor D to yield the active enzyme, Ba. The enzyme Ba then cleaves more C3 to C3b, producing extensive deposition of C3b-Ba complexes on the activating surface. When a second C3b is deposited, forming a C3b-C3b-Ba complex, the enzyme can then cleave C5 and trigger activation of the terminal pathway.

[0009] The non-enzymatic terminal pathway, also known as the membrane attack pathway, comprises the components C5, C6, C7, C8 and C9. Activation of this membrane attack pathway results when the C5 component is enzymatically cleaved by either the classical or alternative pathway to yield the small C5a polypeptide (9 kDa) and the large C5b fragment (200 kDa). The C5a polypeptide binds to a 7 transmembrane G-protein coupled receptor which was originally described on leukocytes and is now known to be expressed on a variety of tissues including hepatocytes (Haviland et al. (1995) J. Immunol. 154:1861) and neurons (Gasque et al. (1997) Am. J. Pathol. 150:31). The C5a molecule is the primary chemotactic component of the human Complement System and can trigger a variety of biological responses including leukocyte chemotaxis, smooth muscle contraction, activation of intracellular signal transduction pathways, neutrophil-endothelial adhesion (Mulligan et al. (1997) J. Immunol. 158:1857), cytokine and lipid mediator release and oxidant formation. The larger C5b fragment binds sequentially to later components to form the C5b-9 membrane attack complex (MAC). The C5b-9 MAC can directly lyse erythrocytes, and in greater quantities is lytic for leukocytes and is damaging to tissues such as muscle, epithelial and endothelial cells (Stahl et al. (1997) Circ. Res. 76:575). In sublytic amounts the MAC can stimulate upregulation of adhesion molecules, intracellular calcium increase and cytokine release (Ward (1996) Am. J. Pathol. 149:1079). In addition, the C5b-9 MAC can stimulate cells such as endothelial cells and platelets without causing cell lysis. The non-lytic effects of C5a and the C5b-9 MAC are sometimes quite similar.

[0010] The Complement System has an important role in defense against bacterial and viral infection, and possibly in immune surveillance against tumors. This is demonstrated most clearly in humans who are deficient in complement components. Individuals deficient in early components (C1, C4, C2 or C3) suffer from recurrent infections, while individuals deficient in late components (C5 through C9) are susceptible to nisseria infection. Complement classical pathway is activated on bacteria by antibodies, by binding of CRP or SAP, or by direct activation through LPS. Complement alternative pathway is activated through binding of C3 to the cell coat. Complement can be activated by viruses through antibodies, and can also be activated on viral infected cells because these are recognized as foreign. In a similar way, transformed cells can be recognized as foreign and can be lysed by the Complement System or targeted for immune clearance.

[0011] Activation of the Complement System can and has been used for therapeutic purposes. Antibodies which were produced against tumor cells were then used to activate the Complement System and cause tumor rejection. Also, the Complement System is used together with polyclonal or monoclonal antibodies to eliminate unwanted lymphocytes. For example, anti-lymphocyte globulin or monoclonal anti-T-cell antibodies are used prior to organ transplantation to eliminate lymphocytes which would otherwise mediate rejection.

[0012] Although the Complement System has an important role in the maintenance of health, it has the potential to cause or contribute to disease. The Complement System has been implicated in numerous renal, rheumatological, neurological, dermatological, hematological, vascular/pulmonary, allergy, infectious, biocompatibility/shock and other diseases or conditions (Morgan (1995) Crit. Rev. in Clin Lab. Sci. 32(3 :265-298; Matis and Rollins (1995) Nature Medicine 1(8):839-842). The Complement System is not necessarily the only cause of the disease state, but it may be one of several factors, each of which contributes to pathogenesis.

[0013] Several pharmaceuticals have been developed that inhibit the Complement System in vivo, however, many cause toxicity or are poor inhibitors (Morgan (1995) Crit. Rev. in Clin Lab. Sci. 32(3):265-298). Heparins, K76COOH and nafamstat mesilate have been shown to be effective in animal studies (Morgan (1995) Crit. Rev. in Clin Lab. Sci. 32(3):265-298). Recombinant forms of naturally occurring inhibitors of the Complement System have been developed or are under consideration, and these include the membrane regulatory proteins Complement Receptor 1 (CR1), Decay Accelerating Factor (DAF), Membrane Cofactor Protein (MCP) and CD59.

[0014] C5 is an attractive target for the development of a Complement System inhibitor, as both the classical and alternative pathways converge at component C5 (Matis and Rollins (1995) Nature Medicine 1(8):839-842). In addition, inhibition of C5 cleavage blocks both the C5a and the C5b effects on leukocytes and on tissue such as endothelial cells

(Ward (1996) Am. J. Pathol. 149:1079); thus C5 inhibition can have therapeutic benefits in a variety of diseases and situations, including lung inflammation (Mulligan et al. (1998) J. Clin. Invest. 98:503), extracorporeal complement activation (Rinder et al. (1995) J. Clin. Invest. 96:1564) or antibody-mediated complement activation (Biesecker et al. (1989) J. Immunol. 142:2654). Matis and Rollins ((1995) Nature Medicine 1(8):839-842) have developed C5-specific monoclonal antibodies as an anti-inflammatory biopharmaceutical. Both C5a and the MAC have been implicated in acute and chronic inflammation associated with human disease, and their role in disease states has been confirmed in animal models. C5a is required for complement- and neutrophil-dependent lung vascular injury (Ward (1997) J. Lab. Clin. Med. 129:400; Mulligan et al. (1998) J. Clin. Invest. 98:503), and is associated with neutrophil and platelet activation in shock and in burn injury (Schmid et al. (1997) Shock 8:119). The MAC mediates muscle injury in acute autoimmune myasthenia gravis (Biesecker and Gomez (1989) J. Immunol. 142:2654), organ rejection in transplantation (Baldwin et al. (1995) Transplantation 59:797; Brauer et al. (1995) Transplantation 59:288; Takahashi et al. (1997) Immunol. Res. 16:273) and renal injury in autoimmune glomerulonephritis (Biesecker (1981) J. Exp. Med. 39:1779; Nangaku (1997) Kidney Int. 52:1570). Both C5a and the MAC are implicated in acute myocardial ischemia (Homeister and Lucchesi (1994) Annu. Rev. Pharmacol. Toxicol. 34:17), acute (Bednar et al. (1997) J. Neurosurg. 86:139) and chronic CNS injury (Morgan (1997) Exp. Clin. Immunogenet. 14:19), leukocyte activation during extracorporeal circulation (Sun et al. (1995) Nucleic Acids Res. 23:2909; Spycher and Nydegger (1995) Infushionsther. Transfusionsmed. 22:36) and in tissue injury associated with autoimmune diseases including arthritis and lupus (Wang et al. (1996) Immunology 93:8563). Thus, inhibiting cleavage of C5 prevents generation of two potentially damaging activities of the Complement System. Inhibiting C5a release eliminates the major Complement System chemotactic and vasoactive activity, and inhibiting C5b formation blocks assembly of the cytolytic C5b-9 MAC. Furthermore, inhibition of C5 prevents injury by the Complement System while leaving intact important Complement System defense and clearance mechanisms, such as C3 and C1q phagocytic activity, clearance of immune complexes and the innate immune response (Carrol (1998) Ann. Rev. Immunol. 16:545).

[0015] C3 is an attractive target for the development of a Complement System inhibitor, as it is common to both pathways. Inhibition of C3 using recombinant versions of a natural inhibitors (Kalli et al. (1994) Springer Semin. Immunopathol. 15:417) can prevent cell-mediated tissue injury (Mulligan et al. (1992) J. Immunol. 148:1479) and this has been shown to have therapeutic benefit in diseases such as myocardial infarction (Weisman et al. (1990) Science 249:146) and liver ischemia/reperfusion (Chavez-Cartaya et al. (1995) Transplantation 59:1047). Controlling C3 limits most biological activities of the Complement System. Most natural inhibitors, including DAF, MCP, CR1 and Factor H target C3.

[0016] SELEX™

[0017] A method for the in vitro evolution of Nucleic Acid molecules with highly specific binding to target molecules has been developed. This method, Systematic Evolution of Ligands by EXponential enrichment, termed the SELEX process, is described in U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, entitled "Systematic Evolution of Ligands by Exponential Enrichment," now abandoned; U.S. patent application Ser. No. 07/714,131, filed Jun. 10, 1991, entitled "Nucleic Acid Ligands," now U.S. Pat. No. 5,475,096; U.S. patent application Ser. No. 07/931,473, filed Aug. 17, 1992, entitled "Methods for Identifying Nucleic Acid Ligands," now U.S. Pat. No. 5,270,163 (see also WO 91/19813), each of which is herein specifically incorporated by reference in its entirety. Each of these applications, collectively referred to herein as the SELEX Patent Applications, describes a fundamentally novel method for making a Nucleic Acid Ligand to any desired Target molecule.

[0018] The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of Nucleic Acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the Target under conditions favorable for binding, partitioning unbound Nucleic Acids from those Nucleic Acids which have bound specifically to Target molecules, dissociating the Nucleic Acid-Target complexes, amplifying the Nucleic Acids dissociated from the Nucleic Acid-Target complexes to yield a ligand-enriched mixture of Nucleic Acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity Nucleic Acid Ligands to the Target molecule.

[0019] The basic SELEX method has been modified to achieve a number of specific objectives. For example, U.S. patent application Ser. No. 07/960,093, filed Oct. 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure," now abandoned (see also U.S. Pat. No. 5,707,796), describes the use of the SELEX method in conjunction with gel electrophoresis to select Nucleic Acid molecules with specific structural characteristics, such as bent DNA. U.S. patent application Ser. No. 08/123,935, filed Sep. 17, 1993, entitled "Photoselection of Nucleic Acid Ligands," now abandoned, (see also U.S. Pat. No. 5,763,177) describes a SELEX-based method for selecting Nucleic Acid Ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a Target molecule. U.S. patent application Ser. No. 08/134,028, filed Oct. 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine," now abandoned (see also U.S. Pat. No. 5,580,737), describes a method for identifying highly specific Nucleic Acid Ligands able to discriminate between closely related molecules, termed Counter-SELEX. U.S. patent application Ser. No. 08/143,564, filed Oct. 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX," now abandoned, (see also U.S. Pat. No. 5,567,588) and U.S. patent application Ser. No. 08/792,075, filed Jan. 31, 1997, entitled "Flow Cell SELEX," now U.S. Pat. No. 5,861,254, describe SELEX-based methods which achieve highly efficient partitioning between oligonucleotides having high and low affinity for a Target molecule. U.S. patent application Ser. No. 07/964,624, filed Oct. 21, 1992, entitled "Nucleic Acid Ligands to HIV-RT and HIV-1

Rev," now U.S. Pat. No. 5,496,938, describes methods for obtaining improved Nucleic Acid Ligands after the SELEX process has been performed. U.S. patent application Ser. No. 08/400,440, filed Mar. 8, 1995, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX," now U.S. Pat. No. 5,705,337, describes methods for covalently linking a ligand to its Target.

[**0020**] The SELEX method encompasses the identification of high-affinity Nucleic Acid Ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified Nucleic Acid Ligands containing modified nucleotides are described in U.S. patent application Ser. No. 08/117,991, filed Sep. 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," now abandoned, (see also U.S. Pat. No. 5,660,985) that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. U.S. patent application Ser. No. 08/134,028, now U.S. Pat. No. 5,580,737, supra, describes highly specific Nucleic Acid Ligands containing one or more nucleotides modified with 2'-amino (2'-NH_2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). U.S. patent application Ser. No. 08/264,029, filed Jun. 22, 1994, entitled "Novel Method of Preparation of Known and Novel 2' Modified Nucleosides by Intramolecular Nucleophilic Displacement," now abandoned, describes oligonucleotides containing various 2'-modified pyrimidines.

[**0021**] The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in U.S. patent application Ser. No. 08/284,063, filed Aug. 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX," now U.S. Pat. No. 5,637,459 and U.S. patent application Ser. No. 08/234,997, filed Apr. 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX," now U.S. Pat. No. 5,683,867, respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

BRIEF SUMMARY OF THE INVENTION

[**0022**] The present invention includes methods of identifying and producing Nucleic Acid Ligands to Complement System Proteins and homologous proteins and the Nucleic Acid Ligands so identified and produced. By homologous proteins it is meant a degree of amino acid sequence identity of 80% or more. Exemplified herein is a method of identifying and producing Nucleic Acid Ligands to C1q, C3 and C5, and the Nucleic Acid Ligands so produced. Nucleic Acid Ligand sequences are provided that are capable of binding specifically to C1q, C3 and C5. In particular, RNA sequences are provided that are capable of binding specifically the C1q, C3 and C5. Specifically included in the invention are the RNA ligand sequences shown in Tables 2-6, 8, 10 and 12-13 and FIGS. **5**A-B (SEQ ID NOS: 5-155 and 160-196). Also included in the invention are Nucleic Acid Ligands that inhibit the function of proteins of the Complement System. Specifically included in the invention herein are RNA ligands that inhibit the function of C1 q, C3 and C5. Also included are Nucleic Acid Ligands that inhibit and/or activate the Complement System.

[**0023**] Further included in this invention is a method of identifying Nucleic Acid Ligands and Nucleic Acid Ligand sequences to Complement System Proteins comprising the steps of (a) preparing a Candidate Mixture of Nucleic Acids, (b) contacting the Candidate Mixture of Nucleic Acids with a Complement System Protein, (c) partitioning between members of said Candidate Mixture on the basis of affinity to said Complement System Protein, and (d) amplifying the selected molecules to yield a mixture of Nucleic Acids enriched for Nucleic Acid sequences with a relatively higher affinity for binding to said Complement System Protein.

[**0024**] Also included in this invention is a method of identifying Nucleic Acid Ligands and Nucleic Acid Ligand sequences to C1q, C3 and C5, comprising the steps of (a) preparing a Candidate Mixture of Nucleic Acids, (b) contacting the Candidate Mixture of Nucleic Acids with C1q, C3 or C5, (c) partitioning between members of said Candidate Mixture on the basis of affinity to C1q, C3 or C5, and (d) amplifying the selected molecules to yield a mixture of Nucleic Acids enriched for Nucleic Acid sequences with a relatively higher affinity for binding to C1q, C3 or C5.

[**0025**] More specifically, the present invention includes the RNA ligands to C1q, C3 and C5 identified according to the above-described method, including RNA ligands to C1q, including those ligands shown in Table 2 (SEQ ID NOS:5-20) and Table 6 (SEQ ID NOS: 84-155), RNA ligands to C3, including those sequences shown in Table 3 (SEQ ID NOS:21-46), and RNA ligands to C5, including those sequences shown in Table 4 (SEQ ID NOS:47-74), Table 5 (SEQ ID NOS:76-83), Table 8 (SEQ ID NOS:75, 160-162), Table 10 (SEQ ID NOS:163-189), Table 12 (SEQ ID NOS:190-192), Table 13 (SEQ ID NOS:194-196) and FIGS. **5**A-B (SEQ ID NOS:160 and 193). Also included are RNA ligands to C1q, C3 and C5 that are substantially homologous to any of the given ligands and that have substantially the same ability to bind C1q, C3 or C5, and inhibit the function of C1q, C3 or C5. Further included in this invention are Nucleic Acid Ligands to C1q, C3 and C5 that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind C1q, C3 or C5 and inhibit the function of C1q, C3 or C5.

[**0026**] The present invention also includes modified nucleotide sequences based on the RNA ligands identified herein and mixtures of the same.

BRIEF DESCRIPTION OF THE FIGURES

[**0027**] **FIG. 1** shows the results of an inhibition assay in which 2'-F RNA ligands C 12 (SEQ ID NO:59), A6 (SEQ ID NO:48), K7 (SEQ ID NO:50), C9 (SEQ ID NO:58), E5c (SEQ ID NO:47) and F8 (SEQ ID NO:49) to human C5 were incubated with antibody-coated sheep erythrocytes and whole human serum. The results are presented as optical density (OD) versus concentration of ligand in nM.

[**0028**] **FIG. 2** shows the % C5a generation as a function of concentration of clone C6 (SEQ ID NO:51).

[**0029**] **FIG. 3A** shows a sequencing gel of 5'-kinase-labeled clone C6 (SEQ ID NO:51) after alkaline hydrolysis or digestion with T_1 nuclease. The 3'-sequence (5'-end labeled) is aligned with the alkaline hydrolysis ladder. On the left is the T_1 ladder and on the right are RNA selected with 5× and 1× concentrations of C5. The boundary where removal of a base eliminates binding is shown by the arrow. The asterisk shows a G which is hypersensitive to T_1.

[**0030**] **FIG. 3B** shows a sequencing gel of 3'-pCp-ligated clone C6 after alkaline hydrolysis or digestion with T_1 nuclease. The 5'-sequence (3'-end-labled) is aligned with the alkaline hydrolysis ladder. The T_1 and protein lanes, boundary and hypersensitive G nucleotides are as described for **FIG. 3A**.

[**0031**] **FIG. 4** shows the results of the 2'-O-methyl interference assay. Positions where 2'-OH purines can be substituted with 2'-O-methyl were determined from binding interference. Plotted is the ratio of (the intensity of bands selected by protein)/(the band intensity for oligonucleotides not selected by protein) with a linear curve fit (open circles). The same ratio for mixed 2'-OH:2'-OMe nucleotides is also plotted (closed circles).

[**0032**] **FIG. 5A** shows the proposed structure of the 38 mer truncate (SEQ ID NO:160) of clone C6 (SEQ ID NO:51) together with alternative bases.

[**0033**] **FIG. 5B** shows the 2'-O-methyl substitution pattern of a 38 mer truncate (SEQ ID NO: 193 of clone C6 (SEQ ID NO:51). Positions where 2'-OMe substitutions can be made are shown in bold. Positions which must be 2'-OH are underlined.

[**0034**] **FIG. 6** shows the % hemolysis verses concentration of nucleic acid ligand (μm) for a 38 mer truncate of clone YL-13 (SEQ ID NO: 175) without 2'-OMe substitution (SEQ ID NO:194; open circles), with a 2'-OMe substitution at position 20 (SEQ ID NO:195; closed triangles) and with 2'-OMe substitutions at positions 2, 7, 8, 13, 14, 15, 20, 21, 22, 26, 27, 28, 36 and 38 (SEQ ID NO:196; closed circles).

DETAILED DESCRIPTION OF THE INVENTION

[**0035**] This application describes Nucleic Acid Ligands to Complement System Proteins identified generally according to the method known as SELEX. As stated earlier, the SELEX technology is described in detail in the SELEX Patent Applications which are incorporated herein by reference in their entirety. Certain terms used to describe the invention herein are defined as follows:

[**0036**] "Nucleic Acid Ligand" as used herein is a non-naturally occurring Nucleic Acid having a desirable action on a Target. A desirable action includes, but is not limited to, binding of the Target, catalytically changing the Target, reacting with the Target in a way which modifies/alters the Target or the functional activity of the Target, covalently attaching to the Target as in a suicide inhibitor, and facilitating the reaction between the Target and another molecule. In the preferred embodiment, the desirable action is specific binding to a Target molecule, such Target molecule being a three dimensional chemical structure other than a polynucleotide that binds to the Nucleic Acid Ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein the Nucleic Acid Ligand is not a Nucleic Acid having the known physiological function of being bound by the Target molecule. Nucleic Acid Ligands include Nucleic Acids that are identified from a Candidate Mixture of Nucleic Acids, said Nucleic Acid Ligand being a ligand of a given Target by the method comprising: a) contacting the Candidate Mixture with the Target, wherein Nucleic Acids having an increased affinity to the Target relative to the Candidate Mixture may be partitioned from the remainder of the Candidate Mixture; b) partitioning the increased affinity Nucleic Acids from the remainder of the Candidate Mixture; and c) amplifying the increased affinity Nucleic Acids to yield a ligand-enriched mixture of Nucleic Acids.

