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(54) **Title:** NOVEL FAS RNAi THERAPEUTICS AND USES THEREOF

(57) **Abstract:** The present invention relates to novel therapeutic compounds, known as RNAi agents, that decrease expression of the FAS receptor (expressed by the FAS gene), thereby decreasing expression of FAS mRNA and protein expression. Such RNAi agents are useful in the treatment of diseases involving the regulation of FAS expression and function, such as autoimmune hepatitis.

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NOVEL FAS RNAi THERAPEUTICS AND USES THEREOF

BACKGROUND

[001] The present invention relates to novel therapeutic compounds, known as RNAi agents, that decrease expression of the FAS (expressed by the FAS gene), thereby decreasing expression of FAS mRNA and FAS protein. Such RNAi agents are useful in the treatment of diseases involving the regulation of FAS expression and function, such as autoimmune hepatitis.

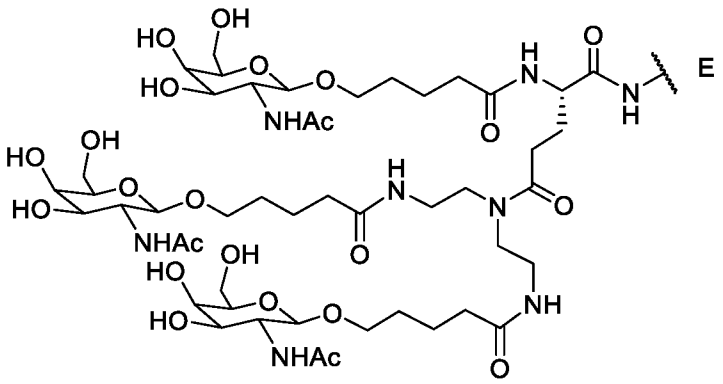
[002] FAS, the Fas cell death receptor, and its ligand, FASL, are members of the TNFR superfamily. Binding of FASL to FAS results in downstream death-inducing signaling involving caspases (e.g., caspase 8 and 10) and Fas-associated death domain protein (FADD), which form a complex. Caspase autoproteolysis in the complex results in caspase cascade, and leads to apoptosis. NF-kappaB, MAPK3/ERK1, and MAPK8/JNK are also known to be activated by FAS signaling, and such activation is thought to result in proliferation in normal diploid fibroblast and T cells. These play an important role in regulation of the immune response involving cells that express FAS, which includes hepatocytes.

[003] Autoimmune hepatitis (AIH) is a chronic inflammation of the liver with no identifiable cause such as a viral infection. AIH patients have increased FAS levels in hepatocytes. Genetics, the environment (such as an environmental trigger), and native immune system dysregulation are thought to play a part in the progression of the disease from inflammation to liver fibrosis. AIH often first presents as patients reach their teen years.

[004] Treatment options are limited and include high dose steroid treatment, often in combination with azathioprine, another immunosuppressive agent. Treatment is correlated with a downregulation of FAS and patients can achieve near remission of inflammation biochemically. However, steroid treatment, especially when taken long term and/or in high doses, can cause a wide range of serious side effects, as can other immunosuppressive agent treatment such azathioprine. Serious side effects can include onset of diabetes, thinning bones (osteoporosis), broken bones (osteonecrosis), high blood pressure, cataracts, glaucoma, and weight gain. If treatment is removed, patients often experience recurrence, and some patients experience disease progression that requires a liver transplant. Accordingly, there is a need for improved treatments for AIH.

SUMMARY OF INVENTION

[005] In one aspect, provided herein are RNAi agents for reducing FAS gene expression, wherein the RNAi agent comprises a delivery moiety of Formula I conjugated to R, wherein R is a double stranded RNA (dsRNA) comprising an antisense strand and a sense strand:



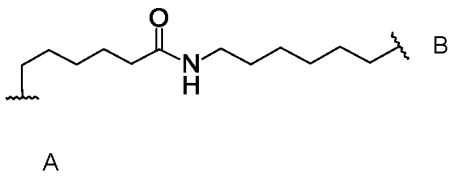
Formula I,

wherein R is conjugated to connection point E of Formula I, optionally via a linker, wherein the sense strand and the antisense strand form a duplex region, and wherein the antisense strand comprises a region of complementarity to a FAS mRNA target sequence of SEQ ID NO: 1, and wherein the sense and antisense strand each optionally comprise one or more modified nucleotides and one or more modified internucleotide linkages. In some embodiments, Formula I is conjugated to the sense strand, optionally via a linker. In some embodiments, Formula I is conjugated to the 3' terminal nucleotide of the sense strand, optionally via a linker.

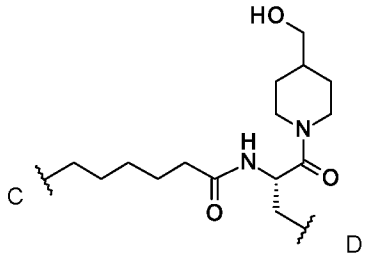
[006] In some embodiments, the antisense strand is 15 to 50 nucleotides in length. In some embodiments, the sense strand is 15 to 50 nucleotides in length. In some embodiments, the antisense strand is between 18 and 23 nucleotides in length. In some embodiments, the sense strand is between 18 and 21 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 21 nucleotides in length.

[007] In some embodiments, the sense strand or the antisense strand comprises a sequence selected from Table 2, 3A, 3B, 4A, 4B, 7, or 8 disclosed herein. In some embodiments, the sense strand and the antisense strand comprises a sequence selected from Table 2, 3A, 3B, 4A, 4B, 7, or 8 disclosed herein.

[008] In some embodiments, R is conjugated to Formula I via a linker. In further embodiments, the linker comprises a linker of Formula II having connection points A and B or the linker comprises Formula III having connection points C and D, and wherein:



Formula II;



Formula III;

a. Formula I conjugated to Formula II at connection point A and Formula II is conjugated to a phosphate group at connection point B, and the phosphate group is further conjugated to R; or

b. Formula I conjugated to Formula III at connection point C and Formula III is conjugated to a phosphate group at connection point D, and the phosphate group is further conjugated to R.

[009] In another aspect, provided herein are pharmaceutical composition comprising the FAS RNAi agent described herein and one or more pharmaceutically acceptable excipients.

[0010] In another aspect, provided herein are methods of treating autoimmune hepatitis (AIH) in a patient in need thereof, comprising administering to the patient a FAS RNAi agent or pharmaceutical composition thereof described herein.

[0011] In another aspect, provided herein are FAS RNAi agent for use in a therapy. Also provided herein are FAS RNAi agent for use in in the treatment of AIH. Also provided herein are uses of FAS RNAi agent in the manufacture of a medicament for the treatment of AIH.

DETAILED DESCRIPTION

[0012] FAS siRNAs and ASOs have been described, but none have progressed for treatment in patients, including for the treatment of AIH. Using the FAS RNAi agents herein to decrease expression of FAS can be employed to treat AIH in patients in need thereof. Such siRNAs may exhibit one or more of, e.g., as compared to other liver targeted siRNAs such as FAS siRNAs comprising a different delivery ligand, a different sequence, a differently modified sequence, or as compared to treatment with a vehicle control: improved knockdown in the liver; improved tissue exposure, improved exposure in liver hepatocytes; an improved durable response; an improved pharmacokinetic profile; fewer off target effects; and/or an improved toxicity profile. Other embodiments of the FAS RNAi agents herein may include one or more of fewer side effects as compared to steroids or other standard of care; an improved toxicity profile; an improved safety profile; improved tolerability or compliance; and/or improved liver function tests. Still other siRNAs herein may have other benefits, e.g., in combination with any of the preceding or as a stand-alone benefit, including improved and/or simplified synthesis, synthetic processes with fewer degradation products; or any combination thereof.

[0013] The RNAi agents herein comprise a sense strand and an antisense strand, wherein each is an oligonucleotide. In some embodiments, the RNAi agent described herein also comprise a delivery moiety. As used herein, “nucleotide” means an organic compound having a nucleoside (a nucleobase such as, for example, adenine, cytosine, guanine, thymine, or uracil; and a pentose sugar such as, for example, ribose or 2'-deoxyribose) and a phosphate group. A “nucleotide” can serve as a monomeric unit of nucleic acid polymers such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

[0014] As used herein, “oligonucleotide” means a short nucleic acid compound (e.g., less than about 100 nucleotides in length). An oligonucleotide may be single-stranded (ss) or double stranded (ds). An oligonucleotide may or may not have duplex regions. As a set of non-limiting examples, an oligonucleotide may be, but is not limited to, a small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), Dicer substrate interfering RNA (DsiRNA), or antisense oligonucleotide (ASO).

[0015] As used herein, “ribonucleotide” means a nucleotide having a ribose as its pentose sugar, which contains a hydroxyl group at its 2' position. A modified ribonucleotide is a ribonucleotide having one or more modifications or substitutions of atoms other than

hydrogen at the 2' position, including modifications or substitutions in or of the nucleobase, sugar, or phosphate group.

[0016] As used herein, "modified internucleotide linkage" means an internucleotide linkage having one or more chemical modifications when compared with a reference internucleotide linkage having a phosphodiester bond. A modified internucleotide linkage can be a non-naturally occurring linkage.

[0017] As used herein, "modified nucleotide" refers to a nucleotide having one or more chemical modifications when compared with a corresponding reference nucleotide selected from: adenine ribonucleotide, guanine ribonucleotide, cytosine ribonucleotide, uracil ribonucleotide, adenine deoxyribonucleotide, guanine deoxyribonucleotide, cytosine deoxyribonucleotide, and thymidine deoxyribonucleotide. A modified nucleotide can be a non-naturally occurring nucleotide. A modified nucleotide can have, for example, one or more chemical modification in its sugar, nucleobase, and/or phosphate group. Additionally, or alternatively, a modified nucleotide can have one or more chemical moieties conjugated to a corresponding reference nucleotide.

[0018] The term "percentage sequence identity" with respect to a reference nucleic acid sequence is defined as the percentage of nucleotides, nucleosides, or nucleobases in a candidate sequence that are identical with the nucleotides, nucleosides, or nucleobases in the reference nucleic acid sequence, after optimally aligning the sequences and introducing gaps or overhangs, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software programs, for example, those described in Current Protocols in Molecular Biology (Ausubel et al., eds., 1987, Supp. 30, section 7.7.18, Table 7.7.1), and including BLAST, BLAST-2, ALIGN, Clustal W2.0 or Clustal X2.0 or Megalign (DNASTAR) software. In one embodiment herein, sequence identity is calculated use Clustal W2.0 or Clustal X2.0. In another embodiment, sequence identity is calculated using Clustal W2.0. In another embodiment, sequence identity is calculated using Clustal X2.0. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Percentage of "sequence identity" can be determined by comparing two optimally aligned sequences over a comparison window,

where the fragment of the nucleic acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage can be calculated by determining the number of positions at which the identical nucleotide, nucleoside, or nucleobase occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. The output is the percent identity of the subject sequence with respect to the query sequence. In some embodiments, percent sequence identity is the percent of nucleotide residues that are identical between two strands using the PID3 calculation, which is the number of identical nucleotide residues divided by the total number of nucleotides of the shortest of the two sequences, multiplied by 100. See, e.g., Raghava, G., Barton, G.J. Quantification of the variation in percentage identity for protein sequence alignments. BMC Bioinformatics 7, 415 (2006).

[0019] As used herein, "phosphate analog" means a chemical moiety that mimics the electrostatic and/or steric properties of a phosphate group. In some embodiments, a phosphate analog is positioned at the 5' terminal nucleotide of an oligonucleotide in place of a 5'-phosphate. A 5' phosphate analog can include a phosphatase-resistant linkage. Examples of phosphate analogs include, but are not limited to, 5' phosphonates, such as 5' methylene phosphonate (5'-MP) and 5'-(E)-vinylphosphonate (5'-VP). An oligonucleotide can have a phosphate analog at a 4'-carbon position of the sugar (referred to as a "4'-phosphate analog") at a 5'-terminal nucleotide. An example of a 4'-phosphate analog is oxymethylphosphonate, in which the oxygen atom of the oxymethyl group is bound to the sugar moiety (e.g., at its 4'-carbon) or analog thereof. See, e g., Intl. Patent Application Publication No. WO 2018/045317. Other modifications have been developed for the 5' end of oligonucleotides (see, e.g., Intl. Patent Application No. WO 2011/133871; US Patent No. 8,927,513; and Prakash et al. (2015) Nuc. Acids Res. 43:2993-3011).

[0020] As used herein, "region of complementarity" means a nucleotide sequence of a nucleic acid (e.g., a double stranded oligonucleotide) that is sufficiently complementary to an antiparallel nucleotide sequence to permit hybridization between the two sequences of nucleotides under appropriate hybridization conditions (e.g., in a phosphate buffer, in a

cell, etc.). In some embodiments, an oligonucleotide herein includes a targeting sequence having a region of complementary to a mRNA target sequence.

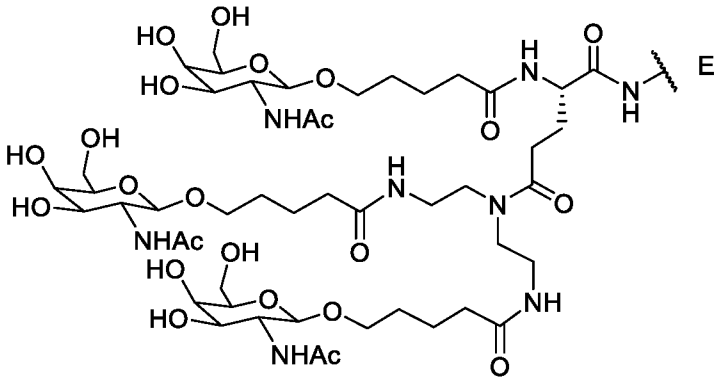
[0021] As used herein, "duplex," in reference to nucleic acids or oligonucleotides, such as a sense strand or an antisense strand means a structure formed through hydrogen bonds of complementary base pairing of two antiparallel sequences of nucleotides under suitable conditions to promote such a structure. A duplex may form despite not having full complementarity between the two strands, or when an abasic nucleotide is present.

[0022] RNA interference is a specialized cellular process that utilizes RISC for degrading RNA in a sequence dependent manner. As used herein, "RNAi agent" means an agent comprising either (a) a double stranded oligonucleotide having a sense strand (passenger) and antisense strand (guide), in which the antisense strand or part of the antisense strand is used by the Argonaute 2 (Ago2) endonuclease in the cleavage of a target mRNA or (b) a single stranded oligonucleotide having a single antisense strand, where that antisense strand (or part of that antisense strand) is used by the Ago2 endonuclease in the cleavage of a target mRNA. In some embodiments, the RNAi agent described herein also comprise a delivery moiety.

[0023] As used herein, "treatment" or "treating" refers to all processes wherein there may be a slowing, controlling, delaying, or stopping of the progression of the disorders or disease disclosed herein, or ameliorating disorder or disease symptoms, and need not indicate a total elimination of all disorder or disease symptoms. Treatment includes administration of an RNAi agent or pharmaceutical composition thereof for treatment of a disease or condition in a mammal including a human.

[0024] An "effective amount" refers to an amount necessary (for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount of a RNAi agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the RNAi agent to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the RNAi agent are outweighed by the therapeutically beneficial effects.

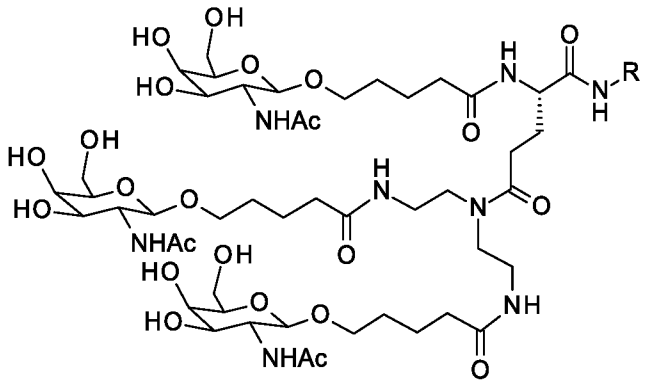
[0025] Provided herein are RNAi agents for reducing FAS gene expression, wherein the RNAi agent comprises a delivery moiety of Formula I conjugated to R, wherein R is a double stranded RNA (dsRNA) comprising an antisense strand and a sense strand:



Formula I,

wherein R is conjugated to connection point E of Formula I, optionally via a linker, wherein the sense strand and the antisense strand form a duplex region, and wherein the antisense strand comprises a region of complementarity to a FAS mRNA target sequence of SEQ ID NO: 1, and wherein the sense and antisense strand each optionally comprise one or more modified nucleotides and one or more modified internucleotide linkages.

[0026] Also provided here are RNAi agents for reducing FAS gene expression, wherein the RNAi agent comprises a delivery moiety of Formula Ia conjugated to R, wherein R comprises an antisense strand and a sense strand:



Formula Ia,

wherein R is optionally conjugated to Formula Ia via a linker, wherein the sense strand and the antisense strand form a duplex region, and wherein the antisense strand comprises a region of complementarity to a FAS mRNA target sequence of SEQ ID NO: 1, and wherein the sense and antisense strand each optionally comprise one or more modified nucleotides and one or more modified internucleotide linkages.

[0027] Disclosed herein are RNAi agents for reducing FAS gene expression, wherein the RNAi agents comprise a dsRNA comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, and wherein the antisense strand comprises a region of complementarity of at least 15 nucleotides to the sequence as set forth in SEQ ID NO: 1, and wherein the sense strand and/or the antisense strand each optionally comprise one or more modified nucleotides and/or modified internucleotide linkages. In further embodiments, the antisense strand comprises at least 15 nucleotides of a sequence in Table 2. In further embodiments, the antisense strand comprises at least 18 nucleotides of a sequence in Table 2. In further embodiments, the RNAi agent reduces FAS gene expression by about 50% or greater in a cell expressing FAS, as compared to a control. In further embodiments, the RNAi agent reduces FAS gene expression by reducing the level of FAS mRNA transcript, the level of FAS protein, or both.

[0028] In further embodiments, the antisense strand is 15 to 50 nucleotides in length, and/or the sense strand is 15 to 50 nucleotides in length. In further embodiments, the sense and/or sense strand is independently 15 to 30 nucleotides in length. In further embodiments, the antisense strand is between 18 and 23 nucleotides in length. In further embodiments, the sense strand is between 18 and 21 nucleotides in length.

[0029] In further embodiments, the RNAi agent comprises an antisense strand that comprises at least 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2-112. In still further embodiments, the antisense strand comprises at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2-112.

[0030] In other further embodiments, the antisense strand comprises at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 224 to 334, 337, 338, 573, and 577.

[0031] In other further embodiments, the sense strand comprises at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 113 to 223, 335, 336, 572, and 576.

[0032] In further embodiments, the antisense strand of the RNAi agent is 23 nucleotides in length. In still further embodiments, the sense strand is 21 nucleotides in length. In

another embodiment, the sense and antisense strand comprise a sequence selected from the sequences set forth in Table 3A.

[0033] The sense strand and the antisense strand of the RNAi agents disclosed herein do not require full complementarity. Accordingly, in the RNAi agents disclosed herein, the duplex region between the sense strand and the antisense strand comprises 0, 1, 2, or 3 mismatches between the sense strand and the antisense strand. In further embodiments, the duplex region between the sense strand and the antisense strand consists of 0, 1, 2, or 3 mismatches between the sense strand and the antisense strand.

[0034] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 129, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 240;
b. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 116, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 227;
c. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 151, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 262;
d. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 128, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 239; and
e. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 155, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 266.

[0035] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 129, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 240;
b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 116, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 227;
c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 151, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 262;
d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 128, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 239; and
e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 155, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 266.

[0036] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence comprises SEQ ID NO: 129, and the second nucleic acid sequence comprises SEQ ID NO: 240;
b. the first nucleic acid sequence comprises SEQ ID NO: 116, and the second nucleic acid sequence comprises SEQ ID NO: 227;
c. the first nucleic acid sequence comprises SEQ ID NO: 151, and the second nucleic acid sequence comprises SEQ ID NO: 262;
d. the first nucleic acid sequence comprises SEQ ID NO: 128, and the second nucleic acid sequence comprises SEQ ID NO: 239; and
e. the first nucleic acid sequence comprises SEQ ID NO: 155, and the second nucleic acid sequence comprises SEQ ID NO: 266.

[0037] In further embodiments, the sense strand and the antisense strand each independently comprise one or more modified nucleotides, such as 2' fluoro modified nucleotides or 2'-O-methyl modified nucleotides. In still further embodiments of the RNAi

agents disclosed herein, each nucleotide of the sense strand and each nucleotide of the antisense strand is a modified nucleotide. In further embodiments, each nucleotide is a 2' fluoro modified nucleotide or a 2'-O-methyl modified nucleotide.

[0038] In further embodiments of the RNAi agents disclosed herein, the antisense strand is 23 nucleotides in length, each nucleotide of the antisense strand is a modified nucleotide, and 2' fluoro modified nucleotides are present at

a. Positions 2, 3, 7, 14, and 16 from the 5' end of the antisense strand; or
b. Positions 2, 5, 7, 14, and 16 from the 5' end of the antisense strand; or
c. Positions 2, 3, 8, 14, and 16 from the 5' end of the antisense strand; or
d. Positions 2, 5, 8, 14, and 16 from the 5' end of the antisense strand; or
e. Positions 2, 6, 14, and 16 from the 5' end of the antisense strand.

In further embodiments, the nucleotides that are not 2' fluoro modified nucleotides are 2'-O-methyl modified nucleotides.

[0039] In further embodiments of the RNAi agents disclosed herein, the sense strand and antisense strand each independently comprise one or more modified internucleotide linkages, and each modified internucleotide linkage is a phosphorothioate linkage. In further embodiments, the sense strand and antisense strand each independently comprise four phosphorothioate linkages. In still further embodiments, the two terminal nucleotides at each of the 5' and 3' ends of each of the sense and antisense strand are phosphorothioate linkages.

[0040] In other embodiments, the 5' nucleotide of the antisense strand comprises a phosphate group or a phosphate analog. As used herein, "phosphate analog" means a chemical moiety that mimics the electrostatic and/or steric properties of a phosphate group. In some embodiments, a phosphate analog is positioned at the 5' terminal nucleotide of an oligonucleotide in place of a 5'-phosphate. A 5' phosphate analog can include a phosphatase-resistant linkage. Examples of phosphate analogs include, but are not limited to, 5' phosphonates, such as 5' methylene phosphonate (5'-MP) and 5'-(E)-vinylphosphonate (5'-VP). An oligonucleotide can have a phosphate analog at a 4'-carbon position of the sugar (referred to as a "4'-phosphate analog") at a 5'-terminal nucleotide. An example of a 4'-phosphate analog is oxymethylphosphonate, in which the oxygen atom of the oxymethyl group is bound to the sugar moiety (e.g., at its 4'-carbon) or analog

thereof. See, e g., Intl. Patent Application Publication No. WO 2018/045317. Other modifications have been developed for the 5' end of oligonucleotides (see, e.g., Intl. Patent Application No. WO 2011/133871; US Patent No. 8,927,513; and Prakash et al. (2015) Nuc. Acids Res. 43:2993-3011).

[0041] In further embodiments of the RNAi agents disclosed herein, the antisense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 563, 566, 567, 569, 570, 571, 575, 579, 581, 583, 585, or a sequence having at least 90% sequence identity thereto, wherein the 5’ terminal nucleotide of the antisense strand comprises a vinyl phosphonate, a phosphate, or a hydroxyl group. In other embodiments, the phosphate group listed at the 5’ end of the recited SEQ ID NO: is removed and replaced with an OH. In other embodiments, the phosphate group listed at the 5’ end of the recited SEQ ID NO: is replaced with a 5’ vinylphosphonate.

[0042] In further embodiments of the RNAi agents disclosed herein, the antisense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 813, 815, 817, 819, or a sequence having at least 90% sequence identity thereto.

[0043] In further embodiments the sense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467,

469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 564, 565, 568, 574, 578, 580, 582, 584, or a sequence having at least 90% sequence identity thereto.

[0044] In further embodiments the sense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 809, 810, 811, 812, 814, 816, 818, or a sequence having at least 90% sequence identity thereto.

[0045] In still further embodiments of the RNAi agents disclosed herein, the sense strand and antisense strand are a pair of oligonucleotide sequences selected from Table 4A or 4B, or a sequence that is at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to the sequence in Table 4A or 4B. In further embodiments, 1, 2, or 3 mismatches are introduced into the sense strand of the pair in Table 4A, Table 4B or Table 7. In further embodiments, 1, 2, or both terminal nucleotides of 5' end of the antisense strand are changed.

[0046] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 339, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 340;
b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 341, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 342;

c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 343, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 344;
d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO:345, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 346;
e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 347, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 348;
f. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 349, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 350;
g. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 353, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 354;
h. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 363 and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 364; and
i. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 381, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 382.

[0047] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 564 or 809, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 571;
b. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 568 or 811, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 567;

c. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 580 or 814, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 582 or 816, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 584 or 818, and the second nucleic acid sequence has at least 90% sequence identity to SEQ ID NO: 585 or 819.

[0048] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 564 or 809, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 571;
b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 568 or 811, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 567;
c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 580 or 814, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 582 or 816, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 584 or 818, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 585 or 819.

[0049] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence,

wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

a. the first nucleic acid sequence comprises SEQ ID NO: 564 or 809, and the second nucleic acid sequence comprises SEQ ID NO: 571;
b. the first nucleic acid sequence comprises SEQ ID NO: 568 or 811, and the second nucleic acid sequence comprises SEQ ID NO: 567;
c. the first nucleic acid sequence comprises SEQ ID NO: 580 or 814, and the second nucleic acid sequence comprises SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence comprises SEQ ID NO: 582 or 816, and the second nucleic acid sequence comprises SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence comprises SEQ ID NO: 584 or 818, and the second nucleic acid sequence comprises SEQ ID NO: 585 or 819.