[**0037**] "Candidate Mixture" is a mixture of Nucleic Acids of differing sequence from which to select a desired ligand. The source of a Candidate Mixture can be from naturally-occurring Nucleic Acids or fragments thereof, chemically synthesized Nucleic Acids, enzymatically synthesized Nucleic Acids or Nucleic Acids made by a combination of the foregoing techniques. In a preferred embodiment, each Nucleic Acid has fixed sequences surrounding a randomized region to facilitate the amplification process.

[**0038**] "Nucleic Acid" means both DNA, RNA, single-stranded or double-stranded and any chemical modifications thereof. Modifications include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and fluxionality to the Nucleic Acid Ligand bases or to the Nucleic Acid Ligand as a whole. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil, backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping.

[**0039**] "SELEX™" methodology involves the combination of selection of Nucleic Acid Ligands which interact with a Target in a desirable manner, for example binding to a protein, with amplification of those selected Nucleic Acids. Iterative cycling of the selection/amplification steps allows selection of one or a small number of Nucleic Acids which interact most strongly with the Target from a pool which contains a very large number of Nucleic Acids. Cycling of the selection/amplification procedure is continued until a selected goal is achieved. In the present invention, the SELEX methodology is employed to obtain Nucleic Acid Ligands to C1q, C3 and C5. The SELEX methodology is described in the SELEX Patent Applications.

[**0040**] "Target" means any compound or molecule of interest for which a ligand is desired. A Target can be a protein, peptide, carbohydrate, polysaccharide, glycoprotein, hormone, receptor, antigen, antibody, virus, substrate, metabolite, transition state analog, cofactor, inhibitor, drug, dye, nutrient, growth factor, etc. without limitation. In this application, the Target is a Complement System Protein, preferably C1q, C3 and C5.

[**0041**] "Complement System Protein" means any protein or component of the Complement System including, but not limited to, C1, C1q, C1r, C1s, C2, C3, C3a, C3b, C4, C4a, C5, C5a, C5b, C6, C7, C8, C9, Factor B (B), Factor D (D), Factor H (H) and receptors thereof, and other soluble and membrane inhibitors/control proteins.

[**0042**] "Complement System" is a set of plasma and membrane proteins that act together in a regulated cascade system to attack extracellular forms of pathogens or infected or transformed cells, and in clearance of immune reactants or cellular debris. The Complement System can be activated spontaneously on certain pathogens or by antibody binding to the pathogen. The pathogen becomes coated with Complement System Proteins (opsonized) for uptake and destruction. The pathogen can also be directly lysed and killed. Similar mechanisms target infected, transformed or damaged cells. The Complement System also participates in clearance of immune and cellular debris.

[**0043**] The SELEX process is described in U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, entitled "Systematic Evolution of Ligands by EXponential Enrichment," now abandoned; U.S. patent application Ser. No. 07/714,131, filed Jun. 10, 1991, entitled "Nucleic Acid Ligands," now U.S. Pat. No. 5,475,096; U.S. patent application Ser. No. 07/931,473, filed Aug. 17, 1992, entitled "Methods for Identifying Nucleic Acid Ligands," now U.S. Pat. No. 5,270,163 (see also WO 91/19813). These applications, each specifically incorporated herein by reference, are collectively called the SELEX Patent Applications.

[**0044**] In its most basic form, the SELEX process may be defined by the following series of steps:

[**0045**] 1) A Candidate Mixture of Nucleic Acids of differing sequence is prepared. The Candidate Mixture generally includes regions of fixed sequences (i.e., each of the members of the Candidate Mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the Target, or (c) to enhance the concentration of a given structural arrangement of the Nucleic Acids in the Candidate Mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

[**0046**] 2) The Candidate Mixture is contacted with the selected Target under conditions favorable for binding between the Target and members of the Candidate Mixture. Under these circumstances, the interaction between the Target and the Nucleic Acids of the Candidate Mixture can be considered as forming Nucleic Acid-Target pairs between the Target and those Nucleic Acids having the strongest affinity for the Target.

[**0047**] 3) The Nucleic Acids with the highest affinity for the Target are partitioned from those Nucleic Acids with lesser affinity to the Target. Because only an extremely small number of sequences (and possibly only one molecule of Nucleic Acid) corresponding to the highest affinity Nucleic Acids exist in the Candidate Mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the Nucleic Acids in the Candidate Mixture (approximately 5-50%) are retained during partitioning.

[**0048**] 4) Those Nucleic Acids selected during partitioning as having the relatively higher affinity to the Target are then amplified to create a new Candidate Mixture that is enriched in Nucleic Acids having a relatively higher affinity for the Target.

[**0049**] 5) By repeating the partitioning and amplifying steps above, the newly formed Candidate Mixture contains fewer and fewer weakly binding sequences, and the average degree of affinity of the Nucleic Acids to the Target will generally increase. Taken to its extreme, the SELEX process will yield a Candidate Mixture containing one or a small number of unique Nucleic Acids representing those Nucleic Acids from the original Candidate Mixture having the highest affinity to the Target molecule.

[**0050**] The SELEX Patent Applications describe and elaborate on this process in great detail. Included are Targets that can be used in the process; methods for partitioning Nucleic Acids within a Candidate Mixture; and methods for amplifying partitioned Nucleic Acids to generate enriched Candidate Mixture. The SELEX Patent Applications also describe ligands obtained to a number of target species, including both protein Targets where the protein is and is not a Nucleic Acid binding protein.

[**0051**] The SELEX method further encompasses combining selected Nucleic Acid Ligands with lipophilic or Non-Immunogenic, High Molecular Weight compounds in a diagnostic or therapeutic complex as described in U.S. patent application Ser. No. 08/434,465, filed May 4, 1995, entitled "Nucleic Acid Ligand Complexes," now U.S. Pat. No. 6,011,020. VEGF Nucleic Acid Ligands that are associated with a Lipophilic Compound, such as diacyl glycerol or dialkyl glycerol, in a diagnostic or therapeutic complex are described in U.S. patent application Ser. No. 08/739,109, filed Oct. 25, 1996, entitled "Vascular Endothelial Growth Factor (VEGF) Nucleic Acid Ligand Complexes," now U.S. Pat. No. 5,859,228. VEGF Nucleic Acid Ligands that are associated with a Lipophilic Compound, such as a glycerol lipid, or a Non-Immunogenic, High Molecular Weight Compound, such as polyalkylene glycol, are further described in U.S. patent application Ser. No. 08/897,351, filed Jul. 21, 1997, entitled "Vascular Endothelial Growth Factor (VEGF) Nucleic Acid Ligand Complexes," now U.S. Pat. No. 6,051,698. VEGF Nucleic Acid Ligands that are associated with a Non-Immunogenic, High Molecular Weight compound or a lipophilic compound are also further described in PCT/US97/18944, filed Oct. 17, 1997, entitled "Vascular Endothelial Growth Factor (VEGF) Nucleic Acid Ligand Complexes." Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

[**0052**] Certain embodiments of the present invention provide a complex comprising one or more Nucleic Acid Ligands to a Complement System Protein covalently linked with a Non-Immunogenic, High Molecular Weight compound or lipophilic compound. A complex as used herein describes the molecular entity formed by the covalent linking of the Nucleic Acid Ligand of a Complement System Protein to a Non-Immunogenic, High Molecular Weight compound. A Non-Immunogenic, High Molecular Weight compound is a compound between approximately 100 Da to 1,000,000 Da, more preferably approximately 1000 Da to

500,000 Da, and most preferably approximately 1000 Da to 200,000 Da, that typically does not generate an immunogenic response. For the purposes of this invention, an immunogenic response is one that causes the organism to make antibody proteins. In one preferred embodiment of the invention, the Non-Immunogenic, High Molecular Weight compound is a polyalkylene glycol. In the most preferred embodiment, the polyalkylene glycol is polyethylene glycol (PEG). More preferably, the PEG has a molecular weight of about 10-80K. Most preferably, the PEG has a molecular weight of about 20-45K. In certain embodiments of the invention, the Non-Immunogenic, High Molecular Weight compound can also be a Nucleic Acid Ligand.

[0053] Another embodiment of the invention is directed to complexes comprised of a Nucleic Acid Ligand to a Complement System Protein and a lipophilic compound. Lipophilic compounds are compounds that have the propensity to associate with or partition into lipid and/or other materials or phases with low dielectric constants, including structures that are comprised substantially of lipophilic components. Lipophilic compounds include lipids as well as non-lipid containing compounds that have the propensity to associate with lipids (and/or other materials or phases with low dielectric constants). Cholesterol, phospholipid, and glycerol lipids, such as dialkyl glycerol, diacyl glycerol, and glycerol amide lipids are further examples of lipophilic compounds. In a preferred embodiment, the lipophilic compound is a glycerol lipid.

[0054] The Non-Immunogenic, High Molecular Weight compound or lipophilic compound may be covalently bound to a variety of positions on the Nucleic Acid Ligand to a Complement System Protein, such as to an exocyclic amino group on the base, the 5-position of a pyrimidine nucleotide, the 8-position of a purine nucleotide, the hydroxyl group of the phosphate, or a hydroxyl group or other group at the 5' or 3' terminus of the Nucleic Acid Ligand to a Complement System Protein. In embodiments where the lipophilic compound is a glycerol lipid, or the Non-Immunogenic, High Molecular Weight compound is polyalkylene glycol or polyethylene glycol, preferably the Non-Immunogenic, High Molecular Weight compound is bonded to the 5' or 3' hydroxyl of the phosphate group thereof. In the most preferred embodiment, the lipophilic compound or Non-Immunogenic, High Molecular Weight compound is bonded to the 5' hydroxyl of the phosphate group of the Nucleic Acid Ligand. Attachment of the Non-Immunogenic, High Molecular Weight compound or lipophilic compound to the Nucleic Acid Ligand of the Complement System Protein can be done directly or with the utilization of linkers or spacers.

[0055] A linker is a molecular entity that connects two or more molecular entities through covalent bonds or non-covalent interactions, and can allow spatial separation of the molecular entities in a manner that preserves the functional properties of one or more of the molecular entities. A linker can also be referred to as a spacer.

[0056] The complex comprising a Nucleic Acid Ligand to a Complement System Protein and a Non-Immunogenic, High Molecular Weight compound or lipophilic compound can be further associated with a lipid construct. Lipid constructs are structures containing lipids, phospholipids, or derivatives thereof comprising a variety of different structural arrangements which lipids are known to adopt in aqueous suspension. These structures include, but are not limited to, lipid bilayer vesicles, micelles, liposomes, emulsions, lipid ribbons or sheets, and may be complexed with a variety of drugs and components which are known to be pharmaceutically acceptable. In a preferred embodiment, the lipid construct is a liposome. The preferred liposome is unilamellar and has a relative size less than 200 nm. Common additional components in lipid constructs include cholesterol and alpha-tocopherol, among others. The lipid constructs may be used alone or in any combination which one skilled in the art would appreciate to provide the characteristics desired for a particular application. In addition, the technical aspects of lipid constructs and liposome formation are well known in the art and any of the methods commonly practiced in the field may be used for the present invention.

[0057] The methods described herein and the Nucleic Acid Ligands identified by such methods are useful for both therapeutic and diagnostic purposes. Therapeutic uses include the treatment or prevention of diseases or medical conditions in human patients, specifically diseases or conditions caused by activation of the Complement System. The Complement System does not have to be the only cause of the disease state, but it may be one of several factors, each of which contributes to pathogenesis. Such diseases or conditions include, but are not limited to, renal diseases, such as lupus nephritis and membranoproliferative glomerulonephritis (MPGN), membranous nephritis, IgA nephropathy; rheumatological diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), Behcet's syndrome, juvenile rheumatoid arthritis, Sjögren's syndrome and systemic sclerosis; neurological diseases, such as myasthenia gravis, multiple sclerosis, cerebral lupus, Guillain-Barré syndrome and Alzheimer's disease; dermatological diseases, such as Pemphigus/pemphigoid, phototoxic reactions, vasculitis and thermal bums; hematological diseases, such as paroxysmal nocturnal hemoglobinuria (PNH), hereditary erythroblastic multinuclearity with positive acidified serum lysis test (HEMPAS) and idiopathic thrombocytopenic purpura (ITP); biocompatibility/shock diseases, such as post-bypass syndrome, adult respiratory distress syndrome (ARDS), catheter reactions, anaphylaxis, transplant rejection, pre-eclampsia, hemodialysis and platelet storage; vascular/pulmonary diseases, such as atherosclerosis, myocardial infarction, stroke and reperfusion injury; allergies, such as anaphylaxis, asthma and skin reactions; infection, such as septic shock, viral infection and bacterial infection; and other conditions, such as atheroma, bowel inflammation, thyroiditis, infertility, paroxysmal nocturnal hemoglobinuria (PNH) and hemolytic anemia.

[0058] The Complement System can be inhibited at several points in the activation cascade by targeting different components. Inhibition of C1q would block the initiation by either antibody or non-antibody mechanisms. Antibodies activate C1q in many diseases including SLE, myasthenia gravis and arthritis. Non-antibody Complement System activation occurs in many diseases including Alzheimer's disease, myocardial infarction and septic shock. Blocking C1q could prevent the complement-mediated tissue injury in these diseases.

[0059] The Complement can also be activated in the absence of antibodies directly at the C3 stage. Activating surfaces including bacteria, virus particles or damaged cells can trigger Complement System activation that does not require C1q. An inhibitor of C3 could prevent Complement System activation and damage in these situations.

[0060] In other instances the inhibition of C5 is most useful. Activation of the Complement System by either C1q or C3 mechanisms both lead to activation of C5, so that inhibition of C5 could prevent Complement System-mediated damage by either pathway. However, both C1q and C3 are important in normal defense against microorganisms and in clearance of immune components and damaged tissue, while C5 is mostly dispensable for this function. Therefore, C5 can be inhibited either for a short term or a long term and the protective role of Complement System would not be compromised, whereas long term inhibition of C1q or C3 is not desirable. Finally, the C5 fragments C5a and C5b directly cause the majority of tissue injury and disease associated with unwanted Complement System activation. Therefore, inhibition of C5 is the most direct way of producing therapeutic benefit.

[0061] In other instances, the activation of the Complement System is desirable in the treatment or prevention of diseases or medical conditions in human patients. For example, the activation of the Complement System is desirable in treating bacterial or viral infections and malignancies. In addition, the activation of the Complement System on T-cells prior to transplantation could prevent rejection of an organ or tissue by eliminating the T-cells that mediate the rejection.

[0062] Furthermore, Nucleic Acid Ligands that bind to cell surface Targets could be made more efficient by giving them the ability to activate the Complement System. Nucleic Acid binding would then both inhibit a Target function and also eliminate the cell, for example, by membrane attack complex lysis and cell clearance through opsonization. Nucleic Acid Ligands could activate the Complement System through either the classical or the alternative pathways. C1q Nucleic Acid Ligands can be conjugated to other structures that target a cell surface component. For example, C1q Nucleic Acid Ligands can be conjugated to antibodies to cell targets, cytokines, growth factors or a ligand to a cell receptor. This would allow the C1q Nucleic Acid Ligands to multimerize on the targeted cell surface and activate the Complement System, thereby killing the cell.

[0063] The prototype classical pathway activators are immune aggregates, which activate the Complement System through binding to globular head groups on the C1q component. Generally, binding of two or more Fc domains to C1q is required; pentameric IgM is an especially efficient activator. In contrast, Nucleic Acid Ligands can activate through binding at a separate site on the C1q collagen-like tail region. This site also binds to a variety of other non-antibody activators including C-reactive protein, serum amyloid protein, endotoxin, β-amyloid peptide 1-40 and mitochondrial membranes. As with immunoglobulin, these non-antibody activators need to be multimerized to activate.

[0064] Nucleic Acid Ligands that bind to sites on the collagen-like region of C1q may also become activators when aggregated. Such a Complement System-activating aggregate may be lytic if formed on a cell surface, such as binding to a tumor-specific antigen (TSA) or to a leukocyte antigen. The extent of Nucleic Acid Ligand-mediated activation increases with the extent of Nucleic Acid Ligand aggregation (i.e., multiplicity of Nucleic Acid Ligand-C1q interaction). The Complement System-mediated killing is especially specific if the Nucleic Acid Ligands circulate as monomers which do not activate, but become activators when they are multimerized on the targeted cell surface.

[0065] As with any Complement System activation, the extent and specificity is determined by the amount of C3 deposited onto the targeted cell. Deposited C3 forms an enzyme convertase that cleaves C5 and initiates membrane attack complex formation. C3 is also the classical serum opsonin for targeting phagocytic ingestion. The prototype alternative pathway activators are repeating carbohydrate units including bacterial and yeast cell walls, fucoidin and Sepharose, or glycolipids such as endotoxin or the glycocalyx. Nucleic Acid Ligands could activate the alternative pathway by aggregating the C3 component on the cell surface. Depositing C3 on a cell promotes Factor B binding and alternative pathway C3 convertase formation. Binding of a Nucleic Acid Ligand to C3 blocks binding of the inhibitor Factor H and prevents C3b decay. This would also increase C3 convertase formation and alternative path activation. Nucleic Acid Ligands to C3 may have this activity since heparin binds activated C3 and can promote alternative pathway activation. Binding of Nucleic Acid Ligands to C3 blocks binding to C3 of the membrane-associated inhibitors CR1, CR2, MCP and DAF, preventing C3b convertase decay and stimulating alternative pathway activation. This alternative pathway mechanism can be as efficient as C1q-dependent activation in cell killing and lysis.

[0066] Nucleic Acid Ligand-mediated Complement System cell killing could be employed in several ways, for example, by: a) direct killing of tumor cells; b) lysis of targeted microorganisms or infected cells; and c) elimination of lymphocytes or lymphocyte subsets. Nucleic Acid Ligands could replace antibodies currently used for these purposes.

[0067] Diagnostic utilization may include either in vivo or in vitro diagnostic applications. The SELEX method generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic applications. The SELEX method identifies Nucleic Acid Ligands that are able to bind targets with high affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek in a diagnostic ligand.

[0068] The Nucleic Acid Ligands of the present invention may be routinely adapted for diagnostic purposes according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt any Nucleic Acid Ligand by procedures known in the art to incorporate a labeling tag in order to track the presence of such ligand. Such a tag could be used in a number of diagnostic procedures. The Nucleic Acid Ligands to C1q, C3 and C5 described herein may specifically be used for identification of the C1q, C3 or C5 protein.

[0069] The SELEX process provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of Nucleic Acids research. The present invention applies the SELEX procedure to the specific target C1q, which is part of the first component (C1) of the classical pathway of Complement System activation, to the specific target C3, which is part of both the classical and alternative pathway, and to the specific target C5, which is part of the terminal pathway. In the Example section below, the experimental parameters used to isolate and identify the Nucleic Acid Ligands to C1q, C3 and C5 are described.

[0070] In order to produce Nucleic Acids desirable for use as a pharmaceutical, it is preferred that the Nucleic Acid Ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the Nucleic Acid Ligand have the highest possible affinity to the Target.

[0071] Pharmaceutical agents, which include, but are not limited to, small molecules, antisense oligonucleotides, nucleosides, and polypeptides can activate the Complement System in an undesirable manner. Nucleic Acid Ligands to Complement System Proteins could be used as a prophylactic by transiently inhibiting the Complement System, so that a pharmaceutical agent could be administered and achieve a therapeutically effective amount without eliciting the undesirable side effect of activating the Complement System.

[0072] In co-pending and commonly assigned U.S. patent application Ser. No. 07/964,624, filed October 21, 1992, now U.S. Pat. No. 5,496,938, (the '938 Patent), methods are described for obtaining improved Nucleic Acid Ligands after SELEX has been performed. The '938 Patent, entitled "Nucleic Acid Ligands to HIV-RT and HIV-1 Rev," is specifically incorporated herein by reference in its entirety.

[0073] In the present invention, SELEX experiments were performed in order to identify RNA with specific high affinity for C1q, C3 and C5 from a degenerate library containing 30 or 50 random positions (30N or 50N). This invention includes the specific RNA ligands to C1q shown in Table 2 (SEQ ID NOS:5-20) and Table 6 (SEQ ID NOS:84-155), identified by the method described in Examples 2 and 6, the specific RNA ligands to C3 shown in Table 3 (SEQ ID NOS:21-46), identified by method described in Example 3, and the specific RNA ligands to C5 shown in Table 4 (SEQ ID NOS:47-74), Table 5 (SEQ ID NOS:76-83), Table 8 (SEQ ID NOS:75, 160-162), Table 10 (SEQ ID NOS:163-189), Table 12 (SEQ ID NOS:190-192), Table 13 (SEQ ID NOS:194-196) and FIGS. **5**A-B (SEQ ID NOS:160 and 193) identified by methods described in Examples 4, 9, 10 and 11. This invention further includes RNA ligands to C1q, C3 and C5 which inhibit the function of C1q, C3 and C5. The scope of the ligands covered by this invention extends to all Nucleic Acid Ligands of C1q, C3 and C5, modified and unmodified, identified according to the SELEX procedure. More specifically, this invention includes Nucleic Acid sequences that are substantially homologous to the ligands shown in Tables 2-6, 8, 10 and 12-13 and FIGS. **5**A-B (SEQ ID NOS:5-155 and 160-196). By substantially homologous, it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%, and even more preferably in excess of 90%, 95% or 99%. The percentage of homology as described herein is calculated as the percentage of nucleotides found in the smaller of the two sequences which align with identical nucleotide residues in the sequence being compared when 1 gap in a length of 10 nucleotides may be introduced to assist in that alignment. A review of the sequence homologies of the ligands of C1q shown in Table 2 (SEQ ID NOS:5-20) and Table 6 (SEQ ID NOS:84-155) shows that sequences with little or no primary homology may have substantially the same ability to bind C1q. Similarly, a review of the sequence homologies of the ligands of C3 shown in Table 3 (SEQ ID NOS:21-46) shows that sequences with little or no primary homology may have substantially the same ability to bind C3. Similarly, a review of the sequence homologies of the ligands of C5 shown in Table 4 (SEQ ID NOS:47-74), Table 5 (SEQ ID NOS:76-83), Table 8 (SEQ ID NOS:75, 160-162), Table 10 (SEQ ID NOS:163-189), Table 12 (SEQ ID NOS:190-192), Table 13 (SEQ ID NOS:194-196) and FIGS. **5**A-B (SEQ ID NOS:160 and 193) shows that sequences with little or no primary homology may have substantially the same ability to bind C5. For these reasons, this invention also includes Nucleic Acid Ligands that have substantially the same structure and ability to bind C1q as the Nucleic Acid Ligands shown in Table 2 (SEQ ID NOS:5-20) and Table 6 (SEQ ID NOS:84-155), Nucleic Acid Ligands that have substantially the same structure and ability to bind C3 as the Nucleic Acid Ligands shown in Table 3 (SEQ ID NOS:21-46) and Nucleic Acid Ligands that have substantially the same structure and ability to bind C5 as the Nucleic Acid Ligands shown in Table 4 (SEQ ID NOS:47-74), Table 5 (SEQ ID NOS:76-83), Table 8 (SEQ ID NOS:75, 160-162), Table 10 (SEQ ID NOS:163-189), Table 12 (SEQ ID NOS:190-192), Table 13 (SEQ ID NOS:194-196) and FIGS. **5**A-B (SEQ ID NOS:160 and 193). Substantially the same ability to bind C1q, C3 or C5 means that the affinity is within one or two orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence—substantially homologous to those specifically described herein—has substantially the same ability to bind C1q, C3 or C5.

[0074] The invention also includes Nucleic Acid Ligands that have substantially the same postulated structure or structural motifs. Substantially the same structure or structural motifs can be postulated by sequence alignment using the Zukerfold program (see Zucker (1989) Science 244:48-52). As would be known in the art, other computer programs can be used for predicting secondary structure and structural motifs. Substantially the same structure or structural motif of Nucleic Acid Ligands in solution or as a bound structure can also be postulated using NMR or other techniques as would be known in the art.

[0075] One potential problem encountered in the therapeutic, prophylactic and in vivo diagnostic use of Nucleic Acids is that oligonucleotides in their phosphodiester form may be quickly degraded in body fluids by intracellular and extracellular enzymes such as endonucleases and exonucleases before the desired effect is manifest. Certain chemical modifications of the Nucleic Acid Ligand can be made to increase the in vivo stability of the Nucleic Acid Ligand or to enhance or to mediate the delivery of the Nucleic Acid Ligand. See, e.g., U.S. patent application Ser. No. 08/117,991, filed Sep. 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," now abandoned (see also U.S. Pat. No. 5,660,985) and U.S. patent application Ser. No. 08/434,465, filed May 4, 1995, entitled

"Nucleic Acid Ligand Complexes," which are specifically incorporated herein by reference in their entirety. Modifications of the Nucleic Acid Ligands contemplated in this invention include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and fluxionality to the Nucleic Acid Ligand bases or to the Nucleic Acid Ligand as a whole. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil, backbone modifications, phosphorothioate or alkyl phosphate modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping.

[0076] Where the Nucleic Acid Ligands are derived by the SELEX method, the modifications can be pre- or post-SELEX modifications. Pre-SELEX modifications yield Nucleic Acid Ligands with both specificity for their SELEX Target and improved in vivo stability. Post-SELEX modifications made to 2'-OH Nucleic Acid Ligands can result in improved in vivo stability without adversely affecting the binding capacity of the Nucleic Acid Ligand. The preferred modifications of the Nucleic Acid Ligands of the subject invention are 5' and 3' phosphorothioate capping and/or 3'-3' inverted phosphodiester linkage at the 3' end. In one preferred embodiment, the preferred modification of the Nucleic Acid Ligand is a 3'-3' inverted phosphodiester linkage at the 3' end. Additional 2'-fluoro (2'-F) and/or 2'-amino (2'-NH_2) and/or 2'-O-methyl (2'-OMe) modification of some or all of the nucleotides is preferred. Described herein are Nucleic Acid Ligands that were 2'-NH_2 modified or 2'-F modified and incorporated into the SELEX process. Further described herein are 2'-F modified Nucleic Acid Ligands derived from the SELEX process which were modified to comprise 2'-OMe purines in post-SELEX modifications.

[0077] Other modifications are known to one of ordinary skill in the art. Such modifications may be made post-SELEX (modification of previously identified unmodified ligands) or by incorporation into the SELEX process.

[0078] As described above, because of their ability to selectively bind C1q, C3 and C5, the Nucleic Acid Ligands to C1q, C3 and C5 described herein are useful as pharmaceuticals. This invention, therefore, also includes a method for treating Complement System-mediated diseases by administration of a Nucleic Acid Ligand capable of binding to a Complement System Protein or homologous proteins. Certain diseases or conditions such as Alzheimer's disease or myocardial infarction activate C1q through the collagen-like region. In Alzheimer's disease, β-amyloid activates C1q. Structures in heart muscle that are exposed during myocardial infarction such as intermediate filaments, mitochondrial membranes or actin activate C1q. Nucleic Acid Ligands to C3 or to C5 could also inhibit Complement System activation in Alzheimer's disease or myocardial infarction, whether the Complement System is activated through C1q by antibody or non-antibody mechanisms, or independent of C1q through the alternative pathway. Thus, the Nucleic Acid Ligands of the present invention may be useful in treating Alzheimer's disease or myocardial infarction.

[0079] Therapeutic compositions of the Nucleic Acid Ligands may be administered parenterally by injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment, it is envisioned that the carrier and the Nucleic Acid Ligand constitute a physiologically-compatible, slow release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release or absorption of the ligand. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dose or multi-dose form.

[0080] Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The manner of administering formulations containing Nucleic Acid Ligands for systemic delivery may be via subcutaneous, intramuscular, intravenous, intranasal or vaginal or rectal suppository.

[0081] The following Examples are provided to explain and illustrate the present invention and are not intended to be limiting of the invention. These Examples describe the use of SELEX methodology to identify high affinity RNA ligands to C1q, C3 and C5. Example 1 describes the various materials and experimental procedures used in Examples 2, 3, 4 and 6. Example 2 describes the generation of 2'-NH_2 RNA ligands to C1q. Example 3 describes the generation of 2'-F Nucleic Acid Ligands of Complement System Protein C3. Example 4 describes the generation of 2'-F Nucleic Acid Ligands of Complement System Protein C5. Example 5 describes the activation of the Complement System through C1q ligands. Example 6 describes the generation of 2'-F RNA ligands to C1q. Example 7 describes an assay for hemolytic inhibition for 2'-F RNA ligands to C5. Example 8 describes an assay for inhibition of C5a release by a Nucleic Acid Ligand (clone C6) to Human C5. Example 9 describes boundary experiments performed to determine the minimum binding sequence for Nucleic Acid Ligands to Human C5. Example 10 describes a Biased SELEX experiment performed to improve Nucleic Acid Ligand affinity, using a 42 mer truncated sequence of clone C6 as the random sequence in the template. Example 11 describes the results of 2'-OMe purine substitutions in a Human C5 Nucleic Acid Ligand in an interference assay. Example 12 describes the structure of a 38 mer truncate of a Nucleic Acid Ligand to human C5. Example 13 describes a hemolytic assay of 2'-OMe purine substituted Nucleic Acid Ligands to human C5.

Example 1

[0082] Experimental Procedures

[0083] This example provides general procedures followed and incorporated in Examples 2, 3, 4 and 6 for the identification of 2'-NH_2 and 2'-F RNA ligands to C1q, and 2'-F ligands to C3 and C5.

[0084] A. Biochemicals

[0085] C1q, C3, C5 and C4-deficient guinea pig sera were obtained from Quidel (San Diego, Calif.). Bovine serum albumin (BSA), rabbit anti-BSA, CRP, SAP and β-amyloid peptides 1-40 and 1-42 were obtained from Sigma (St. Louis, Mo.). Nucleotides GTP, ATP and deoxynucleotides were obtained from Pharmacia (Uppsala, Sweden). Taq polymerase was obtained from Perkin-Elmer (Norwalk, Conn.). Modified nucleotides 2'-NH_2-CTP and 2'-NH_2-UTP, and 2'-F-CTP and 2'-F-UTP, were prepared as described in Jellinek et al. (1995) Biochem. 34:11363. Avian reverse transcriptase was obtained from Life Sciences (St. Petersburg, Fla.) and T7 RNA polymerase from USB (Cleveland, Ohio.). Nitrocellulose filters were obtained from Millipore (Bedford, Mass.). All chemicals were the highest grade available.

[0086] B. RNA SELEX Procedures

[0087] The SELEX procedure has been described in detail in the SELEX Patent Applications (see also Jellinek et al. (1995) Biochem. 34:11363; Jellinek et al. (1994) Biochem. 33:10450). Briefly, a DNA template was synthesized with a 5' fixed region containing the T7 promoter, followed by a 30N or a 50N stretch of random sequence, and then with a 3'-fixed region (Table 1; SEQ ID NOS:1 and 156). For the initial round of the SELEX process, 1 nmole (~10^{14} unique sequences) of RNA (Table 1; SEQ ID NOS:2 and 157) was in vitro transcribed by T7 polymerase (Milligan et al. (1987) Nucleic Acids Res. 12:785) using mixed GTP/ATP and 2'-NH_2-CTP/UTP or 2'-F-CTP/UTP nucleotides, and with the addition of α-[^{32}P]-ATP. For this and subsequent rounds of the SELEX process, the RNA was purified by electrophoresis on 8% acrylamide gels with 7 M urea, 10 mM Tris-Borate, 2 mM EDTA, pH 8.3 running buffer. After autoradiography, the band containing labeled, modified RNA transcript was excised and frozen at −70° C., then 400 μL of 100 mM NaCl, 2 mM EDTA was added, the gel was mashed, and the slurry was spun through 2 cm of glass-wool (Rnase-free—Alltech Associates, Deerfield, Ill.) and two nitrocellulose filters. The RNA was precipitated by addition of ⅕ vol of 6.6 M NH_4OAc, pH 7.7, plus 2 vol of ethanol. The pellet was washed twice with 80% ethanol, and taken to dryness. The dry RNA pellet was dissolved in phosphate buffered saline (Sambrook et al. (1989) *Molecular Cloning. A laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) containing 1 mM $MgCl_2$ (MgPBS).

[0088] For each round of the SELEX process, the RNA was incubated with C1q, C3 or C5 in MgPBS for 10 minutes at 37° C. Then the sample was filtered through a 43 mm nitrocellulose filter, and the filter was washed with 10 mL of MgPBS. For some rounds, the diluted RNA was pre-soaked with nitrocellulose filters overnight to reduce background. Four samples were run in parallel for most rounds with lesser amounts (chosen to be in suitable range to measure binding) of both RNA and C1q, C3 or C5 to measure binding Kd for each sample. In addition, at each round, a sample of RNA was filtered without protein to determine background.

[0089] Filters were air-dried, sliced into strips, counted, and then extracted for 60 minutes at 37° C. with 400 μL of 1% SDS, 0.5 mg/mL Proteinase K (Boehringer Mannheim, Indianapolis, Ind.), 1.5 mM DTT, 10 mM EDTA, 0.1 M Tris, pH 7.5, with addition of 40 μg tRNA carrier. The aqueous RNA was extracted with phenol, phenol/chloroform (1:1), and chloroform and then precipitated following addition of NH_4OAc/EtOH as above. The RNA was reverse transcribed in a volume of 50 μL for between 1 hour and overnight. The DNA was PCR amplified with specific primers (Table 1; SEQ ID NOS:3-4) in a volume of 500 μL for 12-14 cycles, and then phenol/chloroform extracted and NaOAc/EtOH precipitated. The DNA pellet was taken up in H_2O, and an aliquot was T7 transcribed for the next round of the SELEX process.

[0090] C. Cloning DNA from the 12th or the 14th round was PCR amplified with primers which also contained a ligation site to facilitate cloning. The DNA was cloned into a pUC9 vector, and colonies were picked for overnight growth and plasmid mini-preps (PERFECTprep, 5'-3', Boulder, Colo.). The purified plasmids were PCR amplified with original 3' and 5' primers (as above), and products were analyzed by agarose gel electrophoresis (Sambrook et al. (1989) *Molecular Cloning. A laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). DNA was T7 transcribed with α-[^{32}P]-ATP to prepare radiolabeled RNA for binding analysis and without radiolabel to prepare RNA for inhibition studies.

[0091] D. Sequencing Plasmids purified using the PERFECTprep kit were sequenced with ABI dRhodamine Terminator cycling kit (Perkin-Elmer). Samples were sequenced on the ABI Prism 377 DNA Sequencer.

[0092] E. Binding Assays

[0093] Individual cloned DNA was T7 transcribed with α-[^{32}P]-ATP and the full length [^{32}P]-2'-NH_2-RNA or 2'-F-RNA was gel-purified (as above). RNA was suspended at approximately 5,000 cpm per 30 μL sample (<10 pM), and aliquots were incubated with various concentrations of C1q, C3 or C5 in MgPBS for 10 minutes at 37° C. Samples were then filtered through nitrocellulose, the filters washed with buffer and dried under an infrared lamp, and counted with addition of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Ga.). A background sample of RNA alone was run in parallel. To measure inhibition of ligand binding to C1q, the RNA Nucleic Acid Ligand plus C1q plus inhibitor (e.g., the A-chain residue 14-26 site, SAP, β-amyloid peptide, CRP) were incubated for 10 minutes at 37° C., and then filtered. Filters were washed and counted.

[0094] RNA ligand binding to C1q was also measured in the presence of immune-complexes, which would block the binding of ligands to C1q head-groups. Immune complexes (IC) were formed by mixing 620 μg BSA at equivalence with 1 mL of rabbit anti-BSA (Sigma, St. Louis, Mo.) plus PEG

8000 added to 1% final concentration, and then the samples were incubated overnight at 4° C. The IC were pelleted by microfugation at 12,000 rpm for 10 minutes, washed five times with PBS, and suspended in 1 mL of MgPBS. For measurement of C1q RNA clone binding to C1q-immune complexes (C1q-IC), 20 μL of the purified [^{32}P]-RNA plus 20 μL of the IC were mixed with 20 μL of C1q at various concentrations at between 10^{-11} and 10^{-7} M in MgPBS plus 1% Triton. Samples were incubated for 30 minutes at room temperature, microfuged, and the pellets and supernatants counted.

[0095] F. Hemolytic Assays

[0096] Complement System consumption was measured by C4 hemolytic assay as described (Gaither et al. (1974) J. Immunol. 113:574). All samples were diluted and the assay run in veronal-buffered saline containing calcium, magnesium and 1% gelatin (GVB^{++}-complement buffer). For measurement of C4 consumption by β-amyloid peptide consumption, the peptide was added at 250 μg/mL to a 1/8 dilution of whole human serum and then incubated for 60 minutes at 37° C. The sample was then diluted for assay of C4 hemolytic activity. For assay of inhibition of β-amyloid peptide mediated complement consumption by C1 q 2'-NH$_2$-RNA clones, the C1q RNA Nucleic Acid Ligand was included in the initial β-amyloid peptide-whole human serum incubation mixture, and then C4 amounts assayed as above.

[0097] Complement System inhibition by C5 Nucleic Acid Ligands was measured using human serum and antibody-coated sheep red blood cells. The red blood cells were incubated with a 1:40 dilution of fresh human serum and with serial dilutions of C5 ligand for 30 minutes at 37° C. Dilutions of serum and ligand were made in complement buffer (see previous paragraph). After incubation the samples were then diluted with 4° C. buffer containing EDTA to stop the reaction, and the hemoglobin release was quantitated from the optical density at 412 nm.

Example 2

[0098] 2'-NH$_2$ RNA Ligands to C1q

[0099] A. RNA SELEX

[0100] The pool of random 50N7-2'-NH$_2$ RNA bound to C1q by nitrocellulose filter assay with a K$_d$ of 2.3 μM. For round 1 of the SELEX process, the C1q concentration was between 0.156-1.25 μM and the RNA concentration was 15 μM. Throughout the SELEX process, the RNA concentrations were maintained at approximately 10-fold greater than the concentration of C1q, which was reduced at each round with a final round 14 C1q concentration of 136 pM. Background binding of RNA to nitrocellulose filters remained low throughout the SELEX procedure, in part because RNA was pre-adsorbed with nitrocellulose filters. The binding of pool RNA to C1q improved at each round. The evolved round 14 pool 2'-NH$_2$ RNA bound C1q with a K$_d$=670 pM, yielding an overall improvement in binding K$_d$ of 3400-fold.