[0050] In some embodiments, the RNAi agent comprises a sense strand comprising a first nucleic acid sequence, and an antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:

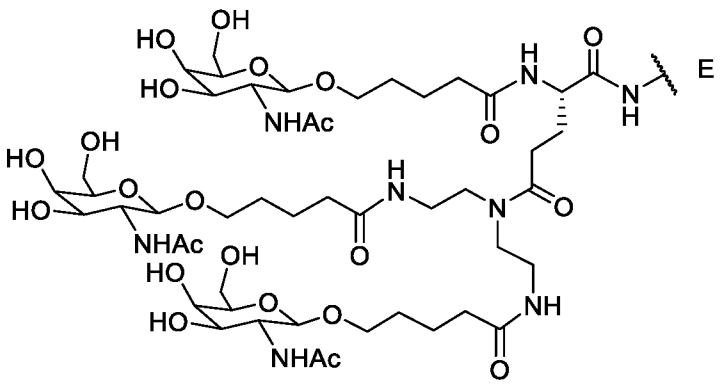
a. the first nucleic acid sequence consists of SEQ ID NO: 564 or 809, and the second nucleic acid sequence consists of SEQ ID NO: 571;
b. the first nucleic acid sequence consists of SEQ ID NO: 568 or 811, and the second nucleic acid sequence consists of SEQ ID NO: 567;
c. the first nucleic acid sequence consists of SEQ ID NO: 580 or 814, and the second nucleic acid sequence consists of SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence consists of SEQ ID NO: 582 or 816, and the second nucleic acid sequence consists of SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence consists of SEQ ID NO: 584 or 818, and the second nucleic acid sequence consists of SEQ ID NO: 585 or 819.

[0051] In further embodiments, the 5' terminal nucleotide of the antisense strand is substituted such that the final sequence contains a vinylphosphonate, a phosphate group, or an OH group. For example, for antisense sequences of SEQ ID NOs: 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382,

384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 563, 566, 567, 569, 570, 571, 575, 579, 581, 583, 585, or a sequence having at least 90% sequence identity thereto, the 5' phosphate group is replaced with an OH group.

[0052]

[0053] In other embodiments disclosed herein are RNAi agents having a delivery moiety of Formula I conjugated to R:

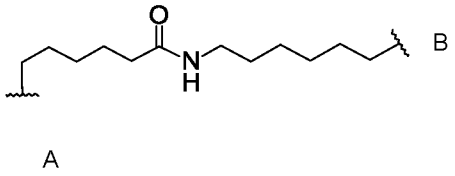


Formula I,

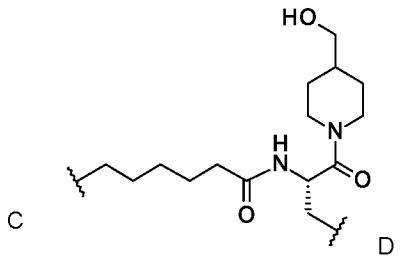
wherein R is a dsRNA comprises a sense strand and an antisense strand, wherein the antisense strand comprises at least 15 contiguous nucleotides that have complementarity to FAS mRNA target sequence of SEQ ID NO:1, and wherein the sense strand and the antisense strand form a region of complementarity of at least 15 nucleotides, and wherein the sense strand and antisense strand are each independently 18 to 23 nucleotides in length, and optionally wherein the sense strand and antisense strand each independently comprise one or more modified nucleotides, and optionally wherein the sense strand and the antisense strand each independently comprise one or more modified internucleotide linkages, and wherein R is optionally conjugated to Formula I via a linker. In further embodiments, the sense or the antisense strand is selected from Table 2, 3A, 3B, 4A, 4B, 7, or 8 disclosed herein. In other embodiments, the antisense or antisense strand of the

RNAi agent has a sequence of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the corresponding sequence selected from Table 2, 3A, 3B, 4A, 4B, 7, or 8 herein.

[0054] In other embodiments, the RNAi agent disclosed herein comprises a linker. In further embodiments, R is conjugated to Formula I via a linker. In other further embodiments R is conjugated to Formula I via a linker. In further embodiments, the linker comprises a linker of Formula II having connection points A and B or the linker comprises Formula III having connection points C and D, and wherein:



Formula II;

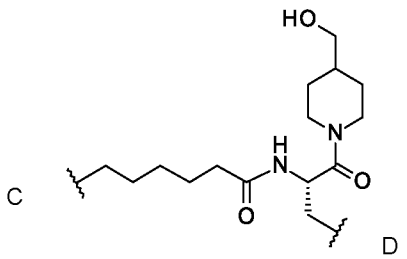


Formula III;

a. Formula I is conjugated to Formula II at connection point A and Formula II is conjugated to a phosphate group at connection point B, and the phosphate group is conjugated to R; or

b. Formula I is conjugated to Formula III at connection point C and Formula III is conjugated to a phosphate group at connection point D, and the phosphate group is further conjugated to R.

[0055] In other embodiments wherein the RNAi agent comprises a linker, R is conjugated to Formula I via a linker, and the linker is a linker comprising Formula III having connection points C and D:



Formula III;

and wherein Formula I is conjugated to Formula III at connection point C and Formula III is conjugated to a phosphate group at connection point D, and the phosphate group is further conjugated to R.

[0056] The sense strand and antisense strand of FAS RNAi agent can be synthesized using any nucleic acid polymerization methods known in the art, for example, solid-phase synthesis by employing phosphoramidite chemistry methodology (e.g., Current Protocols in Nucleic Acid Chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA), H-phosphonate, phosphortriester chemistry, or enzymatic synthesis. Automated commercial synthesizers can be used, for example, MerMade™ 12 from LGC Biosearch Technologies, or other synthesizers from BioAutomation or Applied Biosystems. Phosphorothioate linkages can be introduced using a sulfurizing reagent such as phenylacetyl disulfide or DDTT (((dimethylaminomethylidene) amino)-3H-l,2,4-dithiazaoline-3-thione). It is well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products to synthesize modified oligonucleotides.

[0057] In still other embodiments, the RNAi agent is capable of decreasing expression of the FAS gene in a liver cell. In other embodiments, the RNAi agents disclosed herein are for use in therapy. In further embodiments, the use is for the treatment of AIH.

[0058] The RNAi agents may be formulated into pharmaceutical compositions. Accordingly, disclosed herein are pharmaceutical compositions comprising the RNAi agent disclosed herein, and one or more pharmaceutically acceptable excipients. Pharmaceutical compositions can be prepared by methods well known in the art (e.g., Remington: The Science and Practice of Pharmacy, 23rd edition (2020), A. Loyd et al., Academic Press).

[0059] In other embodiment are uses of the RNAi agents herein for the manufacture of a medicament for the treatment of AIH.

[0060] In other embodiments are methods of treating AIH, in patients in need thereof, comprising administering a FAS RNAi agent disclosed herein, or a pharmaceutical composition thereof.

[0061] The RNAi agent can be administered to the patient intravenously or subcutaneously.

[0062] RNAi dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

[0063] Dosage values may vary with the type and severity of the condition to be alleviated. It is further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

[0064] In other embodiments are methods of decreasing FAS expression in a cell, comprising contacting the cell with an RNAi agent disclosed herein, and incubating the cell for a time sufficient for decreasing the level of FAS mRNA by at least 50% as compared to an untreated or control treated cell.

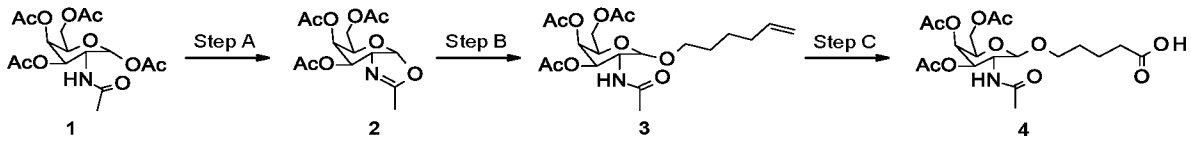
EXAMPLES

[0065] Certain abbreviations are defined as follows: "1,2-DCE" refers to 1,2-dichloroethane; "DCM" refers to dichloromethane; "DIEA" refers to N,N-diisopropylethylamine; "DMF" refers to N,N-dimethylformamide; "DMAP" refers to 4-dimethylaminopyridine; "DMTCl" refers to 4,4'-dimethoxytrityl chloride; "DPP4" refers to dipeptidyl peptidase; "EDC" refers to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; "EtOAc" refers to ethyl acetate; "GalNAc" refers to N-acetylgalactosamine; "HATU" refers to 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; "HBTU" refers to O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate; "HOBt" refers to 1-hydroxybenzotriazole hydrate; "HPRT" refers to hypoxanthine-guanine phosphoribosyltransferase; "IPA" refers to isopropanol and isopropyl alcohol; "LDHA" refers to lactate dehydrogenase-A;

"MeCN" refers to acetonitrile; "MeOH" refers to methanol and methyl alcohol; "MWCO" refers to molecular weight cut-off; "NHS" refers to N-hydroxysuccinimide; "OD" refers to optical density; "PBS" refers to phosphate-buffered saline; "PhSiH3" refers to phenylsilane; "PTS" refers to portable endotoxin testing system; "siRNA" refers to small interfering ribonucleic acid; "TEA" refers to triethylamine; "TFA" refers to trifluoroacetic acid; "THF" refers to tetrahydrofuran; "TLC" refers to thin line chromatography; and "TMP" refers to 2,2,6,6-tetramethylpiperidine.

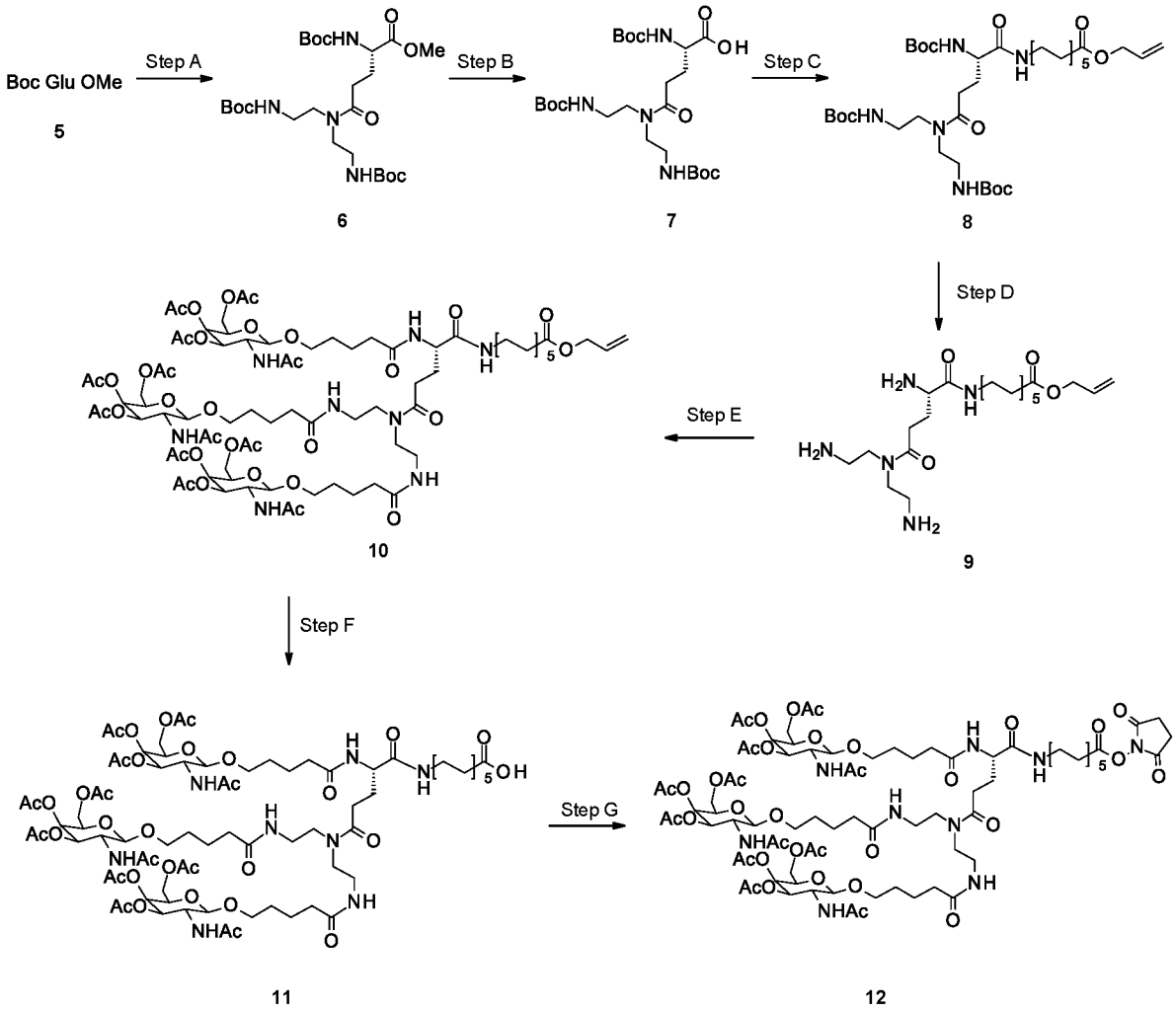
[0066] A delivery moiety comprising Formula I may be made by the following nonlimiting synthetic steps and schemes.

Scheme 1



[0067] Scheme 1, step A, depicts the cyclization of compound (1) using trimethyl trifluoromethanesulfonate in a solvent such as 1,2-DCE to give compound (2). Step B shows the addition of hex-5-en-1-ol to compound (2) using trimethylsilyl trifluoromethanesulfonate in a solvent such as 1,2-DCE to give compound (3). The oxidation of compound (3) using an appropriate oxidizing agent such as sodium periodate with a catalyst such as ruthenium(III) chloride to give compound (4) is shown in step C.

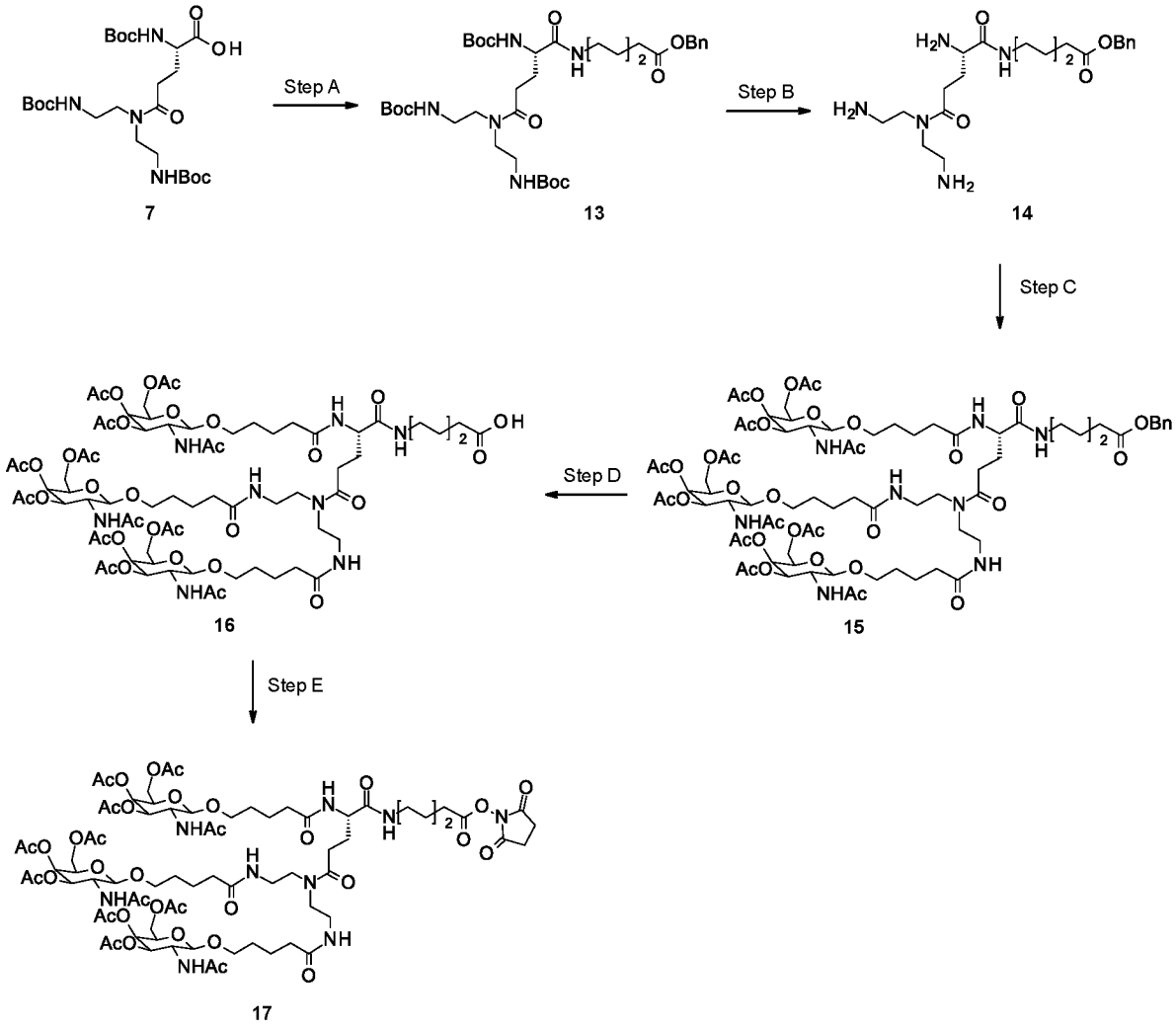
Scheme 2



[0068] Scheme 2, step A, shows an amide coupling between compound (5) and tert-butyl N-[2-[2-(tert-butoxycarbonylamino)ethylamino]ethyl]carbamate using HBTU and HOBt with an appropriate base such as DIEA in a solvent such as DMF to give compound (6). Step B depicts a basic hydrolysis of compound (6) using a base such as aqueous NaOH in a THF and MeOH solvent system to give compound (7). Step C shows an amide coupling between compound (7) and allyl 11-aminoundecanoate hydrochloride using HATU with an appropriate base such as DIEA in a solvent such as DMF to give compound (8). Step D shows the acidic deprotection of compound (8) with TFA in a solvent such as DCM to give compound (9). The amide coupling between compound (9) and compound (4) using EDC and HOBt in a solvent such as DCM to give compound (10)

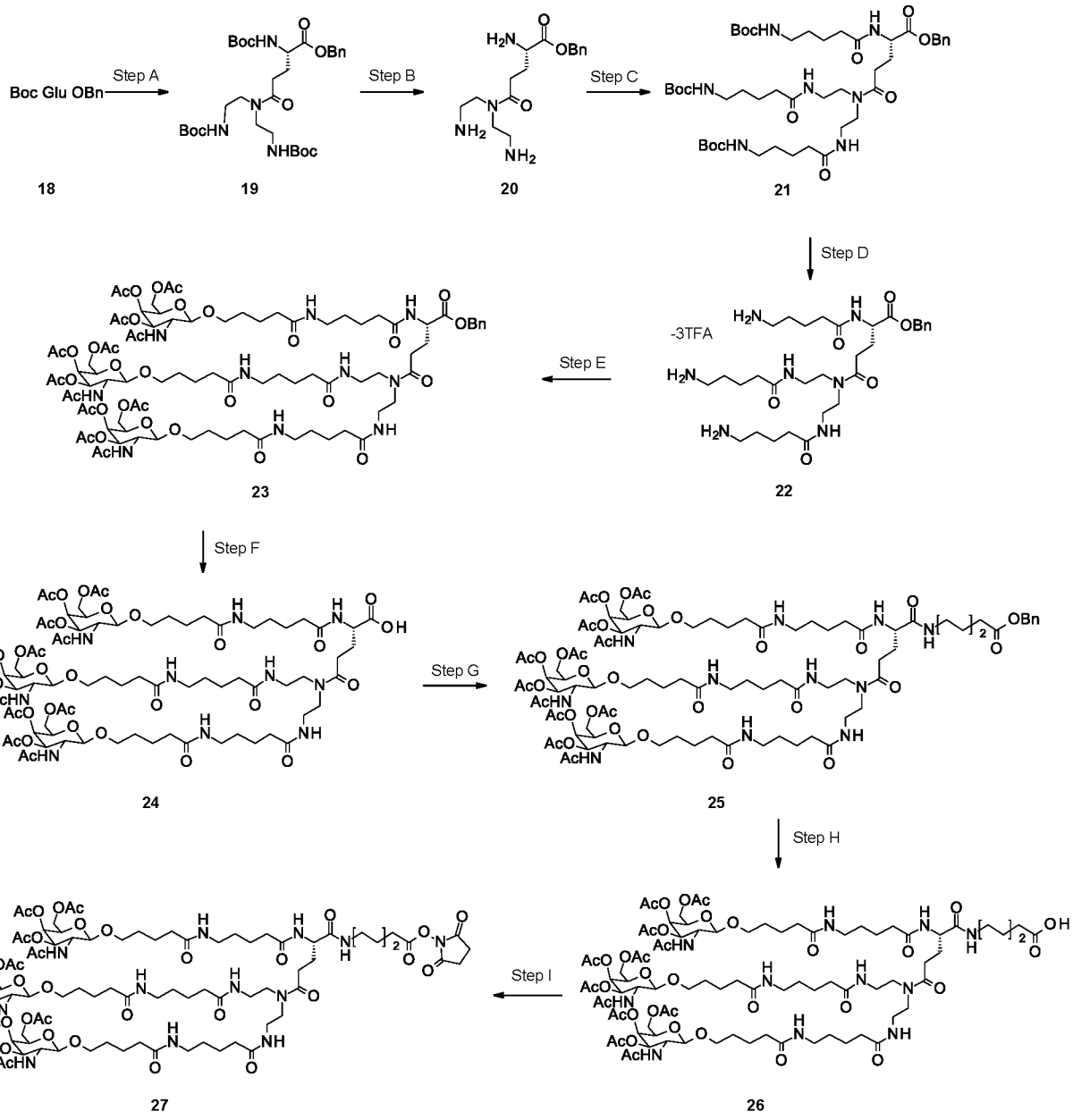
is shown in step E. Step F shows the deprotection of compound (10) with tetrakis(triphenylphosphine)palladium and PhSiH3 in a solvent such as DCM to give compound (11). Step F depicts the coupling of compound (11) with NHS using EDC in a solvent such as DCM to give compound (12).

Scheme 3



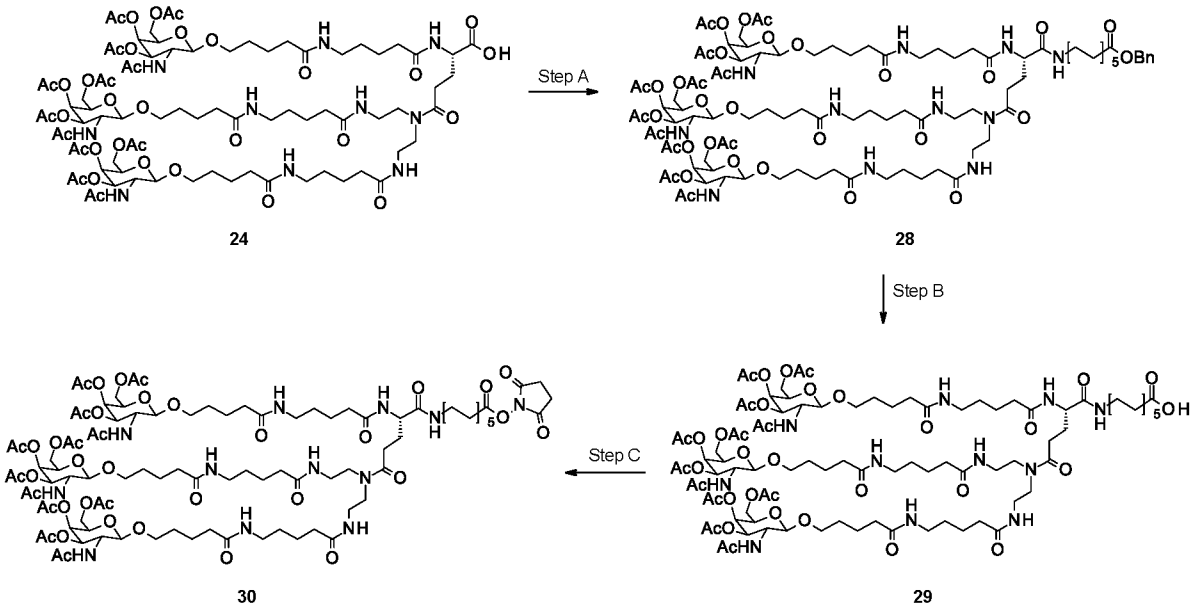
[0069] Scheme 3, steps A-C are essentially analogous to those of scheme 2, steps C-E beginning with compound (7) to give compounds (13), (14), and (15). Step D depicts the hydrogenation of compound (15) using palladium on carbon in a solvent such as MeOH to give compound (16). Step E is essentially analogous to the preparation of scheme 2, step G to give compound (17).

Scheme 4



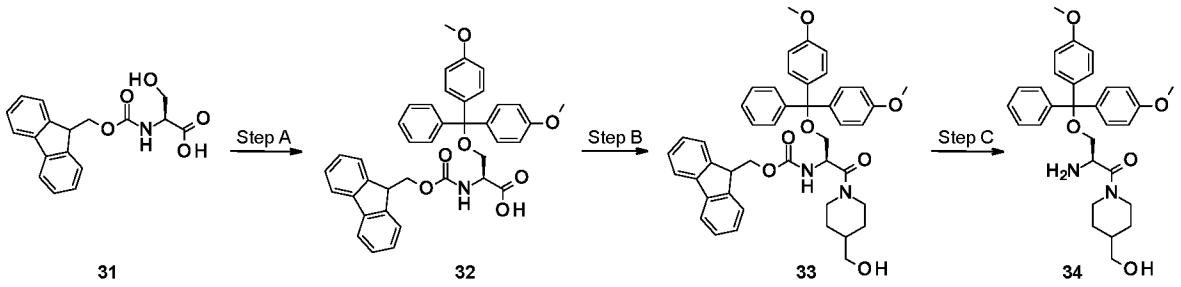
[0070] Scheme 4, steps A-I, are composed of a series of amide couplings and deprotections using methods essentially analogous to those found in schemes 2 and 3 beginning with compound (18) to give compound (27).