[0101] Bulk RNA was then cloned for sequence determination and evaluation of binding. Through comparison of binding at 0.1 and 0.5 nM C1q, individual clones were ranked, and clones with C1q binding above background were sequenced and are shown in Table 2 (SEQ ID NOS:5-20). Family 1 contained 12 of the 19 total sequences. Family 2 contained three sequences. Both Family 3 and Family 4 contained two sequences. Both Family 1 and Family 2 sequences contain G-rich regions and both have the repeated sequence motifs GGAG and GGUG. The identity and homology of Family 1 members is greatest in the 5' half, which is G-rich. The C-rich 3' half has only short stretches of sequence homology, and these are shown only with inclusion of large gap regions. Sequences from all families can be folded to give stem-loop structures with extensive Watson-Crick base-pairing. Full binding curves for the highest affinity ligands yielded a K$_d$ range from 290 pM to 3.9 nM; the high affinity ligands were found in all four sequence families. All of the binding curves were monophasic. The binding maximum is not 100% because of variable amounts of nucleic acid alterations taking place during purification. This is known because usually ligands can be bound to protein, extracted, and then re-bound, and give maximum binding approaching 100% (data not shown).

[0102] B. Competition

[0103] 2'-NH$_2$ RNA ligands from different families interact with the same or overlapping sites on C1q, as shown by cross-competition. This site is on the collagen-like region, at or near the A-chain 14-26 residue site (Jiang et al. (1994) J. Immunol. 152:5050) as shown by two lines of evidence. First, C1q when bound to IC still binds the ligand #50 (SEQ ID NO:12); binding to immunoglobulin Fc would block the head region, but leave the collagen-like tail available, suggesting that nucleic acid ligands derived by the SELEX process are bound to the tail. Second, and more direct, ligand #50 is competed by proteins which are known to bind the A-chain residue 14-26 site, including SAP, β-amyloid peptide and CRP. Finally, ligand #50 is competed by a peptide that has the same amino acid sequence as residues 14-26 on the A-chain. This result is further supported by results for hemolytic inhibition as described below.

[0104] C. Consumption

[0105] Binding of a nucleic acid ligand derived by the SELEX process to the A-chain 14-26 amino acid site could activate C1q or alternatively, SELEX-derived nucleic acid ligands could inhibit the binding of other molecules and prevent C1q activation. This was tested by measuring C4 consumption in serum after incubation with a 2'-NH$_2$ SELEX-derived nucleic acid ligands, or after incubation with a known C1q activator together with a 2'-NH$_2$ nucleic acid ligand. The SELEX-derived nucleic acid ligands when incubated in serum do not consume C4, and thus are not C1q activators. Nor do these ligands at this concentration inhibit serum lysis of antibody-coated sheep erythrocytes, which would occur if ligands bound near the C1q head groups (data not shown). The ligands do inhibit C4 consumption by another C1q activator, the β-amyloid 1-40 peptide. This peptide is known to activate C1q through binding at the A-chain 14-26 residue site; therefore, this inhibition confirms that SELEX-derived nucleic acid ligands bind at this A-chain site. Control ligands from the SELEX process that did not bind C1q by nitrocellulose assay were also ineffective in blocking the β-amyloid 1-40 peptide C1q activation.

Example 3

[0106] 2'-Fluoro Nucleic Acid Ligands of Complement System Protein C3

[0107] In order to generate ligands to complement protein C3, a library of about 10^{14} RNA was generated that contained 30 nucleotides of contiguous random sequence flanked by defined sequences. In this experiment, 30N random nucleotides of the initial Candidate Mixture were comprised of 2'-F pyrimidine bases. The rounds of selection and amplification were carried out as described in Example 1 using art-known techniques. In round 1 the 30N7-2'-F-RNA and C3 were both incubated at 3 μM. There was barely detectable binding at this round. Both the RNA and C3 concentrations were decreased during the SELEX procedure. Sequences derived from the SELEX procedure are shown in Table 3 (SEQ ID NOS:21-46).

Example 4

[0108] 2'-Fluoro Nucleic Acid Ligands of Complement System Protein C5

[0109] In order to generate ligands to human complement protein C5, a library of about 10^{14} RNA was generated that contained 30 nucleotides of contiguous random sequence flanked by defined sequences. In this experiment, the 30N random nucleotides of the initial Candidate Mixture were comprised of 2'-F pyrimidine bases. Briefly, a DNA template was synthesized with a 5'-fixed region containing the T7 promoter, followed by a 30N stretch of random sequence, and then with a 3'-fixed region (Table 1; SEQ ID NO:1). The rounds of selection and amplification were carried out as described in Example 1 using art-known techniques. The initial rounds of the SELEX experiment were set up with high concentrations of 2'-F RNA (7.5 μM) and protein (3 μM), as the binding of C5 to unselected RNA was quite low. The SELEX experiment was designed to promote binding of RNA at the C5a-C5b cleavage site. RNA and C5 were incubated together with small amounts of trypsin, with the reasoning that limited trypsin treatment of C5 produces a single site cleavage and generates C5a-like activity (Wetsel and Kolb (1983) J. Exp. Med. 157:2029). This cleavage led to a slight increase in random RNA binding. Enhanced RNA binding associated structurally with exposure of the C5a-like domain could evolve Nucleic Acid Ligands that bind near the C5 convertase site and could interfere with or inhibit C5 cleavage. The SELEX experiment was performed simultaneously to both the native and to the mildly-trypsinized protein, so that Nucleic Acid Ligand evolution would pick the highest affinity winner. With this procedure the highest affinity winner against the multiple protein species would be evolved, and multiple aptamers and specific aptamers might be obtained out of a single SELEX experiment.

[0110] For each round of the SELEX process, the procedure was performed in parallel in separate tubes with approximately 5-fold excess of RNA either in buffer alone or with addition of trypsin at between 0.3 and 0.0001 mg/mL. Samples were incubated in MgPBS for 45 minutes at 37° C., and then filtered through nitrocellulose. The filters were washed, dried and counted, extracted, reverse-transcribed, then PCR amplified and finally T7 transcribed in vitro into RNA using mixed GTP/ATP and 2'-F-CTP/UTP nucleotides and α-[^{32}P]-ATP. RNA was purified by electrophoresis in 8% acrylamide gels with 7M urea and Tris-Borate EDTA buffer (TBE). RNA was isolated and precipitated with NH_4OAc/ethanol, and then dissolved in phosphate-buffered saline containing 1 nM $MgCl_2$ (MgPBS). Filters with the highest binding were carried forward. At the end of each round, all of the RNA that bound to the protein (either with or without trypsin) was pooled. The protein and RNA concentrations at each round were reduced, with final concentrations of 2.5 nM and 10 nM respectively. Trypsin was added at concentrations between 0.3 and 0.0001 μg/mL. Background binding was monitored at each round, and starting at round four the transcribed RNA was presoaked overnight with nitrocellulose filters prior to the SELEX rounds to reduce background.

[0111] Based on binding of RNA to native C5 by nitrocellulose assay, round twelve DNA was cloned and sequences were obtained as shown in Table 4 (SEQ ID NOS:47-74). Sequences were grouped according to homology and function. Group I sequences are highly homologous and might have arisen by PCR mutation from a single original sequence. Binding affinities of the Group I Nucleic Acid Ligands are very similar and are shown in Table 7. Group II Nucleic Acid Ligands generally bound with similar affinity to Group I Nucleic Acid Ligands, although some weak binders were also present. Group II sequences and length are more diverse than Group I Nucleic Acid Ligands. The C5 Nucleic Acid Ligands do not bind other complement components including C1q, C3, or factors B, H, or D.

[0112] Nucleic Acid Ligands from each family were also assayed for inhibition of rat Complement System activity (Table 5; SEQ ID NOS:76-83). Nucleic Acid Ligands from Family I and Family III inhibited rat complement, whereas a Nucleic Acid Ligand from Family II did not. An inhibitory Nucleic Acid Ligand can be used to inhibit Complement System activity in various rat disease models including, but not limited to, myasthenia gravis, myocardial infarction, glomerulonephritis, ARDS, arthritis and transplantation.

Example 5

[0113] Activation of the Complement System through C1q Nucleic Acid Ligands

[0114] Oligonucleotides can activate both classical and alternative pathways. Particularly, poly-G oligonucleotides which can form G-quartet structures and can interact with the C1q collagen-like region are able to form high molecular weight aggregates, which both bind and activate C1q. Phosphorothioate oligonucleotides, which have increased non-specific binding as compared with phosphodiester oligonucleotides, are also efficient Complement System activators, particularly poly-G containing phosphorothioate oligonucleotides. Results for oligonucleotide activation of solution phase Complement are shown below where classical pathway activation is measure by the release of C4d fragment by ELISA (Quidel, San Diego, Calif.), and alternative pathway activation is measure by Bb ELISA (Quidel, San Diego, Calif.). Although these pathways are separate, there is evidence to suggest that oligonucleotide activation of both pathways is C1q dependent.

Sample		[C4d] μg (Class.)	[Bb] μg (Altern.)
Poly-AG	Random Co-Polymer	8.1	18.9
Poly-G	Random Co-Polymer	1.2	29.3
Poly-I	Random Co-Polymer	0	14.7
Poly-A	Random Co-Polymer	0	0
Poly-U	Random Co-Polymer	0	1.8
Poly-C	Random Co-Polymer	0	2.5
Phosphorothioate Oligonucleotides			
GGC**GGGG**CTACGTACC**GGGG**CTTTGTAAAACCCCGCC SEQ ID NO: 197		−7.1	32.4
CTCTCGCACCCATCTCTCTCCTTCT SEQ ID NO: 198		0.0	3.9
BSA-anti-BSA	Immune Complexes	8.0	11.9
β-Amyloid Peptide		2.7	n/d
Fucoidan	Sulfated Carbohydrate		27
buffer		0.0	0.0

[0115] Complement System activation is also initiated on the erythrocyte membrane and is tested by hemolytic assays. Known activators, including 2'-OH poly-G and phosphorothioate oligonucleotides, as well as potential activators such as multimerized C1q Nucleic Acid Ligands and small (e.g., 15-mer) 2'-F poly-G oligonucleotides are coated on sheep erythrocytes and subsequent lysis of the erythrocytes by serum complement is measured. Methods of coating oligonucleotides and Nucleic Acid Ligands on cells include passive adsorption, chemical conjugation, streptavidin-biotin coupling and specific Nucleic Acid binding. Following treatment with fresh rat or human serum, the deposition of complement components on the cell, membrane damage and lysis are measured by standard methods as would be known by one of skill in the art.

[0116] A. Aggregation of C1q Nucleic Acid Ligands

[0117] C1q Nucleic Acid Ligands are dimerized using chemical cross-linkers of various lengths. Alternatively, Nucleic Acid Ligand monomers are biotinylated and then multimerized with streptavidin. Each of these multimers are tested for complement activation and lysis of erythrocytes.

[0118] The addition of poly-G sequence to C1q Nucleic Acid Ligands provides additional binding ability and increases the ability of the oligonucleotide to activate the Complement System. In addition, short poly-G sequences on individual C1 q Nucleic Acid Ligands can interact to form higher order structures, which serve to multimerize the C1q Nucleic Acid Ligands and cause activation.

[0119] B. Lysis of Erythrocytes and Leukocytes

[0120] Nucleic Acid Ligands that promote erythrocyte lysis are tested on nucleated cells, including lymphocytes and tumor cells. Nucleated cells have mechanisms of complement resistance that erythrocytes lack. For example, nucleated cells can shed antigens, bleb off membrane vesicles containing the complement components and express increased levels of complement inhibitors as compared with erythrocytes and may up-regulate protective mechanisms upon initial complement attack. As high levels of activation are important for cell killing, activators are compared for amount of Complement System component deposition and extent of membrane damage. Also, different types and sources of tumor cells and lymphocytes are tested to determine if susceptibility is cell-type specific.

[0121] Nucleic Acid Ligands can be generated for virtually any target as described in the SELEX Patent Applications. Nucleic Acid Ligands to L-Selectin have been generated (See U.S. patent application Ser. No. 08/479,724, filed Jun. 7, 1995, entitled "High Affinity Nucleic Acid Ligands to Lectins," now U.S. Pat. No. 5,780,228, which is incorporated herein by reference in its entirety). The diversity of lectin mediated functions provides a vast array of potential therapeutic targets for lectin antagonists. For example, antagonists to the mammalian selecting, a family of endogenous carbohydrate binding lectins, may have therapeutic applications in a variety of leukocyte-mediated disease states. Inhibition of selectin binding to its receptor blocks cellular adhesion and consequently may be useful in treating inflammation, coagulation, transplant rejection, tumor metastasis, rheumatoid arthritis, reperfusion injury, stroke, myocardial infarction, bums, psoriasis, multiple sclerosis, bacterial sepsis, hypovolaemic and traumatic shock, acute lung injury and ARDS. The coupling of C1q Nucleic Acid Ligands to L-Selectin Nucleic Acid Ligands makes the L-Selectin Nucleic Acid Ligand more efficient by promoting cell killing at the target. C1q Nucleic Acid Ligands are coupled to L-Selectin Nucleic Acid Ligands, and the conjugates are tested for leukocyte lysis as described above. Also, Nucleic Acid Ligands to other cell surface targets, antibodies to all targets that do not themselves activate complement, cytokines, growth factors, or a ligand to a cell receptor could be coupled to a C1q Nucleic Acid Ligand and used for cell killing.

[0122] C. In Vivo Testing of Complement Activation

[0123] Nucleic Acid Ligand-mediated Complement System activation is tested in animals to evaluate in vivo Nucleic Acid Ligand action. Erythrocytes and/or lymphocytes are coated with Nucleic Acid Ligands and injected into rats to test cell killing and lysis in vivo. Activating Nucleic Acid Ligands are also coupled to a Mab that does not activate the Complement System, where the antibody is directed against a rat cell antigen (e.g., lymphocyte antigen). These cells are then coated with the Nucleic Acid Ligand-antibody conjugate and injected into rats. Alternatively, the

Nucleic Acid Ligand-antibody conjugate is injected directly into the rat and then in vivo leukocyte killing is measured.

[0124] It is also possible that C1q Nucleic Acid Ligands cross-react with non-human C1q, and non-human C1q could be used for in vivo assays. C1q Nucleic Acid Ligands are tested against species such as mouse, rat and rabbit C1q. C1q is purified from serum and cross-reactivity with C1q Nucleic Acid Ligands is tested by nitrocellulose binding assay. Alternatively, C1q is bound to immune complexes which are added to serum and then C1q Nucleic Acid Ligand binding to the aggregate is tested. If Nucleic Acid Ligands are species-specific, then rat serum is depleted of rat C1q by continuous perfusion over a Ig-Sepharose column, and the serum is reconstituted with human C1 q by methods known to one of skill in the art. These reconstituted animals are then used to test C1q Nucleic Acid Ligands for targeted Complement System activation and cell killing.

Example 6

[0125] 2'-Fluoro RNA Ligands of Complement System Protein C1q

[0126] A. RNA SELEX

[0127] The pool of random 30N7-2'-F RNA bound to C1q by nitrocellulose filter assay with a K_d of 2.3 μM. For round 1 of the SELEX process, the C1q concentration was between 0.156-1.25 μM and the RNA concentration was 15 μM. Throughout the SELEX process, the RNA concentrations were maintained at approximately 10-fold greater than the concentration of C1q, which was reduced at each round with a final round 14 C1q concentration of 136 pM. Background binding of RNA to nitrocellulose filters remained low throughout the SELEX procedure, in part because RNA was pre-adsorbed with nitrocellulose filters. The binding of pool RNA to C1q improved at each round. The evolved round 14 pool 2'-F RNA bound C1q with a K_d of 2 nM, yielding an overall improvement in binding K_d of 1-3000-fold.

[0128] Bulk RNA was then cloned for sequence determination and evaluation of binding. Through comparison of binding at 0.1 and 0.5 nM C1q, individual clones were ranked for binding affinity. Sequences of 2'-F RNA ligands are shown in Table 6 (SEQ ID NOS:84-155). The 2'-F-RNA sequences are not easily grouped into families, but these sequences are G-rich and are similar but not homologous with the 2'-NH_2 RNA sequences described in Example 2.

Example 7

[0129] Hemolytic Inhibition for 2'-F RNA Ligands to C5

[0130] The 2'-F RNA Nucleic Acid Ligands to C5 (Example 4) were assayed for hemolytic inhibition by including dilutions in a standard assay for human serum lysis of antibody-coated sheep erythrocytes. Sheep cells were mixed with a 1:40 dilution of serum containing Nucleic Acid Ligand or buffer, and incubated for 30 minutes at 37° C. After quenching with cold EDTA buffer, the samples were spun and supernatants read at OD 412 nm. Group I Nucleic Acid Ligands inhibited almost to background at 1 μM, with a K_i of 60-100 nM. The results are shown in **FIG. 1**. The results of the hemolysis inhibition assay suggested that 2'-F RNA Nucleic Acid Ligands to C5 target a specific site on C5, where they block interaction of C5 with the Complement C5 convertase. These results also confirmed that the 2'-F RNA Nucleic Acid Ligands are stable in serum.

Example 8

[0131] Inhibition of C5a Release

[0132] Nucleic Acid Ligand-C5 interaction that inhibits cleavage of C5 would prevent formation of the C5b and MAC assembly. Inhibition of C5 cleavage should also inhibit C5a release, and this was shown in the following experiment with clone C6 (SEQ ID NO:51) (Example 4). For this experiment, dilutions of clone C6 were incubated with whole human serum in GVB^{++} (veronal-buffered saline containing calcium, magnesium and 1% gelatin) plus addition of zymosan for 30 minutes at 37° C. The samples were then quenched with EDTA-buffer and spun, and supernatants were assayed for C5a by radioimmunoassay (RIA) (Wagner and Hugli (1984) Anal. Biochem. 136:75). The results showed that clone C6 inhibited C5a release with a K_i of approximately 100 nM (**FIG. 2**), whereas control random pool RNA gave no inhibition (data not shown). This assay also demonstrated the serum stability of clone C6.

Example 9

[0133] Boundaries of Clone C6

[0134] Clone C6 (SEQ ID NO:51) (Example 4) was selected for determination of a minimal binding sequence. This was done in the following two ways.

[0135] 1) The minimal RNA sequences (5' and 3' boundaries) required for binding of clone C6 to C5 were determined by partially hydrolyzing clone C6 and determining protein binding (Green et al. (1995) Chem. Biol. 2:683). Briefly, clone C6 was synthesized as either 5'-[^{32}p]-kinase labeled (to determine the 3' boundary) or 3'-[^{32}P]-pCp labeled (to determine the 5' boundary) and the oligonucleotides were purified. Then the oligonucleotides were subjected to alkaline hydrolysis, which cleaves oligonucleotides from the 3' end to purine bases. The partially hydrolyzed RNA was then incubated with C5, and RNA which bound to the C5 protein was partitioned on nitrocellulose and eluted from the protein. The partitioned RNA together with an RNA ladder were run on an 8% acrylamide/7M urea sequencing gel. The boundary where removal of one more base would reduce or eliminate binding was determined by comparison of selected RNA (RNA which bound to C5) versus non-selected RNA (RNA which did not bind to C5).