Scheme 5



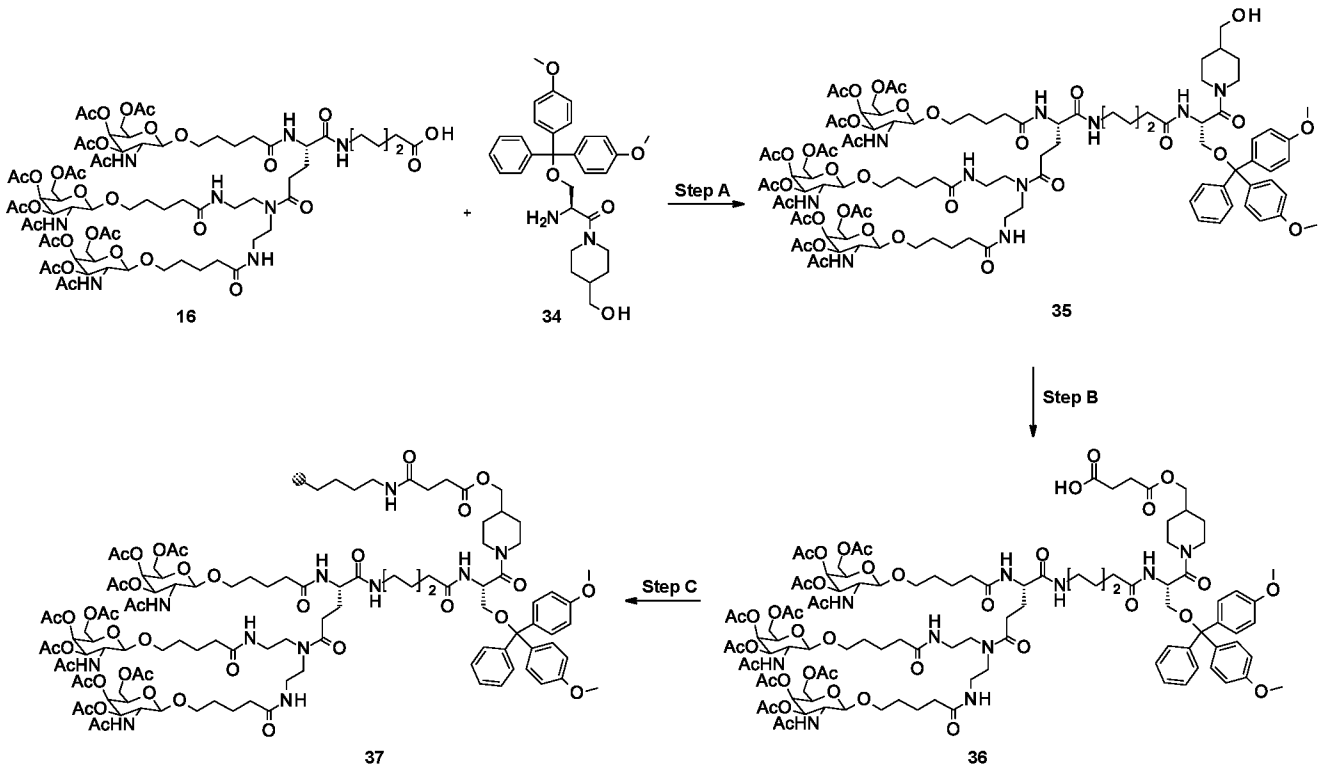
[0071] Scheme 5, steps A-C depict methods essentially analogous to those found in scheme 4, steps G-I beginning with compound (24) to give compound (30).

Scheme 6



[0072] Scheme 6, step A depicts the protection of compound (31) using DMTCl with a suitable base such as DIEA in a solvent such as DCM to give compound (32). Step B shows an amide coupling between compound (32) and piperidin-4-yl methanol using HBTU and HOBt with TMP in a solvent such as DCM to give compound (33). The deprotection of compound (33) with 20% piperidine in DMF to give compound (34) is shown in step C.

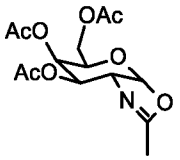
Scheme 7



[0073] Scheme 7, step A is essentially analogous to scheme 2, step A to give compound (35) from the coupling of compounds (16) and (34). Step B shows the formation of compound (36) by adding succinic anhydride to compound (35) in an appropriate solvent such as DCM with a base system of TEA and DMAP. Step C depicts the loading of compound (36) onto resin with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and a base such as DIEA in a solvent system such as MeCN and DCM to give compound (37).

Preparation 1

(6,7-Diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]oxazol-5-yl)methyl acetate



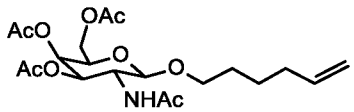
[0074] To a solution of (5-acetamido-3,4,6-triacetoxy-tetrahydropyran-2-yl)methyl acetate (9.00 g, 23.1 mmol) in 1,2-DCE (46 mL) is added trimethylsilyl

trifluoromethanesulfonate (6.5 mL, 35 mmol). The mixture is heated to 50 °C and stirred for 18 hours. After this time, the mixture is diluted with DCM (200 mL), washed with saturated NaHCO3 (200 mL), and saturated aqueous sodium chloride solution (200 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 0-10% MeOH/DCM to give the title compound (6.434 g, 84%). ES/MS m/z 330 (M+H).

[0075]

Preparation 2

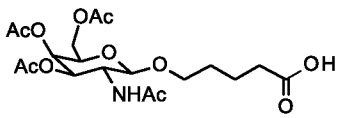
(5-Acetamido-3,4-diacetoxy-6-hex-5-enoxy-tetrahydropyran-2-yl)methyl acetate



[0076] To a solution of (6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]oxazol-5-yl)methyl acetate (30.43 g, 92.42 mmol) in 1,2-DCE (231 mL) is added hex-5-en-1-ol (22.2 mL, 185 mmol) followed by activated powdered 4Å molecular sieves (15.6 g). The suspension is stirred at ambient temperature for 30 minutes and trimethylsilyl trifluoromethanesulfonate (19 mL, 101.9 mmol) is then added. The mixture is stirred at ambient temperature for 18 hours. After this time, the solution is filtered through diatomaceous earth and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 30-100% EtOAc/hexanes to give the title compound (34.76 g, 86%). ES/MS m/z 430.4 (M+H).

Preparation 3

5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoic acid

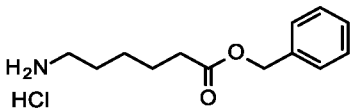


[0077] A solution of (5-acetamido-3,4-diacetoxy-6-hex-5-enoxy-tetrahydropyran-2-yl)methyl acetate (34.76 g, 80.93 mmol) in MeCN (174 mL) and DCM (174 mL) is cooled to 0 °C. A solution of sodium periodate (22.4 g, 104.7 mmol) is added and stirring is continued at 0 °C for 10 minutes. After this time, ruthenium(III) chloride (270 mg, 1.3 mmol) is added and the mixture is stirred while warming to ambient temperature. After

stirring for 2 hours, additional sodium periodate (66 g, 308.4 mmol) is added and stirring is continued for 18 hours. After this time, the mixture is extracted with 3:1 CH3Cl:IPA (2 × 500 mL), washed with saturated aqueous sodium chloride solution (1 L), dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 0-40% MeOH/DCM to give the title compound (29.75 g, 82%). ES/MS m/z 448.4 (M+H).

Preparation 4

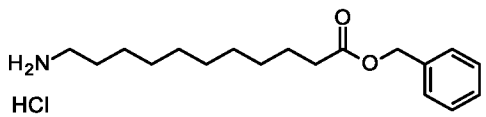
Benzyl 6-aminohexanoate hydrochloride



[0078] To a suspension of 6-aminohexanoic acid (5.00 g, 38.1 mmol) in THF (38 mL) is added benzyl alcohol (47 mL, 453.7 mmol) and the mixture is cooled to 0 °C. Thionyl chloride (8.6 mL, 120 mmol) is added dropwise and the mixture is stirred for 18 hours while warming to ambient temperature. After this time, ether (166 mL) is added and the reaction vessel is transferred to a freezer at -20 °C for 1 hour. After this time, the solid precipitate is collected by filtration to give the title compound (8.57 g, 81%). ES/MS m/z 222 (M+H).

Preparation 5

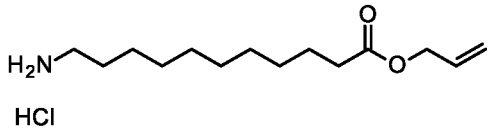
Benzyl 11-aminoundecanoate hydrochloride



[0079] The title compound is prepared from 11-aminoundecanoic acid in a manner essentially analogous to the method of preparation 4. ES/MS m/z 292.2 (M+H).

Preparation 6

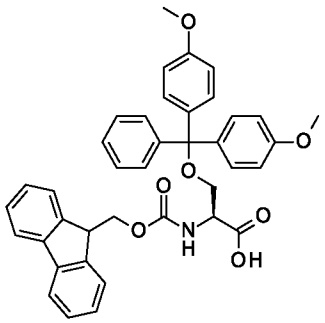
Allyl 11-aminoundecanoate hydrochloride



[0080] A vessel is charged with 11-aminoundecanoic acid (9.00 g, 44.7 mmol) in allyl alcohol (42 mL) and the mixture is cooled to 0 °C. Thionyl chloride (6.5 mL, 89.4 mmol) is added and the mixture is stirred for 18 hours while warming to ambient temperature. After this time, the mixture is concentrated in vacuo and ether (200 mL) is added to the residue to obtain a white suspension. The mixture is stirred at ambient temperature for 10 minutes and the solid precipitate is collected by filtration to obtain the product (12.0 g, 97%). ES/MS m/z 242.2 (M+H).

Preparation 7

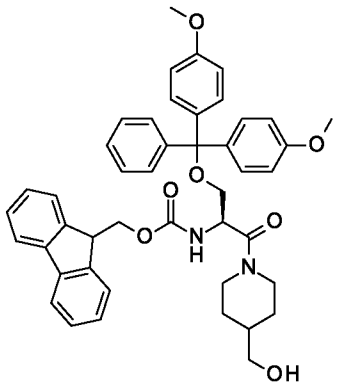
(2S)-3-[Bis(4-methoxyphenyl)-phenyl-methoxy]-2-(9H-fluoren-9ylmethoxycarbonylamino) propanoic acid



[0081] To a stirring solution of (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-propanoic acid (40 g, 0.122 mol) in dry DCM (400 mL) is added DIEA (64 mL, 0.366 mol) at 0 °C under inert atmosphere. To this, a solution of DMTCl (49.6 g, 0.146 mol) in DCM (200 mL) is added slowly. The resulting reaction mixture is brought to ambient temperature and stirred for 16 hours. After this time, the reaction mixture is diluted with water (12.5 vol) and extracted with DCM (25 vol). The organic layer is dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The crude obtained is washed with 10% EtOAc/hexane (12.5 vol) and dried under vacuum to give the title compound as a pale brown solid (62 g, crude). This material was taken to next step without any further purification. TLC: 5% MeOH/ CH2Cl2 (Rf: 0.5) UV, 254 nM.

Preparation 8

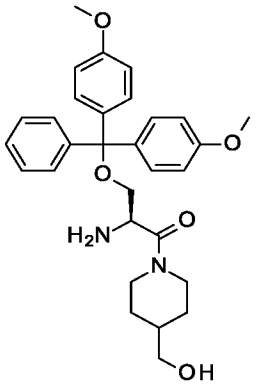
9H-Fluoren-9-ylmethyl N-[(1S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-2-[4-(hydroxymethyl)-1-piperidyl]-2-oxo-ethyl]carbamate



[0082] To a stirring solution of (2S)-3-[bis(4-methoxyphenyl)-phenyl-methoxy]-2-(9H-fluoren-9ylmethoxycarbonylamino) propanoic acid (62 g, 0.103 mol) in DCM (750 mL) are added slowly HBTU (78.3 g, 0.206 mol), HOBt (27.9 g, 0.206 mol), and piperidin-4-yl methanol (15.4 g, 0.134 mol) followed by TMP (15 mL, 0.113 mol) at 0 °C under inert atmosphere. The resulting reaction mixture is brought to ambient temperature and stirred for 4 hours. After this time, the reaction mixture is diluted with water (8 vol) and extracted with DCM (15 vol). The organic layer is dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 20-40% EtOAc/hexane and 1% MeOH/DCM to give the title compound (40 g, 52% over two steps). 1H NMR (DMSO-d6) δ 7.88 (br d, J = 7.5 Hz, 2H), 7.79 - 7.59 (m, 3H), 7.45 - 7.12 (m, 13H), 6.92 - 6.76 (m, 4H), 4.79 - 4.44 (m, 2H), 4.32 (br d, J = 11.4 Hz, 2H), 4.20 (br s, 2H), 3.71 (s, 6H), 3.21 (br s, 4H), 2.99 - 2.79 (m, 1H), 2.69 (br s, 2H), 1.81 - 1.43 (m, 3H), 1.08 - 0.73 (m, 2H).

Preparation 9

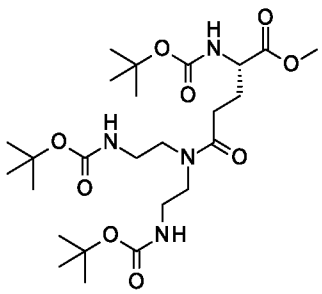
(2S)-2-Amino-3-[bis(4-methoxyphenyl)-phenyl-methoxy]-1-[4-(hydroxymethyl)-1-piperidyl]propan-1-one



[0083] A solution of 20% piperidine in DMF (400 mL) is added slowly to 9H-fluoren-9-ylmethyl N-[(1S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-2-[4 -(hydroxymethyl)-1-piperidyl]-2-oxo-ethyl]carbamate (40 g, 0.055 mol) at 0 °C under inert atmosphere. The resulting reaction mixture is stirred at ambient temperature for 1 hour. After this time, the mixture is diluted with water (15 vol) and extracted with EtOAc (30 vol). The organic layer is dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 1-8% MeOH/DCM to give the title compound as an off white solid (13 g, 47%). ES/MS m/z 1009.5 (2M+H).

Preparation 10

Methyl (2S)-5-[bis[2-(*tert*-butoxycarbonylamino)ethyl]amino]-2-(*tert*-butoxycarbonylamino)-5-oxo-pentanoate

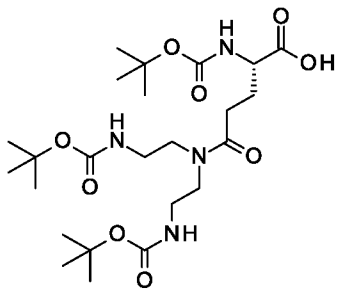


[0084] To a flask containing (S)-4-((tert-butoxycarbonyl)amino)-5-methoxy-5-oxopentanoic acid (7.00 g, 26.8 mmol) and HOBt (4.16 g, 30.8 mmol) are added DMF

(179 mL) and (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (11.7 g, 30.9 mmol). DIEA (14 mL, 80.3 mmol) is added and the mixture is stirred at ambient temperature for 5 minutes. After this time, tert-butyl N-[2-[2-(tert-butoxycarbonylamino)ethylamino]ethyl]carbamate (8.94 g, 29.5 mmol) is added in one portion and stirring is continued at ambient temperature. After stirring for 18 hours, the mixture is diluted with EtOAc (400 mL), washed with water (2 × 400 mL) and saturated aqueous sodium chloride solution (400 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 40-100% EtOAc/hexanes to give the title compound (13.01 g, 89%). ES/MS m/z 547.40 (M+H).

Preparation 11

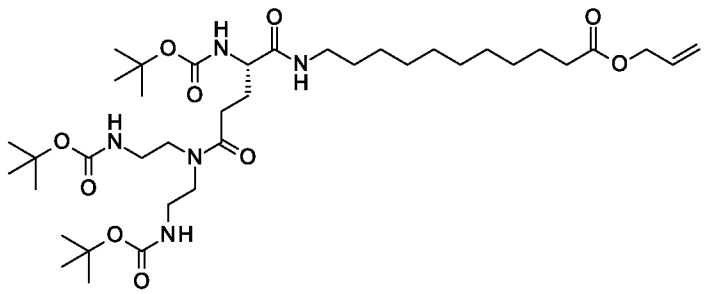
(2S)-5-[Bis[2-(*tert*-butoxycarbonylamino)ethyl]amino]-2-(*tert*-butoxycarbonylamino)-5-oxo-pentanoic acid



[0085] A flask is charged with methyl (2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoate (13.01 g, 23.8 mmol), THF (120 mL), and MeOH (120 mL). 1N NaOH (71 mL, 71 mmol) is added and the mixture is stirred at ambient temperature. After 1 hour, the mixture is concentrated in vacuo and redissolved in water (300 mL). 5N HCl (12 mL) is added to bring the pH to 4. The mixture is extracted with DCM (3 × 300 mL) and the combined organic layers are washed with saturated aqueous sodium chloride solution (1 L), dried over sodium sulfate, filtered, and concentrated to give the title compound (12.41 g, 98%). ES/MS m/z 531.60 (M-H).

Preparation 12

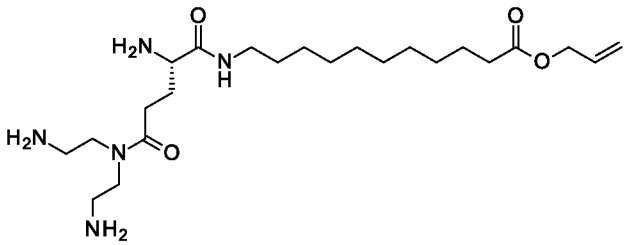
Allyl 11-[[(2S)-5-[bis[2-(*tert*-butoxycarbonylamino)ethyl]amino]-2-(*tert*-butoxycarbonylamino)-5-oxo-pentanoyl]amino]undecanoate



[0086] To a flask containing (2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoic acid (500 mg, 0.94 mmol) and allyl 11-aminoundecanoate hydrochloride (313 mg, 1.13 mmol) is added DMF (6.25 mL) and (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (428 mg, 1.12 mmol). Following addition of DIEA (0.5 mL, 3 mmol) the mixture is stirred at ambient temperature for 18 hours. After this time, the mixture is diluted with EtOAc (200 mL), washed with water (3 × 200 mL) and saturated aqueous sodium chloride solution (200 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 40-100% EtOAc/hexanes to give the title compound (687 mg, 97%). 1H NMR (DMSO-d6) δ 7.78-7.64 (m, 1H), 6.98-6.7 (m, 2H), 5.96-5.84 (m, 1H), 5.31-5.25 (m, 1H), 5.23-5.17 (m, 1H), 4.56-4.50 (m, 2H), 3.88-3.67 (m, 1H), 3.30-3.19 (m, 4H), 3.11-2.91 (m, 6H), 2.35-2.12 (m, 4H), 1.88-1.65 (m, 2H), 1.58-1.47 (m, 2H), 1.46-1.30 (m, 30H), 1.30-1.18 (m, 12H).

Preparation 13

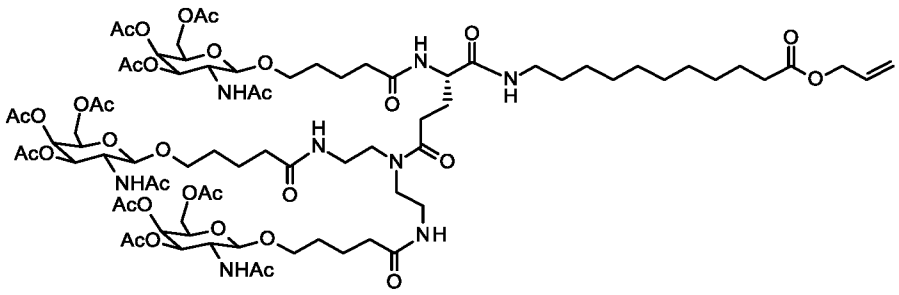
Allyl (*S*)-11-(2-amino-5-(bis(2-aminoethyl)amino)-5-oxopentanamido)undecanoate



[0087] To a solution of allyl 11-[[(2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoyl]amino]undecanoate (687 mg, 0.91 mmol) in DCM (15 mL) is added TFA (15 mL). The mixture is stirred at ambient temperature. After 1.5 hours, the mixture is concentrated in vacuo. The residue is taken up in MeOH and applied to an ion exchange cartridge. The cartridge is eluted with MeOH (150 mL) followed by 7N NH3/MeOH (150 mL). The basic fraction is concentrated in vacuo to give the title compound (410 mg, 99%). ES/MS m/z 456.4 (M+H).

Preparation 14

Allyl 11-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate

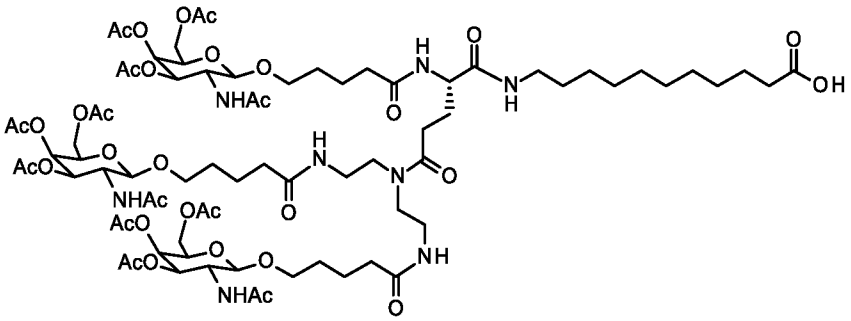


[0088] A flask is charged with 5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoic acid (489 mg, 1.09 mmol) and allyl (S)-11-(2-amino-5-(bis(2-aminoethyl)amino)-5-oxopentanamido)undecanoate (150 mg, 0.33 mmol). DCM (3.35 mL) is added followed by 1-hydroxybenzotriazole monohydrate (164 mg, 1.07 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (206 mg, 1.07 mmol). The mixture is stirred at ambient temperature for 18 hours. After

this time, the solution is diluted with EtOAc (100 mL), washed with saturated NaHCO3 (2 × 100 mL), saturated aqueous NH4Cl (100 mL), and saturated aqueous sodium chloride solution (100 mL). The organic layer is dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 0-10% MeOH/DCM to give the title compound (424 mg, 74%). ES/MS m/z 872.80 (M+2H)/2.

Preparation 15

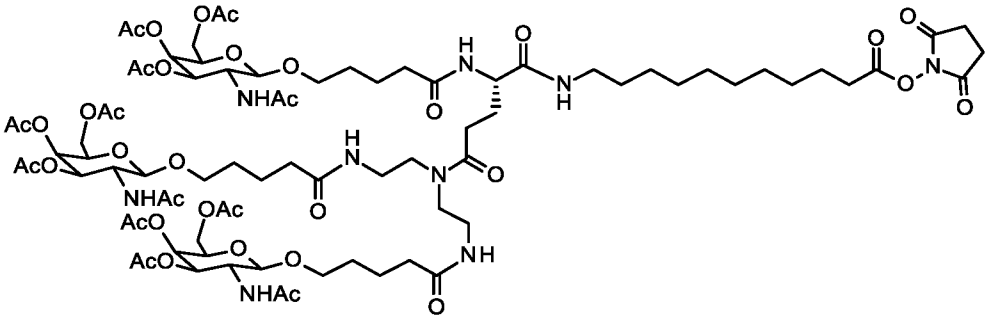
11-[[(2S)-2-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoic acid



[0089] To a solution of allyl 11-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate (354 mg, 0.20 mmol) in DCM (2 mL) is added tetrakis(triphenylphosphine)palladium (29 mg, 0.02 mmol) followed by PhSiH3 (51 uL, 0.41 mmol). The mixture is stirred at ambient temperature for 2 hours, after which it is diluted with saturated aqueous NaHCO3 (100 mL). 1N NaOH (15 mL) is added to bring the pH to about 10. The aqueous solution is washed with DCM (3 × 100 mL) and then acidified with concentrated HCl (5 mL) and then aqueous 5N HCl (15 mL). The aqueous layer is extracted with DCM (100 mL) and the organic layer is dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 0-20% MeOH/DCM to give the title compound (151 mg, 44%). ES/MS m/z 852.60 (M+2H)/2.

Preparation 16

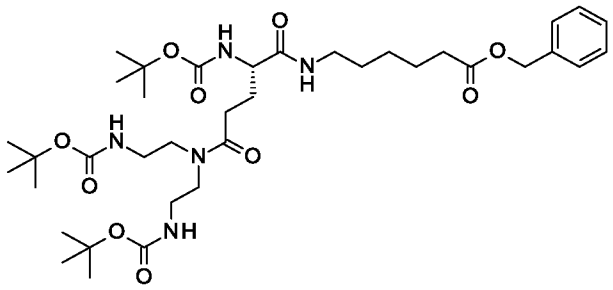
(2,5-Dioxopyrrolidin-1-yl) 11-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate



[0090] To a reaction vial are added 11-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoic acid (50 mg, 0.03 mmol), N-hydroxysuccinimide (5 mg, 0.04 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (8 mg, 0.04 mmol). DCM (0.3 mL) is added and the mixture is stirred at ambient temperature. After 18 hours, the mixture is loaded directly onto a silica gel cartridge and the crude mixture is purified by silica gel flash chromatography eluting with 0-10% MeOH/DCM to give the title compound (49 mg, 93%). ES/MS m/z 901.40 (M+2H)/2.