[0136] The labeled RNA was also digested with T_1 nuclease (which cleaves oligonucleotides from the 3' end to A residues), incubated with C5 and partitioned as above, for a second ladder. **FIG. 3A** shows the results of the digestion of the 5'-kinase-labeled RNA. In this figure, the 3'-sequence (5'-end labeled) is aligned with the alkaline hydrolysis ladder. On the left is the T_1 ladder and on the right are RNA selected with 5× and 1× concentrations of C5. The boundary where removal of a base eliminates binding is shown by the arrow. The asterisk shows a G which is hypersensitive to T_1. Other G nucleotides in the minimal sequences are protected from T_1 digestion. **FIG. 3B** shows the results of the 3'-pCp-ligated RNA. In this figure, the 5'-sequence (3'-end-labled) is aligned with the alkaline hydrolysis ladder. The T_1 and protein lanes, boundary and hypersensitive G nucleotides are as described for **FIG. 3A**.

[0137] 2) In a second experiment, the results obtained from the boundary experiments described above were used to construct synthetic truncated Nucleic Acid Ligands to C5. Several truncates between 34 and 42 nucleotides were synthesized by removing residues at both ends of clone C6 (SEQ ID NO:51), and assayed for C5 binding (Table 8). The shortest oligonucleotide which bound to C5 was a 38 mer (SEQ ID NO:160), which confirms the boundary gel and which provides a preliminary structure for further Nucleic Acid Ligand development. In the minimal 38 mer sequence, 30 bases originated from the random region and eight bases were from the 5' fixed region of clone C6. Removing a base from both 5' and 3' ends of the 38 mer to produce a 36 mer (SEQ ID NO:161) reduced the binding. A 34 mer (SEQ ID NO:162) did not bind. Other truncated oligonucleotides with internal deletions also failed to bind.

Example 10

[0138] Biased SELEX

[0139] A biased SELEX experiment was performed to improve Nucleic Acid Ligand affinity and to further define the structure. The sequence of the 42 mer truncate (SEQ ID NO:75) from Example 9 (Table 8)was used as a template for the Biased SELEX experiment. A synthetic template comprising a 42N random region flanked by new n8 fixed regions (Table 10; SEQ ID NO:163) was constructed and synthesized (Oligos, Etc., CT), where the random region was biased toward the 42 mer truncate of clone C6 from the first SELEX experiment. A 42 mer random region was chosen rather than the minimal 38 mer sequence, as the four extra bases extended a terminal helix. While not wishing to be bound by any theory, the inventors believed that although these four extra bases were not essential for binding, a longer helix was thought desirable to aid in selecting the Nucleic Acid Ligand structure and in minimizing the possible use of fixed regions in the newly selected Nucleic Acid Ligand structure. Each base in the random region was synthesized to contain 0.67 mole fraction of the base corresponding to the base in the 42 mer sequence and 0.125 mole fraction of each of the other three bases. The Biased SELEX experiment was performed as described for the standard SELEX experiment in Example 1. PCR amplification was performed using primers shown in Table 1 (SEQ ID NOS:158-159).

[0140] The Biased SELEX experiment was performed with native C5 protein since clone C6 already inhibits hemolysis and trypsin treatment is not required for binding. The binding of the starting RNA pool to C5 was very low, so the protein and RNA concentrations were started at 2.6 μM and 7.1 μM, respectively, similar to the first SELEX experiment. The binding rapidly improved at round three. RNA and protein concentrations were gradually reduced at each subsequent round to final concentrations by round nine of 62.5 pM and 31 pM, respectively. The binding of the RNA pool to C5 was approximately 5 nM (Table 9), as compared to approximately 100 M for the RNA pool from the first SELEX experiment. Some of the improvements in the affinity of the pool results from absence of lower affinity ligands, size mutants and background binders, which were not allowed to build up to appreciable concentrations during this more rapid SELEX experiment.

[0141] The RNA pool after eight rounds of the Biased SELEX process was improved by 20-50 fold over the round twelve pool from the first SELEX experiment. The overall improvement in K_d from the random pool to pool from eight rounds of the Biased SELEX process is estimated to be greater than 10^5-fold. The isolated and cloned sequences from the Biased SELEX experiment are shown in Table 10 (SEQ ID NOS:164-189). In the sequences shown in Table 10, the two base-pair stem which is dispensable for binding is separated from the minimal 38 mer sequence. These bases show no selective pressure except to maintain the stem. None of the sequences exactly match the original template sequence.

[0142] Clones from the Biased SELEX experiment were assayed and representative binding affinities are shown in Table 11. Most clones bound with a K_d between 10 and 20 nM and are higher affinity binding ligands than the template (SEQ ID NO:163). One of the clones, YL-13 (SEQ ID NO:175), bound approximately five-fold higher affinity than other clones from the Biased SELEX experiment and approximately 10-fold higher affinity than clone C6 (SEQ ID NO:51). None of Nucleic Acid Ligand sequences exactly matched the sequence used for the template in the Biased SELEX experiment. Some bases substitutions are unique to this Biased SELEX experiment sequence set and might account for increased Nucleic Acid Ligand affinity.

Example 11

[0143] 2'-O-Methyl Substitution for Nuclease Protection

[0144] To further stabilize the Nucleic Acid Ligand, positions where 2'-OH-purine nucleotides could be substituted with nuclease-resistant 2'-O-methyl nucleosides were determined. An assay for simultaneously testing several positions for 2'-O-methyl interference was used following the method described in Green et al. (1995) Chem. Biol. 2:683.

[0145] In the 2'-O-methyl interference assay, three sets of oligonucleotides based on a 38 mer truncate of sequence YL-13 (SEQ ID NO:175) from the Biased SELEX experiment were synthesized. These sets of sequences, indicated as M3010 (SEQ ID NO:190), M3020 (SEQ ID NO:191) and M3030 (SEQ ID NO:192) in Table 12 were synthesized on an automated RNA synthesizer in a manner wherein each of the nucleotides indicated by bold underline in Table 12 were synthesized 50% as a 2'-OH-nucleotide and 50% as a 2'-OMe-substituted nucleotide. This resulted in a mixture of 2^5 or 32 different sequences for each of sets M3010, M3020 and M3030.

[0146] The partially substituted 2'-OMe oligonucleotides were 5'-[^{32}P]-kinase-labeled. The oligonucleotides were selected at 100 nM and 10 nM C5 and the binding to protein was greater than 10-fold over background filter binding. The oligonucleotides were eluted from the protein, alkaline hydrolyzed and then run on a 20% acrylamide/7 M urea/TBE sequencing gel. On adjacent tracks were run oligonucleotides not selected with C5. Band intensities were quantitated with on an InstantImager (Packard, Meriden, Conn.). When these oligonucleotides were separated on an acrylamide gel the mixed OH:OMe positions showed up at 50% intensity of a full 2'-OH position, because the 2'-OMe is resistant to hydrolysis. 2'-F pyrimidines are also resistant and do not show on the gel.

[0147] For each position, the ratio of (the intensity of the bands selected by protein binding)/(band intensity for oligonucleotide not selected to protein) was calculated. These ratios were plotted versus nucleotide position and a linear fit determined (**FIG. 4**, open circles). The same calculation was made for mixed 2'-OH/2'-OMe oligonucleotides, and these ratios were compared with previously determined curve (**FIG. 4**, closed circles). Where 2'-OMe substitution did not interfere with binding the ratio was within one standard deviation of the 2'-OH ratio. However, where 2'-OMe substitution interfered with binding, the binding preference for 2'-OH purine increased the ratio. Two nucleotides at positions 16 and 32 were determined to require 2'-OH nucleotides. Separately, residue g5 was determined independently to require 2'-OH and residue G20 was determined to allow 2'-OMe substitution, and these were used to normalize lanes. These results were confirmed by synthesis and assay of 2'-OMe substituted oligonucleotides. The obligate 2'-OH positions are in one of two bulges, or in the loop in the putative folding structure, suggesting these features are involved in the protein interaction. Once the permissible 2'-OMe positions were determined, substituted oligonucleotides were synthesized and relative binding affinities were measured.

Example 12

[0148] Human C5 Nucleic Acid Ligand Structure

[0149] The putative folding and base-pairing, based on truncation experiments, nuclease sensitivity, base substitution patterns from the Biased SELEX experiment, and 2'-OMe substitutions, for the 38 mer truncate of clone C6 together with alternative bases is shown in **FIG. 5A**. The basic sequences is the 38 mer truncate (SEQ ID NO:160). In parentheses are variants from the first SELEX experiment. In brackets are variants from the Biased SELEX experiment. Lower case bases are derived from the 5'-n7 fixed region from the first SELEX experiment. Upper case bases are derived from the original random region.

[0150] The stem-loop structure has between 12 and 14 base-pairs: a) the proposed 5', 3'-terminal base pairs (c1-a3, and U36-G38); b) stem-loop base-pairs (U11-U14 and G24-A27) are supported by covariant changes during the Biased SELEX procedure; and c) the middle stem (g7-C10 and G28-C34), which is generally conserved, U9-A32 which is invariant and g8-C33 conserved during the Biased SELEX procedure. The u4→c4 change improves binding, and this change is found in all clones from the Biased SELEX experiment, and G29, A29 variants are found only in clones from the Biased SELEX experiment.

[0151] The UUU bulge is generally conserved. One original sequence contained two U bases, with no reduction in binding, and two Nucleic Acid Ligands with a single base substitution were found during Biased SELEX experiment. The C10-G28 base-pair following the UUU-bulge is conserved. This region with a conserved bulge and stem is likely involved in protein interaction. The stem-loop G15 to U23 is highly conserved, except for bases 19.

[0152] The 2'-OMe substitution pattern is consistent with this structure (SEQ ID NO:193; **FIG. 5B**). Positions where 2'-OMe substitutions can be made are shown in bold. The three positions which must be 2'-OH are shown as underlined. The obligate 2'-OH bases at g5, G17 and A32 are in bulge or loop regions which might form unique three-dimensional structures required for protein binding. Allowed positions for 2'-OMe substitution occur in stem regions where a standard helical structure is more likely.

Example 13

[0153] Hemolytic Assay of 2'-OMe-Substitued Nucleic Acid Ligands to Human C5

[0154] Three oligonucleotides were synthesized based on clone YL-13 from the Biased SELEX experiment to compare the effect of 2'-OMe substitution on hemolytic inhibition: (1) a 38 mer truncate B2010 (SEQ ID NO:194), in which all of the nucleotides were 2'-OH; (2) a 38 mer in which one nucleotide (position 20) was a 2'-OMe-G (B2070; SEQ ID NO:195); and (3) a 38 mer in which the maximum number of allowable positions (positions 2, 7, 8, 13, 14, 15, 20, 21, 22, 26, 27, 28, 36 and 38) were synthesized as 2'-OMe-G and 2'-OMe-A (M6040; SEQ ID NO:196) as shown in Table 13. These were assayed in the hemolytic assay as described in Example 7. The results are shown in **FIG. 6**. As shown in **FIG. 6**, the K_i decreased with increased 2'-O-Me substitution. The K_d was marginally better (data not shown). This experiment showed that nucleic acid ligand stability is increased with 2'-OMe substitution, and that long term in vivo inhibition of the complement system is feasible.

TABLE 1

SEQ ID NO.	
	Synthetic DNA Template:
1 and 156	5'-TAATACGACTCACTATAGGGAGGACGATGCGG-$[N]_{30\text{ or }50}$-CAGACGACTCGCCCGA-3'
	Starting random sequence RNA pool:
2 and 157	5'-GGGAGGACGAUGCGG-$[N]_{30\text{ or }50}$-CAGACGACUCGCCCGA-3'
	Primer Set for Standard SELEX:
3	5'-PRIMER: 5'-TAATACGACTCACTATAGGGAGGACGATGCGG-3'
4	3'-PRIMER: 5'-TCGGGCGAGTCGTCTG-3'
	Primer Set for Biased SELEX:
158	5'-PRIMER: 5'-TAATACGACTCACTATAGGGAGATAAGAATAAACGCTCAA-3'
159	3' PRIMER: 5' GCCTGTTGTGAGCCTCCTGTCGAA-3'

[0155]

TABLE 2

2'-NH$_2$ RNA Ligands of Complement System Protein Clq*

Clone No.	NO:	SEQ ID Kd(nM)	
	Family 1		
3	gggaggacgaugcggGAGGAGUGGAGGUAAACAAUAGGUCGGUAGCGACUCCCACUAACAGGCCUcagacgacucgcccga	5	
12	gggaggacgaugcggGUGGAGUGGAGGUAAACAAUAGGUCGGUAGCGACUCCCAGUAACGGCCUcagacgacucgcccga	6	
23	cgggaggacgaugcaaGUGGAGUGGAGGUAUAACGGCCGGUAGGCAUCCCACUCGGGCCUAGCUcagacgacucgcccga	7	
30	gggaggacgaugcggGUGGAGUGGGGAUCAUACGGCUGGUAGCACGAGCUCCCUAACAGCGGUcagacgacucgcccga	8	
36	gggaggacgaugcggGAGGAGUGGAGGUAAACAAUAGGCCGGUAGCGACUCCCACUAACAGCCUcagacgacucgcccga	9	0.29
45	gggaggacgaugcggUGGAGUGGAGGUAUACCGGCCGGUAGCGCAUCCCACUCGGGUCUGUGCUcagacgacucgcccga	10	1.38
47	gggaggacgaugcggGUGGAGCGGAGGUUUAUACGGCUGGUAGCUCGAGCUCCCUAACACGCGGUagacgacucgcccga	11	
50	gggaggacgaugcggGUGGAGUGGAGGUAUAACGGCCGGUAGCGCAUCCCACUCGGGUCUGUGCUagacgacucgcccga	12	0.979
78	gggaggacgaugcggGUGGAGUGGAGGGUAAACAAUGGCUGGUGGCAUUCGGAAUCUCCCAACGUagacgacucgcccga	13	
	Family 2		
33	gggaggacgaugcggGUUGCUGGUAGCCUGAUGUGGGUGGAGUGAGUGGAGGGUUGAAAAAUGcagacgacucgcccga	14	3.85
40	gggaggacgaugcggCUGGUAGCAUGUGCAUUGAUGGGAGGAGUGGAGGUCACCGUCAACCGUcagacgacucgcccga	15	
43	gggaggacgaugcggUUUCUCGGCCAGUAGUUUGCGGGUGGAGUGGAGGUAUAUCUGCGUCCUCGcagacgacucgcccga	16	
	Family 3		
14	gggaggacgaugcggCACCUCACCUCCAUAUUGCCGc3UUAUCGCGUAGGGUGAGCCCAGACACGAcagacgacucgcccga	17	2.4
23	gggaggacgaugcggCACUCACCUUCAUAUUGGCCGCCAUCCCCAGGGUUGAGCCCAGACACAGcagacgacucgcccga	18	23
	Family 4		
22	gggaggacgaugcggGCAUAGUGGGCAUCCCAGGGUUGCCUAACGGCAUCCGGGGUUGUUAUUGGcagacgacucgcccga	19	
67	gggaggacgaugcggCAGACGACUCGCCCGAGGGGAUCCCCCGGGCCUGCAGGAAUUCGAUAUcagacgacucgcccga	20	

*Lower case letters represent the fixed region.

[0156]

TABLE 3

2'-F RNA Ligands of Complement System Protein of Human C3*

Clone No.				SEQ ID NO:
C3c 10	gggaggacgaugcgg	AACUCAAUGGGCCUACUUUUUCCGUGGUCCU	cagacgacucgcccga	21
C3C 16	gggaggacgaugcgg	AACUCAAUGGGCCUACUUUUCCGUGGUCCU	cagacgacucgcccga	22
C3C 186	gggaggacgaugcgg	AACUCAAUGGGCCGACUUUUUCCGUGUCCU	cagacgacucgcccg	23
C3C 162	gggaggacgaugcgg	AACUCAAUGGGCCGACUUUCCGUGGUCCU	cagacgacucgcccga	24
C3C 141	gggaggacgaugcgg	AACUCAAUGGGCNUACUUUUCCGUGGUCCU	cagacgacucgcccga	25
C3c 32	gggaggacgaugcgg	AACUCAAUGGGCCGACUUUUCCGUGGUCCU	cagacgacucgcccga	26
27C3B143	gggaggacgaugcgg	AACUCAAUGGGCCGACUUUUCCGUGGUCCU	cagacgacugcccga	27

TABLE 3-continued

2'-F RNA Ligands of Complement System Protein of Human C3*

Clone No.				SEQ ID NO:
30C3B149	gggaggacgaugcgg	ACGCAGGGGAUGCUCACUUUGACUUUUAGGC	cagacgacucgcccg	28
c3a 29c	gggaggacgaugcgg	ACUCGGCAUUCACUAACUUUUGCGCUCGU	cagacgacucgcccga	29
C3B 25	gggaggacgaugcgg	AUAACGAUUCGGCAUUCACUAACUUCUCGU	cagacgacucgcccga	30
C3c 3	gggaggacgaugcgg	AUGACGAUUCGGCAUUCACUAACUUCUCGU	cagacacucgcccga	31
C3C 155	gggaggacgaugcgg	AUGACGAUUCGGCAUUCACUAACUUCUCAU	cagacgacucgcccga	32
C3C 109	gggaggacgaugcgg	AUGACGAUUCGGCAUUCACUAACUUCUACU	cagacgacucgcccga	33
C3-A 18c	gggaggacgaugcgg	AUCUGAGCCUAAAGUCAUUGUGAUCAUCCU	cagacgacucgcccga	34
C3c 35	gggaggacgaugcggg	CGUUGGCGAUUCCUAAGUGUCGUUCUCGU	cagacgacucgcccga	35
C3B 41	gggaggacgaugcgg	CGUCUCGAGCUCUAUGCGUCCUCUGUGGU	cagacgacucgcccga	36
C3B 108	gggaggacgaugcgg	CGUCACGAGCUUUAUGCGUUCUCUGUGGU	cagacgacucgcccga	37
C3c 77	gggaggacgaugcgg	CUUAAAGUUGUUUAUGAUCAUUCCGUACGU	cagacgacucgcccga	38
C3B 102	gggaggacgaugcgg	GCGUUGGCGAUUGGUAAGUGUCGUUCUCGU	cagacgacucgcccga	39
c3a 9c	gggaggacgaugcgg	GCGUCUCGAGCUUUAUGCGUUCUCUGUGGU	cagacgacucgcccga	40
C3B 138	gggaggacgaugcgg	GCGUCUCGAGCUCUAUGCGUUCUCUGUGGU	cagacgacucgcccga	41
c3-8c	ggaggacgaugcgg	GGCCUAAAGUCAAGUGAUCAUCCCCUGCGU	cagacganucgcccga	42
C3-230	gggaggacgaugcgg	GUGGCGAUUCCAAGUCUUCCGUGAACAUGGU	cagacgacucgcccg	43
C3c 36	gggaggacgaugcgg	GUGACUCGAUAUCUUCCAAUCUGUACAUGGU	cagacgacucncccga	44
188	gggaggacgaugcgg	UGGCGAUUCCAAGUCUUCCGTGAACATGGT	cagacgacucgcccga	45
C3B 23	gggaggacgaugcgg	TGGCGATTCCAAGTCTTCCGTGAACAT	cagacgacucgcccga	46

*Lower case letters represent the fixed region.