Preparation 17

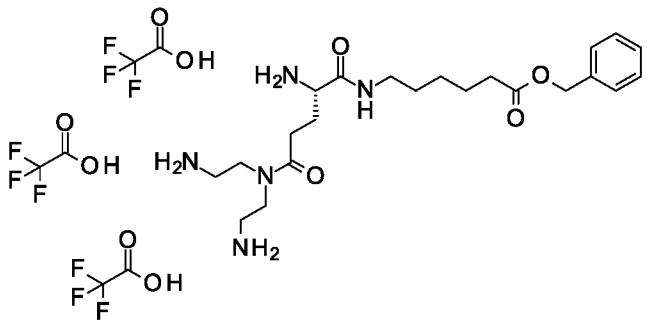
Benzyl 6-[[(2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoyl]amino]hexanoate



[0091] The title compound is prepared from (2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoic acid and benzyl 6-aminohexanoate hydrochloride in a manner essentially analogous to the method of preparation 10. ES/MS m/z 736.40 (M+H).

Preparation 18

Benzyl 6-[[(2S)-2-amino-5-[bis(2-aminoethyl)amino]-5-oxo-pentanoyl]amino]hexanoate tris(trifluoroacetic acid)

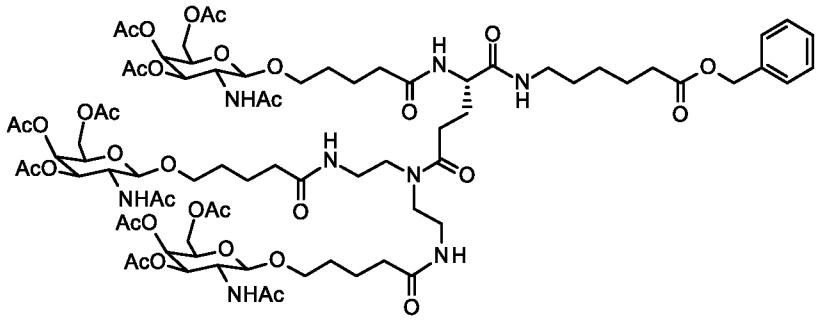


[0092] To a solution of benzyl 6-[[(2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoyl]amino]hexanoate (15.47 g, 21.02 mmol) in DCM (105 mL) is added TFA (16 mL, 210.2 mmol). The mixture is stirred at ambient temperature for 24 hours. After this time, additional TFA (16 mL, 210.2 mmol) is added and stirring is continued for an additional 2 hours. After this time, the mixture is concentrated in vacuo. The resulting residue is azeotroped with toluene (2 × 30 mL). The resulting oil is further dried in a vacuum oven at 40 °C for 4 hours to give the title compound (28.08 g, 58% purity

accounting for residual toluene, 99+%). ES/MS m/z 436.40 (M+H). The compound is dissolved in 70 mL DMF to make a 0.3M solution that is used in the next step.

Preparation 19

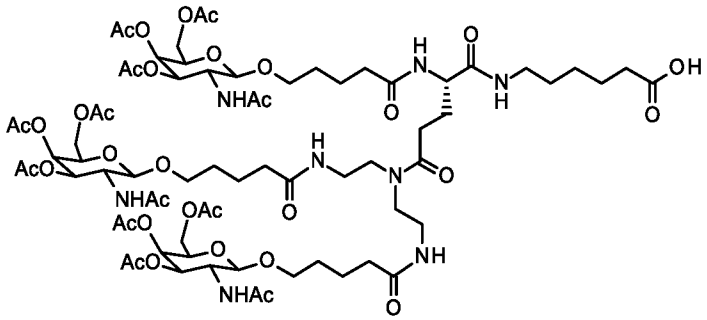
Benzyl 6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate



[0093] The title compound is prepared from 5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoic acid and benzyl 6-[[(2S)-2-amino-5-[bis(2-aminoethyl)amino]-5-oxo-pentanoyl]amino]hexanoate tris trifluoroacetic acid and in a manner essentially analogous to the method of preparation 10. ES/MS m/z 862 (M+2H)/2.

Preparation 20

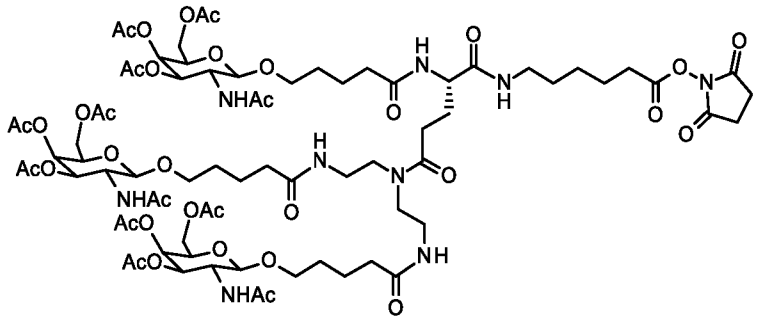
6-[[(2S)-2-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoic acid



[0094] Palladium on carbon (1.90 g, 0.89 mmol, 5 mass%, 50% wet) is placed in a round-bottom flask and the vessel is evacuated and backfilled with nitrogen three times. A solution of benzyl 6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate (15.41 g, 8.94 mmol) in MeOH (178 mL) is added via syringe. The flask is evacuated and backfilled with 1 atm hydrogen and the mixture is stirred at ambient temperature under 1 atm hydrogen for 18 hours. After this time, the mixture is filtered through diatomaceous earth and the filtrate is concentrated in vacuo to give the title compound (13.85 g, 95%). ES/MS m/z 817.2 (M+2H)/2.

Preparation 21

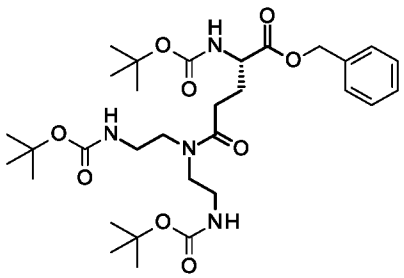
(2,5-Dioxopyrrolidin-1-yl) 6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate



[0095] The title compound is prepared from 6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoic acid in a manner essentially analogous to the method of preparation 16. ES/MS m/z 866.20 (M+2H)/2.

Preparation 22

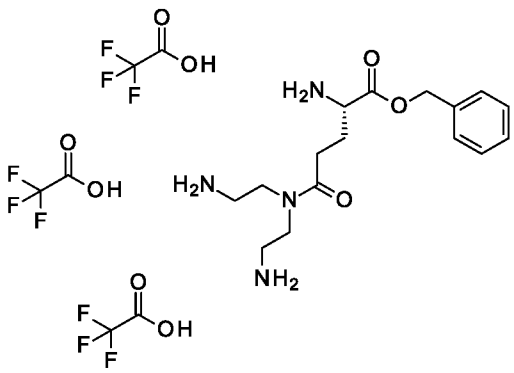
Benzyl (2S)-5-[bis[2-(*tert*-butoxycarbonylamino)ethyl]amino]-2-(*tert*-butoxycarbonylamino)-5-oxo-pentanoate



[0096] The title compound is prepared from tert-butyl N-[2-[2-(tert-butoxycarbonylamino)ethylamino]ethyl]carbamate and (4S)-5-benzyloxy-4-(tert-butoxycarbonylamino)-5-oxo-pentanoic acid in a manner essentially analogous to the method of preparation 12. ES/MS m/z 623.6 (M+H).

Preparation 23

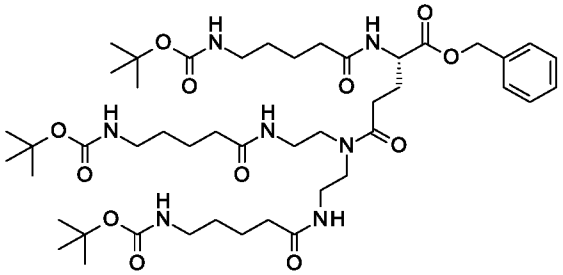
Benzyl (2S)-2-amino-5-[bis(2-aminoethyl)amino]-5-oxo-pentanoate tris(trifluoroacetic acid) salt



[0097] The title compound is prepared from benzyl (2S)-5-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]-2-(tert-butoxycarbonylamino)-5-oxo-pentanoate in a manner essentially analogous to the method of preparation 18. ES/MS m/z 323.2 (M+H).

Preparation 24

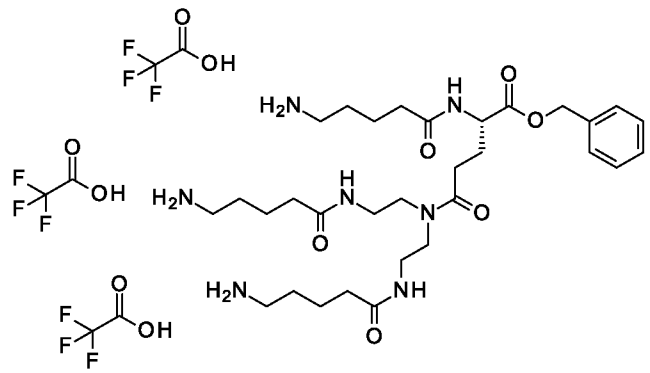
Benzyl (2S)-5-[bis[2-[5-(*tert*-butoxycarbonylamino)pentanoylamino]ethyl]amino]-2-[5-(*tert*-butoxycarbonylamino)pentanoylamino]-5-oxo-pentanoate



[0098] The title compound is prepared from 5-(tert-butoxycarbonylamino)pentanoic acid and benzyl (2S)-2-amino-5-[bis(2-aminoethyl)amino]-5-oxo-pentanoate tris(trifluoroacetic acid) salt in a manner essentially analogous to the method of preparation 10. ES/MS m/z 920.6 (M+H).

Preparation 25

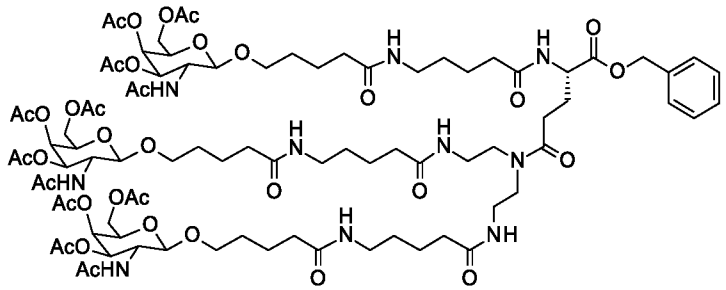
Benzyl (2S)-2-(5-aminopentanoylamino)-5-[bis[2-(5-aminopentanoylamino)ethyl]amino]-5-oxo-pentanoate tris(trifluoroacetic acid) salt



[0099] The title compound is prepared from benzyl (2S)-5-[bis[2-[5-(tert-butoxycarbonylamino)pentanoylamino]ethyl]amino]-2-[5-(tert-butoxycarbonylamino)pentanoylamino]-5-oxo-pentanoate in a manner essentially analogous to the method of preparation 18. ES/MS m/z 620.4 (M+H).

Preparation 26

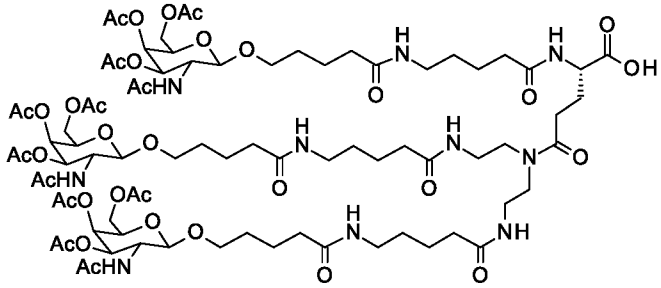
Benzyl (2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoate



[00100] The title compound is prepared from 5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoic acid and benzyl (2S)-2-(5-aminopentanoylamino)-5-[bis[2-(5-aminopentanoylamino)ethyl]amino]-5-oxo-pentanoate tris(trifluoroacetic acid) salt and in a manner essentially analogous to the method of preparation 10. ES/MS m/z 954.80 (M+2H)/2.

Preparation 27

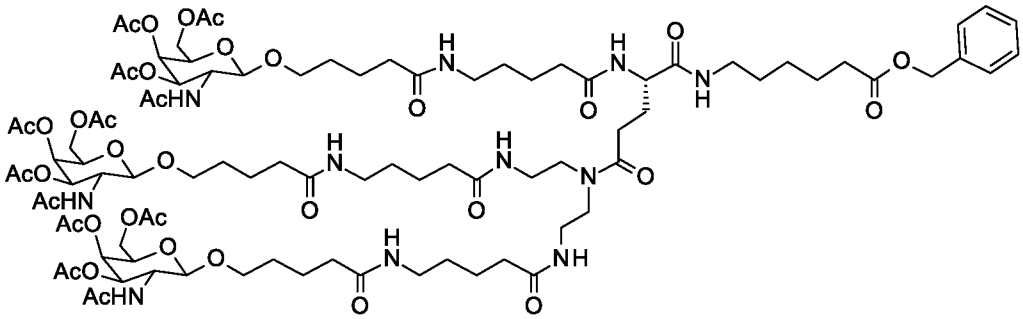
(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoic acid



[00101] A round-bottom flask is charged with palladium on carbon (467 mg, 0.22 mmol, 5 mass%, 50% wet) and the flask is evacuated and backfilled with nitrogen three times. A solution of benzyl (2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoate (4.19 g, 2.20 mmol) in MeOH (44 mL) is added via syringe followed by three drops of acetic acid. The flask is evacuated and backfilled with 1 atm hydrogen and the mixture is stirred at ambient temperature under 1 atm hydrogen. After 2 hours, the mixture is filtered through diatomaceous earth and the filtrate is concentrated in vacuo to give the title compound (3.99 g, 99+%). ES/MS m/z 909.6 (M+2H)/2.