[0157]

TABLE 4

2'-F RNA Ligands of Complement System Protein Human C5*

Clone No:				SEQ ID NO:
		Group I		
E5c/E11	gggaggacgaugcgg	UCCGGCGCGCUGAGUGCCGGUUAUCCUCGU	cagacgacucgcccga	47
A6	gggaggacgaugcgg	UCCGGCGCGCUGAGUGCCGGUUUAUCCUCGU	cagacgacucgcccga	48
F8	gggaggacgaugcgg	UCUCAUGCGCCGAGUGUGAGUUUACCUUCGU	cagacgacucgcccga	49
K7	gggaggacgaugcgg	UCUCAUGCGUCGAGUGUGAGUUUAACUGCGU	cagacgacucgcccga	50
C6	gggaggacgaugcgg	UCUCAUGCGUCGAGUGUGAGUUUACCUUCGU	cagacgacucgcccga	51
G7	gggaggacgaugcgg	UCUGCUACGCUGAGUGGCUGUUUACCUUCGU	cagacgacucgcccga	52
H1	gggaggacgaugcgg	UCGGAUGCGCCGAGUCUCCGUUUACCUUCGU	cagacgacucgcccga	53

TABLE 4-continued

2'-F RNA Ligands of Complement System Protein Human C5*

Clone No:				SEQ ID NO:
		Group II		
F11	gggaggacgaugcgg	UGAGCGCGUAUAGCGGUUUCGAUAGAGCUGCGU	cagacgacucgcccga	54
H2	gggaggacgaugcgg	UGAGCGCGUAUAGCGGUUUCGAUAGAGCCU	cagacgacucgcccga	55
H6	gggaggacgaugcgg	UGAGCGUGGCAAACGGUUUCGAUAGAGCCU	cagacgacucgcccga	56
H8	gggaggacgaugcgg	UGAGCGUGUAAAACGGUUUCGAUAGAGCCU	cagacgacucgcccga	57
C9	gggaggacgaugcgg	UGAGCGUGUAAAACGGUUUCGAUAGAGCCU	cagacgacucgcccga	58
C12	gggaggacgaugcgg	UGGGCGUCAGCAUUUCGAUCUUCGGCACCU	cagacgacucgcccga	59
G9	gggaggacgaugcgg	GAGUUGUUCGGCAUUUAGAUCUCCGCUCCCU	cagacgacucgcccga	60
F7	gggaggacgaugcgg	GCAAAGUUCGGCAUUCAGAUCUCCAUGCCCU	cagacgacucgcccga	61
E9c	gggaggacgaugcgg	GGCUUCUCACAUAUUCUUCUCUUUCCCCGU	cagacgacucgcccga	62
E4c	gggaggaggaucgg	UGUUCAGCAUUCAGAUCUU	cagacgacucgcccga	63
G3	gggaggacgaugcgg	UGUUCAGCAUUCAGN/AUCUUCACGUGUCGU	cagacgacucgcccga	64
F6	gggaggacgaugcgg	UGUUCACCAUUCAGAUCUUCACGUGUCGU	cagacgacucgcccga	65
D9	gggaggacgaugc	UGUUCAGCAUUCAGAUCUUCACGUGUGU	cagacgacucgcccga	66
F4	gggaggacgaugcgg	UUUCGAUAGAGACUUACAGUUGAGCGCGGU	cagacgacucgcccga	67
D3	gggaggacgaugcgg	UUUGUGAUUUGGAAGUGGGGGGGAUAGGGU	cagacgacucgcccga	68
F9	gggaggacgaugcgg	UGAGCGUGGCAAACGGUUUCGAUAGAGCCU	cagacgacucgcccga	69
J1c	ggagggcgaugg	GGUGAGCGUGUAAAAGGUUGCGAUAGAGCCU	cagacgacucgcccga	70
D6	gggaggacgaugcgg	GUAUCUUAUCUUGUUUUCGUUUUUCUGCCCU	cagacgaucgcccga	71
E8x	gggaggacgaugcgg	AGGGUUCUUUUCAUCUUCUUUCUUUCCCCU	cagacgacucgcccga	72
H11	gggaggacgaugcgg	ACGAAGAAGGUGGUGGAGGAGUUUCGUGCU	cagacgacucgcccga	73
G10	gggaggacgaugcgg	ACGAAGAAGGGGGUGGAGGAGUUUCGUGCU	cagacuacucgcccga	74

*Lower case letters represent the fixed region.

[0158]

TABLE 5

Rat C5 2'F- RNA sequences*

Clone No:				SEQ ID NO:
		Family I		
RtC5-116	gggaggacgaugcgg	CGAUUACUGGGACGGACUCGCGAUGUGAGCC	cagacgacucgcccga	76
RtC5-39	gggaggacgaugcgg	CGAUUACUGGGACAGACUCGCGAUGUGAGCU	cagacgacucgcccga	77
RtC5-69	gggaggacgaugcgg	CGACUACUGGGAAGGGUCGCGGUGAGCC	cagacgacucgcccga	78
RtC5-95	gggaggacgaugcgg	CGAUUACUGGGACAGACUCGCGAUGUGAGCU	cagacgacucgcccga	79
RtC5-146	gggaggacgaugcgg	CGACUACUGGGAGAGUACGCGAUGUGUGCC	cagacgacucgcccga	80

TABLE 5-continued

Rat C5 2'F- RNA sequences*

Clone No:				SEQ ID NO:
		Family II		
RtC5-168	gggaggacgaugcgg	GUCCUCGGGGAAAAUUUCGCGACGUGAACCU	cagacgacucgcccga	81
		Family III		
RtC5-74	gggaggacgaugcgg	CUUCUGAAGAUUAUUUCGCGAUGUQAACUUCAGACCCCU	cagacgacucgcccga	82
RtC5-100	gggaggacgaugcgg	CUUCUGAAGAUUAUUUCGCGAUGUGAACUCCAGACCCCU	cagacgacucgcccga	83

*Lower case letters represent the fixed region.

[0159]

TABLE 6

2'-F RNA Ligands of Complement System Protein Clq*

Clone No:				SEQ ID NO:
c1qrdl7-33c	gggaggacgaugcgg	AAAGUGGAAGUGAAUGGCCGACUUGUCUGGU	cagacgacucgcccga	84
C1B100	gggaggacgaugcgg	AAACCAAAUCGUCGAUCUUUCCACCGUCGU	cagacgacucgcecga	85
c1q-a8c	gggaggacgaugcgg	AACACGAAACGGAGGUUGACUCGAUCUGGC	cagacgacucgcccga	86
C1q5	cggaggacgaugcgg	AACACGGAAGACAGUGCGACUCGAUCUGGU	cagacgacucgcccga	87
32.C1B76	cgggaggacgaugcgg	AACAAGGACAAAAGUGCGAUUCUGUCUGG	cagacgacucgcccg	88
c110c	gggaggacgaugcgg	AACAGACGACUCGCGCAACUACUCUGACGU	cagacgacucgcccga	89
C1B121c	gggaggacgaugcgg	AACAGGUAGUUGGGUGACUCUGUGUGACCU	cagacgacucgcccga	90
C1q11c	cggaggacgaugcgg	AACCAAAUCGUCGAUCUUUCCACCGCUCGU	cagacgacucgcccga	91
C15c	cgggaggacgaugcgg	AACCGCUAUUGAAUGUCACUGCUUCGUGCU	cagacgacucgcccga	92
C1Q-A24'c	cgggaggacgaugcgg	AACCGCAUGAGUUAGCCUGGCUCG	93	
C1Q-A5'c	gggaggacgaugcgg	AACCCAAUCGUCUAAUUCGCUGCUCAUCGU	cagacgacucgcccga	94
C121c	gggaggacgaugcgg	AACUCAAUGGGCCUACULTUUCCGUGGUCCU	cagacgacucgcccga	95
c1q-a2C	gggaggacgaugcgg	AAGCGGUGAGUCGUGGCUUUCUCCUCGAUCCUCGU	cagacgacucgcccga	96
c1q-a12C	gggaggacgaugcgg	AAGGAUGACGAGGUGGUUGGGGUUUGUGCU	cagacgacucgcccga	97
c1qrd17-43c	gggaggacgaugcgg	ACAAGACGAGAACGGGGGGAGCUACCUGGC	cagacgacucgcccga	98
CIQ-A7'C	gggaggacgaugcgg	AGACACUAAACAAAUUGGCGACCUGACCGU	cagacgacucgcccga	99
03.C1Q.137c	gggaggacgaugcgg	AGACGCUCAGACGACUCGCCCGACCACGGAUGCGACCU	cagacgacucgcccga	100
14.C1Q156c	gggaggacgaugcgg	AGAUGGAUGGAAGUGCUAGUCUUCUGGGGU	cagacgacucgccc	101
C1B119	cgggaggacgaugcgg	AGAUGGAUGGAAGUGCUAGUCUUUCUGGGGU	cagacgacucgcccga	102
C1Q-A28'C	gggaggacgaugcqg	AGCAGUUGAAAGACGUGCGUUUCGUUUGGU	cagacgacucgcccga	103
15.C1Q.157c	gggaggacgaugcgg	AGCACAAUUUUUUCCUUUUCUUUUCGUCCACGUGCU	cagacgacucgcccga	104
44c1qb60c	gggaggacgaugcgg	AGCUGAUGAAGAUCAUCUCUGACCCCU	cagacgacucgcccga	105

TABLE 6-continued

2'-F RNA Ligands of Complement System Protein Clq*

Clone No:				SEQ ID NO:
06.C1Q.143c	gggaggacgaugcgg	AGCUGAAAGCGAAGUGCGAGGUCUUUGGUC	cagacgacucgcccga	106
C1q4c	ggaggacgaugcgg	AGCGAAAGUGCGAGUGAUUGACCAGGUGCU	cagacgacucgcccga	107
c1qrd17-52c	gggaggacgaugcgg	AGCGUGAGAACAGUUGCGAGAUUGCCUGGU	cagacgacucgcccga	108
C111c	gggaggacgaugcgg	AGGAGAGUGUGGUGAGGGUCGUUUUGAGGGU	cagacgacucgcccga	109
44c1Qb60c	gggaggacgaugcgg	AGGAGCUGAUGAAGAUGAUCUCUGACCCCU	cagacgacucgcccga	110
24c1qb51C	gggaggacgaugcgg	AGUUCCCAGCCGCCUUGAUUUCUCCGUGGU	cagacgacucgcccga	111
31c1qb16	cgggaggacgaugcgg	AUAAGUGCGAGUGUAUGAGGUGCGUGUGGU	cagacgacucgcccga	112
28c1Qb20c	gggaggacgaugcgg	AUCUGAGGAGCUCUUCGUCGUGCUGAGGGU	cagacgacucgcccga	113
c1qrd17-61c	gggaggacgaugcgg	AUCCGAAUCUUCCUUACACGUCCUGCUCGU	cagacgacucgcccga	114
C1q17	cggaggacgaugcgg	AUCCGCAAACCGACAGCUCGAGUUCCGCCU	cagacgacucgcccga	115
34c1qb27c	gggaggacgaugcgg	AUGGUACUUUAGUCUUCCUUGAUUCCGCCU	cagacgacucgcccga	116
C1q7c	cggaggacgaugcgg	AUGAUGACUGAACGUGCGACUCGACCUGGC	cagacgacucgcccga	117
C1q7c	ggaggacgaugcgg	AUGAGGAGGAAGAGUCUGAGGUGCUGGGGU	cagacgacucgcccga	118
C1Q-A22'C	gggaggacgaugcgg	AUUUCGGUCGACUAAAUAGGGGUGGCUCGU	cagacgacucgcccga	119
C122c	gggaggacgaugcgg	CAAGAGGUCAGACGACUGCCCCGAGUCCUCCCCCGGU	cagacgacucgcccga	120
C115c	gggaggacgaugcgg	CAGUGAAAGGCGAGUUUUCUCCUCUCCCU	cagacgacucgcccga	121
09.C1Q.149c	gggaggacgaugcgg	CAUCGUUCAGGAGAAUCCACUUCGCCUCGU	cagacgacucgcccga	122
04.C1Q.138c	gggaggacgaugcgg	CAUCUUCCUUGUUCUUCCAACCCUCCUCCU	cagacgacucgcccga	123
C1Q-A4'C	gggaggacgaugcgg	CAUCGUAAACAAUUUGUUCCAUCUCCGCCU	cagacgacucgcccga	124
c1qrdl7-64c	gggaggacgaugcgg	CAUUGUCCAAGUUUAGCUGUCCGUGCUCGU	cagacgacucgcccga	125
46C1Qb64c	gggaggacgaugcgg	CAUACUCCGGAUACUAGUCACCAGCCUCGU	agacgacucgcccga	126
Cliq6c	gggaggacgaugcgg	CCGUCUCGAUCCUUCUAUGCCUUCGCUCGU	cagacgacucgcccga	127
23C1Qb4x	gggaggacgaugcgg	CGGGAAGUUUGAGGUGUANUACCUGUUGUCUGGU	cagacgacucgcccga	128
c1qrd17-63c	gggaggacgaugcgg	CUCAACUCUCCCACAGACGACUCGCCCGGGCCUCCU	cagacgacucgcccga	129
c1qrd17-47c	gggaggacgaugcgg	GACUCCUCGACCGACUCGACCGGCUCGU	cagacgacucgccga	130
C1g9c	ggaggacgaugcgg	GAACCAAAUCGUCGAUCUUUCCACCGCUCGU	cagacgacucgcccga	131
C1qrd-A63c10	cggaggacgaugcgg	GACCACCUCGAUCCUCAGCGCCAUUGCCCU	cagacgacucgcccga	132
C119c	gggaggacgaugcgg	GAAGUGGAAGGGUAGUUGUGUGACCU	cagacgacucgcccga	133
c1qrd17-42c	cggaggacgaugegg	GCAAACUTUUUCCUUUUCCCUUUAUCUUCCUUGCCCU	cagacgacucgcccga	134
30c1Q24c	gggaggacgaugcgg	GGCCGACGAUUCACCAAUGUUCUCUCUGGU	cagacgacucgcccga	135
C1q10c	ggaggacgaugcgg	GGUUCCUCAAUCACGAUCUCCAUUCCGCUCGU	cagacgacucgcccag	136
C1q20c	ggaggacgaugcgg	GUCGACAUUGAAGCUGCUCUGCCUUGAUCCU	cagacgacucgcccga	137
08.C1Q.147c	gggaggacgaugcgg	UCCAAUUCGUUCUCAUGCCUUUCCGCUCGU	cagacgacucgcccga	138
11.C1Q.152c	gggaggacgaugcgg	UCCGCAACUUUAGCACUCACUGCCUCGU	cagacgacucgcccga	139

TABLE 6-continued

2'-F RNA Ligands of Complement System Protein Clq*

Clone No:				SEQ ID NO:
26c1Qb4c	gggaggacgaugcgg	UCCACAUCGAAUUUUCUGUCCGUUCGU	cagacgacucgeccga	140
C1B115c	gggaggacgaugcgg	UCGAUGUUCUUCCUCACCACUGCUCGUCGCCU	cagacgacucgcccga	141
33c1Q26c	gggaggacgaugcgg	UCGAGCUGAGAGGGGCUACUUGUUCUGGUCA	cagacgacucgcccga	142
01.C1Q.135c	gggaggacgaugcgg	UGGAAGCGAAUGGGCUAGGGUGGGCUGACCUC	cagacgacucgcccga	143
47c1qb65	cgggaggacgaugcgg	UGGACUUCUUUUCCUCUUUCCUCCUUCCGCCGGU	cagacgacucgcccga	144
C1q14c	ggaggacgaugcgg	UUCCAAAUCGUCUAAGCAUCCCUCGCUCGU	cagacgacucgcccag	145
c1qrd17-53c	gggaggacgaugcgg	UUCCACAUCGCAAUUUUCUGUCCGUGCUCGU	cagacgacucgcccga	146
c1q-a6C	gggaggacgaugcgg	UUCCACAUCGAAUUUUCUGUCCGUGUCGU	cagacgacucgcccga	147
C1B114	cgggaggacgaugcgg	UUCCGAUCGACUCCACAUACAUCUGCUCGU	cagacgacucgcccga	148
c1qrd17-56c	gggaggacgaugcgg	UUCCGACAUCGAUGUUGCUCUUCGCCUCGU	cagacgacucgcccga	149
05.C1Q.142c	gggaggacgaugcgg	UUCCGAAGUUCUUCCCCCGAGCCUUCCCCCUC	cagacgacucgcccga	150
30c1q24	cgggaggacgaugcgg	UUCCGACGAUUCUCCAAUGUUCUCUCUGGU	cagacgacucgcccga	151
38c1qb45c	gggaggacgaugcgg	UUCCGACGAUUCUCCAAUCUUCUCUCUGGU	cagacgacucgcccga	152
10.C1Q151c	gggaggacgaugcgg	UUCCGCAAGUUUAGACACUCACUGCCUCGU	cagacgacucgcccga	153
C113x	gggaggacgaugcgg	UUCCGCAAAGUAGAUAUNUCAUCCGCACCU	cagacgacucgcccga	154
10.C1B.134c	gggaggacgaugcgg	UUGAGUGGACAGUGCGAUUCGUUUUGGGGU	cagacgacucgcccga	155

*Lower case letters represent the fixed region.

[0160]

TABLE 7

Binding affinity of C5 nucleic acid ligands

Clone	SEQ ID NO	Kd (nM)
A6	48	35
E11	47	60
E4	63	50
C6	51	30
C9	58	45

TABLE 7-continued

Binding affinity of C5 nucleic acid ligands

Clone	SEQ ID NO	Kd (nM)
G3	64	55
F8	49	30

[0161]

TABLE 8

Effect of truncation of clone C6 on C5 binding

SEQ ID NOS:	Sequence	Length (nts)	Kd (nM)
*75	gA CgAUgCggUCUCAUgCgUCgAgUgUgAgUUUACCUUCg UC	42	
160	CgAUgCggUCUCAUgCgUCgAgUgUgAgUUUACCUUCg	38	20
161	gAUgCggUCUCAUgCgUCgAgUgUgAgUUUACCUUC	36	50
162	AUgCggUCUCAUgCgUCgAgUgUgAgUUUACCUU	34	$>10^6$

*Fragment of SEQ ID NO:51 (Table 4)

[0162]

TABLE 9

Binding of SELEX pools

SELEX pool	Kd
random pool	>1 nM
First SELEX, round 12	100 nM
Biased SELEX, round 8	5 nM

[0163]

TABLE 10

Clones from Biased SELEX

Clone No.	SEQ ID NO:
template gggagataagaataaacgctcaag GA CGATGCGGTCTCATGCGTCGAGTGTGAGTTTACCTTCG TC ttcgacaggaggctcacaacaggc	163
YL-8(10): gggagauaagaauaaacgcucaag UG CGACGCGGUCUCGAGCGCGGAGUUCGAGUUUACCUUCG CA uucgacaggaggcucacaacaggc	164
YL-33(2): gggagauaagaauaaacgcucaag CU CGACGCGGUCCCAGGCGUGGAGUCUGGGUUUACCUUCG AG uucgacaggaggcucacaacaggc	165
YL-79(3): gggagauaagaauaaacgcucaag AA CCACGCGGUCUCAGGCGUAGAGUCUGAGUUUACCUUGG UU uucgacaggaggcucacaacaggc	166
YL-1(2): gggagauaagaauaaacgcucaag AA CCACGCGGUCUCAGGCGUAGAGUCUGUGUUUACCUUGG UU uucgacaggaggcucacaacaggc	167
YL-71: gggagauaagaauaaacgcucaag UG CGACGCGGUCUCGAGCGCGGAGUUCGAGUUCACCUUCG CA uucgacaggaggcucacaacaggc	168
YL-39: gggagauaagaauaaacgcucaag CA CAACGCGGUCUCAUGCGUCGAGUAUGAGUUUACCUUuG UG uucgacaggaggcucacaacaggc	169
YL-60: gggagauaagaauaaacgcucaag GU CCUCGCGGUCUCAUGCGCCGAGUAUGAGUUUACCUAGG AC uucgacaggaggcucacaacaggc	170
YL-9: gggagauaagaauaaacgcucaag GU CGUCGCGGUCUGAUGCGCUGAGUAUCAGUUUACCUACG AC uucgacaggaggcucacaacaggc	171
YL-56: gggagauaagaauaaacgcucaag GU ACACGCGGUCUGACGCGCUGAGUGUCAGUUUACCUUGU AC uucgacaggaggcucacaacaggc	172
YL-63: gggagauaagaauaaacgcucaag AAACCACGCGGUCUCAGGCGCAGAGUCUGAGUUACCUUCG CA uucgacaggaggcucacaacaggc	173
YL-29: gggagauaagaauaaacgcucaag AA CCACGCGGUCUCAGGCGCAGAGUCUGAGUUACCUUGG UU uucgacaggaggcucacaacaggc	174
YL-13: gggagauaagaauaaacgcucaag GA CGCCGCGGUCUCAGGCGCUGAGUCUGAGUUUACCUGCG UC uucgacaggaggcucacaacaggc	175
YL-24: gggagauaagaauaaacgcucaag GC UGACGCGGUCUCAGGCGUGGAGUCUGAGUUUACCUUCG GC uucgacaggaggcucacaacaggc	176
YL-3: gggagauaagaauaaacgcucaag CA UGACGCGGUCUCAGGCGUGGAGUCUGAGUUUACCUUCG UG uucgacaggaggcucacaacaggc	177
YL-67: gggagauaagaauaaacgcucaag GU CGACGCGGUCUCAGGCGUUGAGUCUGUGUUUACCUUCG AC uucgacaggaggcucacaacaggc	178
YL-69: gggagauaagaauaaacgcucaag GU CGACGCGGUCUCAGGCGUUGAGUCUGUGUUUACCUUCG AC uucgacaggaggcucacaacaggc	179

TABLE 10-continued

Clones from Biased SELEX

Clone No.	SEQ ID NO:
YL-81: gggagauaagaauaaacgcucaag GA CGCCGCGGUCUCAGGCGUUGAGUCUGAGUUUACCUGCG UC uucgacaggaggcucacaacaggc	180
YL-15(7): gggagauaagaauaaacgcucaag GA CGACGCGGUCUGAUGCGCUGAGUGUCAGUUUACCUUCG UC uucgacaggaggcucacaacaggc	181
YL-84: gggagauaagaauaaacgcucaag AA CGACGCGGUCUGAUGCGCUGAGUGUCAGUGUACCUUCG UC uuogacaggaggcucacaacaggc	182
YL-4(3): gggagauaagaauaaacgcucaag GU CGACGCGGUCUGAUGCGUAGAGUGUCAGUUUACCUUCG AC uucgacaggaggcucacaacaggc	183
YL-51: gggagauaagaauaaacgcucaag GU CGACGCGGUCUGAUGCGUAGAGUGUCAGUUCACCUUCG AC uucgacaggaggcucacaacaggc	184
YL-14(2): gggagauaagaauaaacgcucaag UA CGACGCGGUCCCGUGCGUGGAGUGCGGGUUUACCUUCG UA uucgacaggaggcucacaacaggc	185
YL-23: gggagauaagaauaaacgcucaag GA CGACGCGGUCUGAUGCGCAGAGUGUCGGUUUACCUUUG UC uucgacaggaggcucacaacaggc	186
YL-59: gggagauaagaauaaacgcucaag GA CGACGCNGUCUGAUGCGCAGAGUGUCAGUUUACCUUCG AC uucgacaggaggcucacaacaggc	187
YL-91: gggagauaagaauaaacgcucaag GA CGACGCGGUCUGAUGCGCAGAGUGUCAGUUUACCUUCG UC uucgacaggaggcucacaacaggc	188
YL-50: gggagauaagaauaaacgcucaag GA CGACGCGGUCGGAUGCGCAGAGUGUCCGUUUACCUUCG UC uucgacaggaggcucacaacaggc	189

*Lower case letters represent the fixed region.

[0164]

TABLE 11

Binding affinity of clones from Biased SELEX experiment

SEQ ID NO:	Clone	Kd (nM)
166	YL-79	15
172	YL-56	12
175	YL-13	6
185	YL-14	25
163	Template	30

[0165]

TABLE 12

Sequences based on YL-13 from Biased SELEX

Clone	Sequence	SEQ ID NO:
M3010	CGC CGC GGU CUC AGG CGC UGA GUC UGA GUU UAC CUG CG	190
M3020	CGC CGC GGU CUC GGG CGC UGA GUC UGA GUU UAC CUG CG	191
M3030	CGC CGC GGU CUC AGGCGC UGA GUC UGA GUU UAC CUG CG	192

G,A = 50% 2'-OH:50% 2'-OMe

[0166]

TABLE 13

Truncates based on YL-13 for hemolytic assay

Clone	Sequence	SEQ ID NO:
YL-13t	CGC CGC GGU CUC AGG CGC UGA GUC UGA GUU UAC CUG CG	194
B2070	CGC CGC GGU CUC AGG CGC U$\underline{G}$A GUC UGA GUU UAC CUG CG	195
M6040	C$\underline{G}$C CGC $\underline{GG}$U CUC$\underline{AGG}$ CGC U$\underline{GA}$ $\underline{G}$UC U$\underline{GA}$ $\underline{G}$UU UAC CU$\underline{G}$C$\underline{G}$	196

$\underline{G}$, $\underline{A}$ = 100% 2'-OMe

[0167]

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 198

<210> SEQ ID NO 1
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (33)..(62)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N's at position 33-62 are
      a or c or g or t

<400> SEQUENCE: 1

taatacgact cactataggg aggacgatgc ggnnnnnnnn nnnnnnnnnn nnnnnnnnnn     60

nncagacgac tcgcccga                                                    78


<210> SEQ ID NO 2
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (16)..(55)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid.  N's at position 16-55
      are a or c or g or u.
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 2

gggaggacga ugcggnnnnn nnnnnnnnnn nnnnnnnnnn nnnnncagac gacucgcccg     60

a                                                                      61


<210> SEQ ID NO 3
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid

<400> SEQUENCE: 3
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-continued

taatacgact cactataggg aggacgatgc gg 32

<210> SEQ ID NO 4
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid

<400> SEQUENCE: 4

tcgggcgagt cgtctg 16

<210> SEQ ID NO 5
<211> LENGTH: 81
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(81)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 5

gggaggacga ugcgggagga guggagguaa acaauagguc gguagcgacu cccacuaaca 60

ggccucagac gacucgcccg a 81

<210> SEQ ID NO 6
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 6

gggaggacga ugcgggugga guggagguaa acaauagguc gguagcgacu cccaguaacg 60

gccucagacg acucgcccga 80

<210> SEQ ID NO 7
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(79)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 7

gggaggacga ugcaagugga guggagguau aacggccggu aggcaucccca cucgggccua 60

gcucagacga cucgcccga 79

<210> SEQ ID NO 8
<211> LENGTH: 79

-continued

<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(79)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 8

gggaggacga ugcgggugga guggggauca uacggcuggu agcacgagcu cccuaacagc 60

ggucagacga cucgcccga 79

<210> SEQ ID NO 9
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 9

gggaggacga ugcgggagga guggagguaa acaauaggcc gguagcgacu cccacuaaca 60

gccucagacg acucgcccga 80

<210> SEQ ID NO 10
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 10

gggaggacga ugcgguggag uggagguaua ccggccggua gcgcauccca cucgggucug 60

ugcucagacg acucgcccga 80

<210> SEQ ID NO 11
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 11

gggaggacga ugcgggugga gcggagguuu auacggcugg uagcucgagc ucccuaacac 60

gcgguagacg acucgcccga 80

<210> SEQ ID NO 12
<211> LENGTH: 80

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 12

gggaggacga ugcgggugga guggagguau aacggccggu agcgcauccc acucgggucu     60

gcgguagacg acucgcccga                                                 80


<210> SEQ ID NO 13
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 13

gggaggacga ugcgggugga guggagggua aacaauggcu gguggcauuc ggaaucuccc     60

gcgguagacg acucgcccga                                                 80


<210> SEQ ID NO 14
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(79)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 14

gggaggacga ugcggguugc ugguagccug augugggugg agugagugga ggguugaaaa     60

augcagacga cucgcccga                                                  79


<210> SEQ ID NO 15
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(79)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 15

gggaggacga ugcggcuggu agcaugugca uugaugggag gaguggaggu caccgucaac     60

cgucagacga cucgcccga                                                  79


<210> SEQ ID NO 16
<211> LENGTH: 81
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(81)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 16

gggaggacga ugcgguuucu cggccaguag uuugcgggug gaguggaggu auaucugcgu     60

ccucgcagac gacucgcccg a                                               81


<210> SEQ ID NO 17
<211> LENGTH: 81
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(81)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 17

gggaggacga ugcggcaccu caccuccaua uugccgguua ucgcguaggg ugagcccaga     60

cacgacagac gacucgcccg a                                               81


<210> SEQ ID NO 18
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(80)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 18

gggaggacga ugcggcacuc accuucauau uggccgccau ccccaggguu gagcccagac     60

acagcagacg acucgcccga                                                 80


<210> SEQ ID NO 19
<211> LENGTH: 81
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(81)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 19

gggaggacga ugcgggcaua gugggcaucc cagguugcc uaacggcauc cgggguuguu     60

auuggcagac gacucgcccg a                                               81


<210> SEQ ID NO 20
<211> LENGTH: 78
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(78)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 20

gggaggacga ugcggcagac gacucgcccg aggggauccc ccgggccugc ggaauucgau     60

aucagacgac ucgcccga                                                   78


<210> SEQ ID NO 21
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 21

gggaggacga ugcggaacuc aaugggccua cuuuuuccgu gguccucaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 22
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 22

gggaggacga ugcggaacuc aaugggccua cuuuuccgug guccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 23
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 23

gggaggacga ugcggaacuc aaugggccga cuuuuuccgu guccucagac gacucgcccg     60


<210> SEQ ID NO 24
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 24

gggaggacga ugcggaacuc aaugggccga cuuuccgugg uccucagacg acucgcccga     60


<210> SEQ ID NO 25
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (52)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N at position 28 is
      a or c or g or u

<400> SEQUENCE: 25

gggaggacga ugcggaacuc aaugggcnua cuuuuccgug guccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 26
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 26

gggaggacga ugcggaacuc aaugggccga cuuuuccgug guccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 27
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 27

gggaggacga ugcggaacuc aaugggccga cuuuuccgug guccucagac gacugcccga     60


<210> SEQ ID NO 28
<211> LENGTH: 60
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 28

gggaggacga ugcggacgca ggggaugcuc acuuugacuu uaggccagac gacucgcccg 60

<210> SEQ ID NO 29
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 29

gggaggacga ugcggacucg gcauucacua acuuuugcgc ucgucagacg acucgcccga 60

<210> SEQ ID NO 30
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 30

gggaggacga ugcggauaac gauucggcau ucacuaacuu cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 31
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 31

gggaggacga ugcggaugac gauucggcau ucacuaacuu cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 32
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely

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      Synthesized  Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 32

gggaggacga ugcggaugac gauucggcau ucacuaacuu cucaucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 33
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 33

gggaggacga ugcggaugac gauucggcau ucacuaacuu cuacucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 34
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 34

gggaggacga ugcggaucug agccuaaagu cauugugauc auccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 35
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 35

gggaggacga ugcgggcguu ggcgauuccu aagugucguu cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 36
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 36

gggaggacga ugcggcgucu cgagcucuau gcguccucug uggucagacg acucgcccga     60


<210> SEQ ID NO 37
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 37

gggaggacga ugcggcguca cgagcuuuau gcguucucug uggucagacg acucgcccga     60


<210> SEQ ID NO 38
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 38

gggaggacga ugcggcuuaa aguuguuuau gaucauuccg uacgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 39
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 39

gggaggacga ugcgggcguu ggcgauuggu aagugucguu cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 40
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
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<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 40

gggaggacga ugcgggcguc ucgagcuuua ugcguucucu guggucagac gacucgcccg   60

a   61

<210> SEQ ID NO 41
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 41

gggaggacga ugcgggcguc ucgagcucua ugcguucucu guggucagac gacucgcccg   60

a   61

<210> SEQ ID NO 42
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (52)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N at position 52 is
      a or c or g or u
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 42

ggaggacgau gcggggccua aagucaagug aucauccccu gcgucagacg anucgcccga   60

<210> SEQ ID NO 43
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 43

gggaggacga ugcggguggc gauuccaagu cuuccgugaa cauggucaga cgacucgccc   60

g   61

<210> SEQ ID NO 44
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (57)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N at position 57 is

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a or c or g or u
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 44

gggaggacga ugcgggugac ucgauaucuu ccaaucugua cauggucaga cgacucnccc 60

ga 62

<210> SEQ ID NO 45
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 45

gggaggacga ugcgguggcg auuccaaguc uuccgugaac auggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 46
<211> LENGTH: 58
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(58)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 46

gggaggacga ugcgguggcg auuccaaguc uuccgugaac aucagacgac ucgcccga 58

<210> SEQ ID NO 47
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 47

gggaggacga ugcgguccgg cgcgcugagu gccgguuauc cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 48
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 48

gggaggacga ugcgguccgg cgcgcugagu gccgguuuau ccucgucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 49
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 49

gggaggacga ugcggucuca ugcgccgagu gugaguuuac cuucgucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 50
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 50

gggaggacga ugcggucuca ugcgucgagu gugaguuuaa cugcgucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 51
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 51

gggaggacga ugcggucuca ugcgucgagu gugaguuuac cuucgucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 52
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 52

gggaggacga ugcggucugc uacgcugagu ggcuguuuac cuucgucaga cgacucgccc 60

ga 62

<210> SEQ ID NO 53
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 53

gggaggacga ugcggucgga ugcgccgagu cuccguuuac cuucgucaga cgacucgccc 60

ga 62

<210> SEQ ID NO 54
<211> LENGTH: 64
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(64)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 54

gggaggacga ugcggugagc gcguauagcg guuucgauag agcugcguca gacgacucgc 60

ccga 64

<210> SEQ ID NO 55
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 55

gggaggacga ugcggugagc gcguauagcg guuucgauag agccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 56
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 56

gggaggacga ugcggugagc guggcaaacg guuucgauag agccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 57
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 57

gggaggacga ugcggugagc guguaaaacg guuucgauag agccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 58
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 58

gggaggacga ugcggugagc guguaaaacg guuucgauag agccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 59
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 59

gggaggacga ugcggugggc gucagcauuu cgaucuucgg caccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 60
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 60

gggaggacga ugcgggaguu guucggcauu uagaucuccg cucccucaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 61
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 61

gggaggacga ugcgggcaaa guucggcauu cagaucucca ugcccucaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 62
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 62

gggaggacga ugcggggcuu cucacauauu cuucucuuuc cccgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 63
<211> LENGTH: 49
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(49)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 63

gggaggagga ucgguguuca gcauucagau cuucagacga cucgcccga                 49


<210> SEQ ID NO 64
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (30)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N at position 30 is
      a or c or g or u
```

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```
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 64

gggaggacga ugcgguguuc agcauucagn aucuucacgu gucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 65
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 65

gggaggacga ugcgguguuc accauucaga ucuucacgug ucgucagacg acucgcccga     60


<210> SEQ ID NO 66
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(57)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 66

gggaggacga ugcuguucag cauucagauc uucacgugug ucagacgacu cgcccga        57


<210> SEQ ID NO 67
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 67

gggaggacga ugcgguuucg auagagacuu acaguugagc gcggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 68
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F
```

```
<400> SEQUENCE: 68

gggaggacga ugcgguuugu gauuuggaag ugggggggau agggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 69
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 69

gggaggacga ugcggugagc guggcaaacg guuucgauag agccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 70
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(59)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 70

ggagggcgau ggggugagcg uguaaaaggu ugcgauagag ccucagacga cucgcccga      59


<210> SEQ ID NO 71
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 71

gggaggacga ugcggguauc uuaucuuguu uucguuuuuc ugcccucaga cgaucgcccg     60

a                                                                     61


<210> SEQ ID NO 72
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 72
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gggaggacga ugcggagggu ucuuuucauc uucuuucuuu ccccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 73
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 73

gggaggacga ugcggacgaa gaagguggug gaggaguuuc gugcucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 74
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 74

gggaggacga ugcggacgaa gaagggggug gaggaguuuc gugcucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 75
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(42)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 75

gacgaugcgg ucucaugcgu cgagugugag uuuaccuucg uc                        42


<210> SEQ ID NO 76
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 76

gggaggacga ugcggcgauu acugggacgg acucgcgaug ugagcccaga cgacucgccc     60
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ga                                                                   62


<210> SEQ ID NO 77
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 77

gggaggacga ugcggcgauu acugggacag acucgcgaug ugagcucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 78
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 78

gggaggacga ugcggcgacu acugggaagg gucgcgaagu gagcccagac gacucgcccg     60

a                                                                    61


<210> SEQ ID NO 79
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 79

gggaggacga ugcggcgauu acugggacag acucgcgaug ugagcucaga cgacucgccc     60

ga                                                                   62


<210> SEQ ID NO 80
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 80

gggaggacga ugcggcgacu acugggagag uacgcgaugu gugcccagac gacucgcccg     60
```

a 61

<210> SEQ ID NO 81
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 81

gggaggacga ugcggguccu cggggaaaau uucgcgacgu gaaccucaga cgacucgccc 60

ga 62

<210> SEQ ID NO 82
<211> LENGTH: 70
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(70)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 82

gggaggacga ugcggcuucu gaagauuauu ucgcgaugug aacuucagac cccucagacg 60

acucgcccga 70

<210> SEQ ID NO 83
<211> LENGTH: 70
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(70)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 83

gggaggacga ugcggcuucu gaagauuauu ucgcgaugug aacuccagac cccucagacg 60

acucgcccga 70

<210> SEQ ID NO 84
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 84

gggaggacga ugcggaaagu ggaagugaau ggccgacuug ucuggucaga cgacucgccc 60

```
ga                                                                  62


<210> SEQ ID NO 85
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 85

gggaggacga ugcggaaacc aaaucgucga ucuuuccacc gucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 86
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 86

gggaggacga ugcggaacac gaaacggagg uugacucgau cuggccagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 87
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 87

ggaggacgau gcggaacacg gaagacagug cgacucgauc uggucagacg acucgcccga     60


<210> SEQ ID NO 88
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(59)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 88

gggaggacga ugcggaacaa ggacaaaagu gcgauucugu cuggcagacg acucgcccg      59
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<210> SEQ ID NO 89
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 89

gggaggacga ugcggaacag acgacucgcg caacuacucu gacgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 90
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 90

gggaggacga ugcggaacag guaguugggu gacucugugu gaccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 91
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 91

ggaggacgau gcggaaccaa aucgucgauc uuuccaccgc ucgucagacg acucgcccga     60


<210> SEQ ID NO 92
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 92

gggaggacga ugcggaaccg cuauugaaug ucacugcuuc gugcucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 93
<211> LENGTH: 61
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 93

gggaggacga ugcggaaccc aaucgucuaa uucgcugcuc aucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 94
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 94

gggaggacga ugcggaaccc aaucgucuaa uucgcugcuc aucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 95
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 95

gggaggacga ugcggaacuc aaugggccua cuuuuccgug guccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 96
<211> LENGTH: 66
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(66)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 96

gggaggacga ugcggaagcg gugagucgug gcuuucuccu cgauccucgu cagacgacuc 60

gcccga 66

<210> SEQ ID NO 97
<211> LENGTH: 61

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 97

gggaggacga ugcggaagga ugacgaggug guuggggguuu gugcucagac gacucgcccg     60

a                                                                      61


<210> SEQ ID NO 98
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 98

gggaggacga ugcggacaag acgagaacgg ggggagcuac cuggccagac gacucgcccg     60

a                                                                      61


<210> SEQ ID NO 99
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 99

gggaggacga ugcggagaca cuaaacaaau uggcgaccug accgucagac gacucgcccg     60

a                                                                      61


<210> SEQ ID NO 100
<211> LENGTH: 69
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(69)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 100

gggaggacga ugcggagagg cucagacgac ucgcccgacc acggaugcga ccucagacga     60

cucgcccga                                                              69


<210> SEQ ID NO 101
<211> LENGTH: 59
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(59)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 101

gggaggacga ugcggagaug gauggaagug cuagucuucu ggggucagac gacucgccc      59


<210> SEQ ID NO 102
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 102

gggaggacga ugcggagaug gauggaagug cuagucuuuc uggggucaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 103
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 103

gggaggacga ugcggagcag uugaaagacg ugcguuucgu uuggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 104
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(67)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 104

gggaggacga ugcggagcac aauuuuuucc uuuucuuuuc guccacgugc ucagacgacu     60

cgcccga                                                               67


<210> SEQ ID NO 105
<211> LENGTH: 58
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(58)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 105

gggaggacga ugcggagcug augaagauga ucucugaccc cucagacgac ucgcccga 58

<210> SEQ ID NO 106
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 106

gggaggacga ugcggagcug aaagcgaagu gcgagguguu ugguccagac gacucgcccg 60

a 61

<210> SEQ ID NO 107
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 107

ggaggacgau gcggagcgaa agugcgagug auugaccagg ugcucagacg acucgcccga 60

<210> SEQ ID NO 108
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 108

gggaggacga ugcggagcgu gagaacaguu gcgagauugc cuggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 109
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:

<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 109

gggaggacga ugcggaggag agugugguga gggucguuug agggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 110
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 110

gggaggacga ugcggaggag cugaugaaga ugaucucuga ccccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 111
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 111

gggaggacga ugcggaguuc ccagccgccu ugauuucucc guggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 112
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 112

gggaggacga ugcggauaag ugcgagugua ugaggugcgu guggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 113
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 113

gggaggacga ugcggaucug aggagcucuu cgucgugcug agggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 114
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 114

gggaggacga ugcggauccg aaucuuccuu acacguccug cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 115
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 115

ggaggacgau gcggauccgc aaaccgacag cucgaguucc gccucagacg acucgcccga     60


<210> SEQ ID NO 116
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 116

gggaggacga ugcggauggu acuuuagucu uccuugauuc cgccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 117
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
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<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 117

ggaggacgau gcggaugaug acugaacgug cgacucgacc uggccagacg acucgcccga 60

<210> SEQ ID NO 118
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 118

ggaggacgau gcggaugagg aggaagaguc ugaggugcug gggucagacg acucgcccga 60

<210> SEQ ID NO 119
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 119

gggaggacga ugcggauuuc ggucgacuaa auaggggugg cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 120
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(68)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 120

gggaggacga ugcggcaaga ggucagacga cugccccgag uccuccccccg gucagacgac 60

ucgcccga 68

<210> SEQ ID NO 121
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 121

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gggaggacga ugcggcagug aaaggcgagu uuucuccucu cccucagacg acucgcccga 60

<210> SEQ ID NO 122
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 122

gggaggacga ugcggcaucg uucaggagaa uccacuucgc cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 123
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 123

gggaggacga ugcggcaucu uccuuguucu uccaaccgug cuccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 124
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 124

gggaggacga ugcggcaucg uaaacaauuu guuccaucuc cgccucagac gacucgcccg 60

a 61

<210> SEQ ID NO 125
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 125

gggaggacga ugcggcauug uccaaguuua gcuguccgug cucgucagac gacucgcccg 60

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a                                                                   61


<210> SEQ ID NO 126
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 126

gggaggacga ugcggcauag uccggauacu agucaccagc cucguagacg acucgcccga     60


<210> SEQ ID NO 127
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 127

gggaggacga ugcggccguc ucgauccuuc uaugccuucg cucgucagac gacucgcccg     60

a                                                                   61


<210> SEQ ID NO 128
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (34)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid. N at position 34 is
      a or c or g or u
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(65)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 128

gggaggacga ugcggcggga aguuugaggu guanuaccug uugucugguc agacgacucg     60

cccga                                                               65


<210> SEQ ID NO 129
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(67)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 129

gggaggacga ugcggcucaa cucucccaca gacgacucgc ccgggccucc ucagacgacu     60
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cgcccga                                                                  67


<210> SEQ ID NO 130
<211> LENGTH: 58
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(58)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 130

gggaggacga ugcgggacuc cucgaccgac ucgaccggcu cgucagacga cucgccga       58


<210> SEQ ID NO 131
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 131

ggaggacgau gcgggaacca aaucgucgau cuuuccaccg cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 132
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 132

gggaggacga ugcgggacca ccucgauccu cagcgccauu gcccucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 133
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(57)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 133

gggaggacga ugcgggaagu ggaaggguag uugugugacc ucagacgacu cgcccga        57
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<210> SEQ ID NO 134
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(67)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 134

cggaggacga ugcgggcaaa cuuuuccuuu ucccuuuauc uuccuugccc ucagacgacu     60

cgcccga                                                               67


<210> SEQ ID NO 135
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 135

gggaggacga ugcggggccg acgauucacc aauguucucu cuggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 136
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 136

ggaggacgau gcgggguucc ucaaugacga ucuccauucc gcucgucaga cgacucgccc     60

ag                                                                    62


<210> SEQ ID NO 137
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 137

ggaggacgau gcgggucgac auugaagcug cucugccuug auccucagac gacucgcccg     60

a                                                                     61
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<210> SEQ ID NO 138
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 138

gggaggacga ugcgguccaa uucguucuca ugccuuuccg cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 139
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(59)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 139

gggaggacga ugcgguccgc aaguuuagca cucacugccu cgucagacga cucgcccga      59


<210> SEQ ID NO 140
<211> LENGTH: 58
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(58)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 140

gggaggacga ugcgguccac aucgaauuuu cuguccguuc gucagacgac ucgcccga       58


<210> SEQ ID NO 141
<211> LENGTH: 63
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(63)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 141

gggaggacga ugcggucgau guucuucuc accacugcuc gucgccucag acgacucgcc      60

cga                                                                   63


<210> SEQ ID NO 142
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 142

gggaggacga ugcggucgag cugagagggg cuacuuguuc uggucacaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 143
<211> LENGTH: 63
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(63)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 143

gggaggacga ugcgguggaa gcgaaugggc uagggugggc ugaccuccag acgacucgcc     60

cga                                                                   63


<210> SEQ ID NO 144
<211> LENGTH: 64
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(64)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 144

gggaggacga ugcgguggac uucuuuuccu cuuccuccuu ccgccgguca gacgacucgc     60

ccga                                                                  64


<210> SEQ ID NO 145
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 145

ggaggacgau gcgguuccaa aucgucuaag caucgcucgc ucgucagacg acucgcccag     60


<210> SEQ ID NO 146
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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```
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(62)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 146

gggaggacga ugcgguucca caucgcaauu uucuguccgu gcucgucaga cgacucgccc     60

ga                                                                    62


<210> SEQ ID NO 147
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 147

gggaggacga ugcgguucca caucgaauuu ucuguccgug ucgucagacg acucgcccga     60


<210> SEQ ID NO 148
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 148

gggaggacga ugcgguuccg aucgacucca cauacaucug cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 149
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 149

gggaggacga ugcgguuccg acaucgaugu ugcucuucgc cucgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 150
<211> LENGTH: 63
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(63)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 150

gggaggacga ugcgguuccg aaguucuucc cccgagccuu cccccuccag acgacucgcc 60

cga 63

<210> SEQ ID NO 151
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 151

gggaggacga ugcgguuccg acgauucucc aauguucucu cuggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 152
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 152

gggaggacga ugcgguuccg acgauucucc aaucuucucu cuggucagac gacucgcccg 60

a 61

<210> SEQ ID NO 153
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 153

gggaggacga ugcgguuccg caaguuuaga cacucacugc cucgucagac gacucgcccg 60

a 61

<210> SEQ ID NO 154
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (33)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely

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```
      Synthesized Nucleic Acid. N at position 33 is
      a or c or g or u
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 154

gggaggacga ugcgguuccg caaaguagau aunucauccg cacgucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 155
<211> LENGTH: 61
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(61)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 155

gggaggacga ugcgguugag uggacagugc gauucguuuu ggggucagac gacucgcccg     60

a                                                                     61


<210> SEQ ID NO 156
<211> LENGTH: 98
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (33)..(72)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid.  N's at positions 33-72
      are a or c or g or t.

<400> SEQUENCE: 156

taatacgact cactataggg aggacgatgc ggnnnnnnnn nnnnnnnnnn nnnnnnnnnn     60

nnnnnnnnnn nnnnnnnnnn nncagacgac tcgcccga                             98


<210> SEQ ID NO 157
<211> LENGTH: 81
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (16)..(65)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid.  N's at positions 16-65
      are a or c or g or u.
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(81)
<223> OTHER INFORMATION: All c's and u's are 2'-NH2

<400> SEQUENCE: 157

gggaggacga ugcggnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn     60

nnnnncagac gacucgcccg a                                               81


<210> SEQ ID NO 158
<211> LENGTH: 40
<212> TYPE: DNA
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid

<400> SEQUENCE: 158

taatacgact cactataggg agataagaat aaacgctcaa                            40


<210> SEQ ID NO 159
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid

<400> SEQUENCE: 159

gcctgttgtg agcctcctgt cgaa                                             24


<210> SEQ ID NO 160
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 160

cgaugcgguc ucaugcgucg agugugaguu uaccuucg                              38


<210> SEQ ID NO 161
<211> LENGTH: 36
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(36)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 161

gaugcggucu caugcgucga gugugaguuu accuuc                                36


<210> SEQ ID NO 162
<211> LENGTH: 34
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(34)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 162

augcggucuc augcgucgag ugugaguuua ccuu                                  34


<210> SEQ ID NO 163
<211> LENGTH: 90
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid

<400> SEQUENCE: 163

gggagataag aataaacgct caaggacgat gcggtctcat gcgtcgagtg tgagtttacc     60

ttcgtcttcg acaggaggct cacaacaggc                                      90


<210> SEQ ID NO 164
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 164

gggagauaag aauaaacgcu caagugcgac gcggucucga gcgcggaguu cgaguuuacc     60

uucgcauucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 165
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 165

gggagauaag aauaaacgcu caagcucgac gcgguccag gcguggaguc uggguuuacc     60

uucgaguucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 166
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 166

gggagauaag aauaaacgcu caagaaccac gcggucucag gcguagaguc ugaguuuacc     60

uugguuuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 167
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 167

gggagauaag aauaaacgcu caagaaccac gcggucucag gcguagaguc uguguuuacc 60

uugguuuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 168
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 168

gggagauaag aauaaacgcu caagugcgac gcggucucga gcgcggaguu cgaguucacc 60

uucgcauucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 169
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 169

gggagauaag aauaaacgcu caagcacaac gcggucucau gcgucgagua ugaguuuacc 60

uuuguguucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 170
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 170

gggagauaag aauaaacgcu caagguccuc gcggucucau gcgccgagua ugaguuuacc 60

uaggacuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 171
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely

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      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 171

gggagauaag aauaaacgcu caaggucguc gcggucugau gcgcugagua ucaguuuacc     60

uacgacuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 172
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 172

gggagauaag aauaaacgcu caagguacac gcggucugac gcgcugagug ucaguuuacc     60

uuguacuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 173
<211> LENGTH: 91
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(91)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 173

gggagauaag aauaaacgcu caagaaacca cgcggucuca ggcgcagagu cugaguuuac     60

cuucgcauuc gacaggaggc ucacaacagg c                                    91


<210> SEQ ID NO 174
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 174

gggagauaag aauaaacgcu caagaaccac gcggucucag gcgcagaguc ugaguuuacc     60

uugguuuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 175
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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```
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 175

gggagauaag aauaaacgcu caaggacgcc gcggucucag gcgcugaguc ugaguuuacc     60

ugcgucuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 176
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 176

gggagauaag aauaaacgcu caaggcugac gcggucucag gcguggaguc ugaguuuacc     60

uucggcuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 177
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 177

gggagauaag aauaaacgcu caagcaugac gcggucucag gcguggaguc ugaguuuacc     60

uucguguucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 178
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 178

gggagauaag aauaaacgcu caaggucgac gcggucucag gcguugaguc uguguuuacc     60

uucgacuucg acaggaggcu cacaacaggc                                      90


<210> SEQ ID NO 179
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
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Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 179

gggagauaag aauaaacgcu caaggucgac gcggucucag gcguugaguc uguguuuacc 60

uucgacuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 180
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 180

gggagauaag aauaaacgcu caaggacgcc gcggucucag gcguugaguc ugaguuuacc 60

ugcgucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 181
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 181

gggagauaag aauaaacgcu caaggacgac gcggucugau gcgcugagug ucaguuuacc 60

uucgucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 182
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 182

gggagauaag aauaaacgcu caagaacgac gcggucugau gcgcugagug ucaguguacc 60

uucgucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 183
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely

-continued

Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 183

gggagauaag aauaaacgcu caaggucgac gcggucugau gcguagagug ucaguuuacc 60

uucgacuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 184
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 184

gggagauaag aauaaacgcu caaggucgac gcggucugau gcguagagug ucaguucacc 60

uucgacuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 185
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 185

gggagauaag aauaaacgcu caaguacgac gcgguccccgu gcguggagug cggguuuacc 60

uucguauucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 186
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 186

gggagauaag aauaaacgcu caaggacgac gcggucugau gcgcagagug ucgguuuacc 60

uuugucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 187
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: unsure

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<222> LOCATION: (33)
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid. N at position 33 is
a or c or g or u
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 187

gggagauaag aauaaacgcu caaggacgac gcngucugau gcgcagagug ucaguuuacc 60

uucgacuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 188
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 188

gggagauaag aauaaacgcu caaggacgac gcggucugau gcgcagagug ucaguuuacc 60

uucgucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 189
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(90)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 189

gggagauaag aauaaacgcu caaggacgac gcggucggau gcgcagagug uccguuuacc 60

uucgucuucg acaggaggcu cacaacaggc 90

<210> SEQ ID NO 190
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'-F. A's and g's at
positions 2, 8, 15, 22 and 28 are 50 % 2'-OH and
50% 2'-O-Methyl

<400> SEQUENCE: 190

cgccgcgguc ucaggcgcug agucugaguu uaccugcg 38

<210> SEQ ID NO 191
<211> LENGTH: 37
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(37)
<223> OTHER INFORMATION: All c's and u's are 2'F.     A's and g's at
      positions 7, 13, 17, 27 and 36 are 50% 2'-OH and
      50% 2'-O-Methyl

<400> SEQUENCE: 191

cgccgcgguu cgggcgcuga gucugaguuu accugcg                              37


<210> SEQ ID NO 192
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'F.  A's and g's at
      positions 15, 21, 26, 32 and 38 are 50% 2'-OH and 50%2'-O-Methyl

<400> SEQUENCE: 192

cgccgcgguc ucaggcgcug agucugaguu uaccugcg                             38


<210> SEQ ID NO 193
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'F.   A's and g's at
      positions 2, 7, 8, 13, 14, 15, 20, 21, 22, 26, 27,
      28, 36 and 38 can be 2'-O-Methyl.   A's and g's
      at positions 5, 17 and 32 must be 2'-OH

<400> SEQUENCE: 193

cgaugcgguc ucaugcgucg agugugaguu uaccuucg                             38


<210> SEQ ID NO 194
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'-F

<400> SEQUENCE: 194

cgccgcgguc ucaggcgcug agucugaguu uaccugcg                             38


<210> SEQ ID NO 195
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'-F.   G at position 20 is
      2'-O-Methyl

<400> SEQUENCE: 195

cgccgcgguc ucaggcgcug agucugaguu uaccugcg                           38


<210> SEQ ID NO 196
<211> LENGTH: 38
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(38)
<223> OTHER INFORMATION: All c's and u's are 2'-F.   A's and g's at
      positions 2, 7, 8, 13, 14, 15, 20, 21, 22, 26, 27,
      28, 36 and 38 are 2'-O-Methyl

<400> SEQUENCE: 196

cgccgcgguc ucaggcgcug agucugaguu uaccugcg                           38


<210> SEQ ID NO 197
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(37)
<223> OTHER INFORMATION: All c's and t's are 2'-F.  All nucleotides are
      bound by a phosphorothioate linkage

<400> SEQUENCE: 197

ggcggggcta cgtaccgggg ctttgtaaaa ccccgcc                            37


<210> SEQ ID NO 198
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Completely
      Synthesized Nucleic Acid
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(25)
<223> OTHER INFORMATION: All c's and t's are 2'-F.  All nucleotides are
      bound by a phosphorothioate linkage

<400> SEQUENCE: 198

ctctcgcacc catctctctc cttct                                         25
```

We claim:

1. A method for treating a Complement System-mediated disease comprising administering to a patient in need thereof a pharmaceutically effective amount of a Nucleic Acid Ligand of a Complement System Protein.

2. The method of claim 1 wherein said Nucleic Acid Ligand is identified according to a method comprising:

a) preparing a candidate mixture of nucleic acids;

b) contacting the candidate mixture of nucleic acids with a Complement System Protein, wherein nucleic acids having an increased affinity to said Complement System Protein relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

d) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding said Complement System Protein, wherein Nucleic Acid Ligands of said Complement System Protein may be identified.

3. The method of claim 1 wherein said Complement System Protein is selected from the group consisting of C1q, C3 and C5.

4. The method of claim 1 wherein said Complement System-mediated disease is selected from the group consisting of myocardial infarction and Alzheimer's disease.

5. A method of treating a Complement System-mediated disease comprising administering to a patient in need thereof a pharmaceutical agent which specifically treats said disease and administering a Nucleic Acid Ligand C1q inhibitor in an amount effective to inhibit activation of the Complement System.

* * * * *