Preparation 28

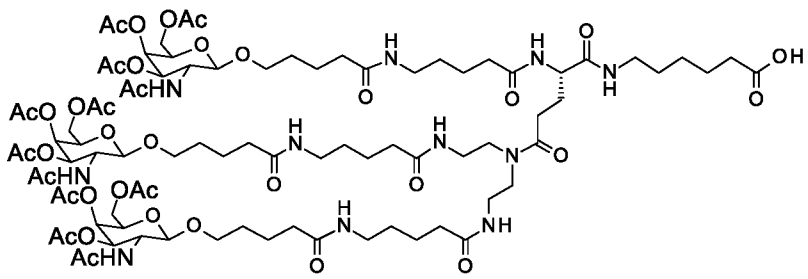
Benzyl 6-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate



[00102] The title compound is prepared from (2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoic acid and benzyl 6-aminohexanoate hydrochloride and in a manner essentially analogous to the method of preparation 10. ES/MS m/z 1011.6 (M+2H)/2.

Preparation 29

6-[[(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoic acid

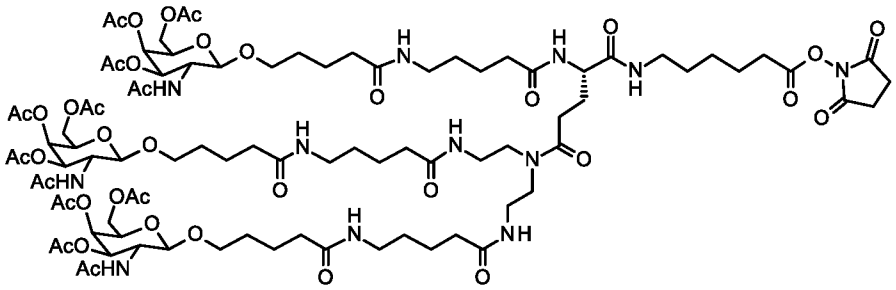


[00103] A round-bottom flask is charged with palladium on carbon (24 mg, 0.01 mmol, 5% by mass, 50% wet) and the flask is evacuated and backfilled with nitrogen. A solution of benzyl 6-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-

(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate (222 mg, 0.11 mmol) in MeOH (2.2 mL) is added via syringe followed by three drops of acetic acid. The flask is evacuated and backfilled with 1 atm hydrogen and the mixture is stirred under 1 atm hydrogen at ambient temperature. After 5 hours, the flask is purged with nitrogen and the mixture is filtered through diatomaceous earth. The filtrate is concentrated *in vacuo* to give the title compound (180 mg, 85%). ES/MS m/z 966.2 (M+2H)/2.

Preparation 30

(2,5-Dioxopyrrolidin-1-yl) 6-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoate

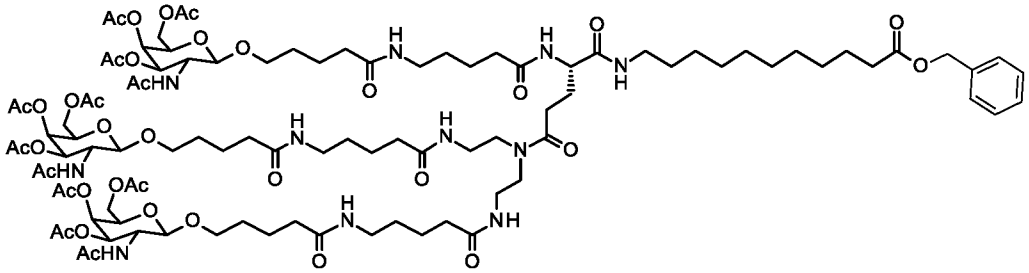


[00104] The title compound is prepared from 6-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoic acid in a manner essentially analogous to the method of preparation 16. ES/MS m/z 1014.6 (M+2H)/2.

Preparation 31

Benzyl 11-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-

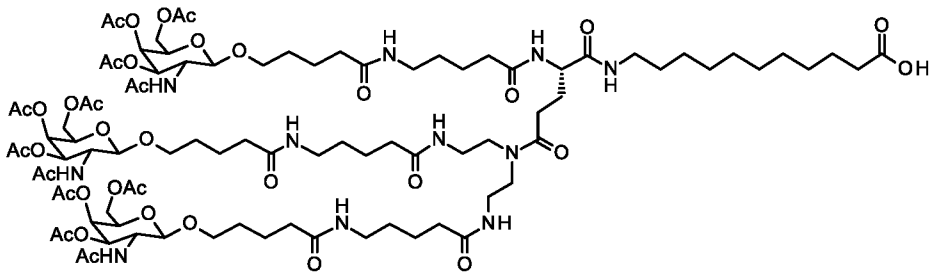
(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate



[00105] The title compound is prepared from (2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoic acid and benzyl 11-aminoudecanoate hydrochloride in a manner essentially analogous to the method of preparation 10. ES/MS m/z 1046.6 (M+2H)/2.

Preparation 32

11-[[(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoic acid

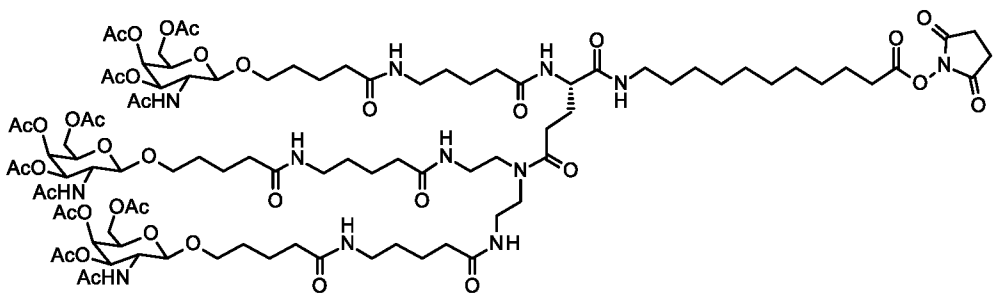


[00106] To a round-bottom flask is added palladium on carbon (35 mg, 0.02 mmol, 5 mass%, 50% wet) and the flask is evacuated and backfilled with nitrogen three times. A solution of benzyl 11-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate (285 mg, 80% purity, 0.11 mmol) is added via syringe. The vessel is evacuated and

backfilled with 1 atm hydrogen and the mixture is then stirred at ambient temperature under 1 atm hydrogen. After stirring for 3 hours, the flask is purged with nitrogen and the mixture is filtered through diatomaceous earth. The filtrate is concentrated to give the title compound (213 mg, 79% purity, 77%). ES/MS m/z 1001.20 (M+2H)/2.

Preparation 33

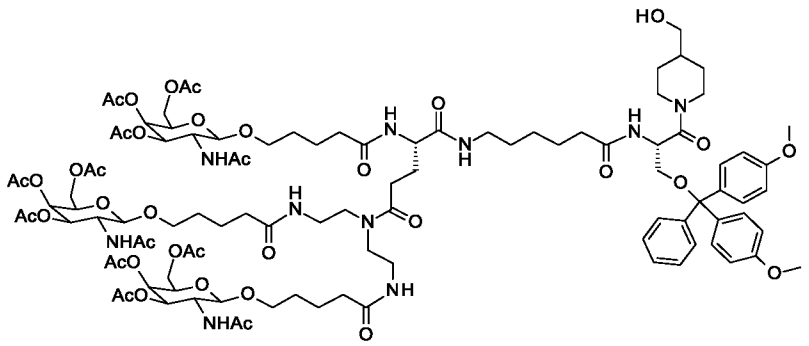
(2,5-Dioxopyrrolidin-1-yl) 11-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoate



[00107] The title compound is prepared from 11-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]undecanoic acid in a manner essentially analogous to the method of preparation 16. ES/MS m/z 1050 (M+2H)/2

Preparation 34

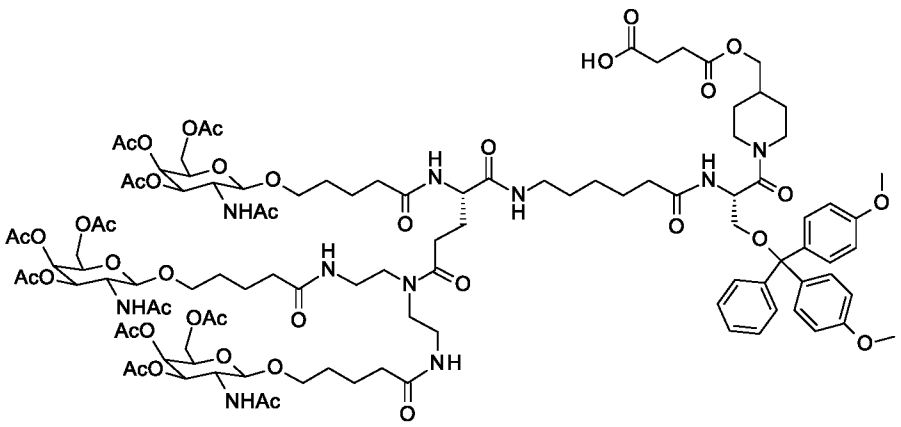
[5-Acetamido-6-[5-[2-[[(4S)-4-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[[6-[[(1S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-2-[4-(hydroxymethyl)-1-piperidyl]-2-oxo-ethyl]amino]-6-oxo-hexyl]amino]-5-oxo-pentanoyl]-[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]ethylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate



[00108] The title compound is prepared from 6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoic acid and (2S)-2-amino-3-[bis(4-methoxyphenyl)-phenyl-methoxy]-1-[4-(hydroxymethyl)-1-piperidyl]propan-1-one in a manner essentially analogous to the method of preparation 10. ES/MS m/z 1059.2 (M-2H)/2.

Preparation 35

4-[[1-[(2S)-2-[6-[[(2S)-2-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoylamino]-3-[bis(4-methoxyphenyl)-phenyl-methoxy]propanoyl]-4-piperidyl]methoxy]-4-oxo-butanoic acid

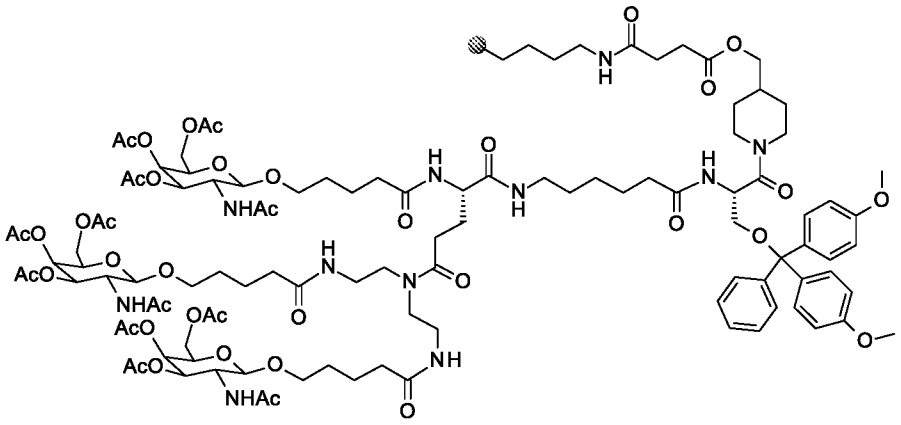


[00109] To a solution of [5-acetamido-6-[5-[2-[[(4S)-4-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[[6-[[(1S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-2-[4-(hydroxymethyl)-1-piperidyl]-2-

oxo-ethyl]amino]-6-oxo-hexyl]amino]-5-oxo-pentanoyl]-[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]ethylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate (1.194 g, 0.56 mmol) in DCM (11 mL) is added succinic anhydride (113 mg, 1.13 mmol), TEA (0.4 mL, 3 mmol) and DMAP (213 mg, 1.69 mmol). The mixture is stirred at ambient temperature for 1 hour. After this time, the mixture is diluted with saturated NH4Cl (200 mL) and extracted with DCM (3 × 200 mL) and 3:1 CHCl3:IPA (200 mL). The organic layers are combined, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue is purified by silica gel flash chromatography eluting with 0-40% MeOH/DCM and the resulting product is dried in a vacuum oven at 40 °C for 3 hours to give the title compound (1.081 g, 86%). ES/MS m/z 1109.60 (M-2H)/2.

Preparation 36

Resin loading

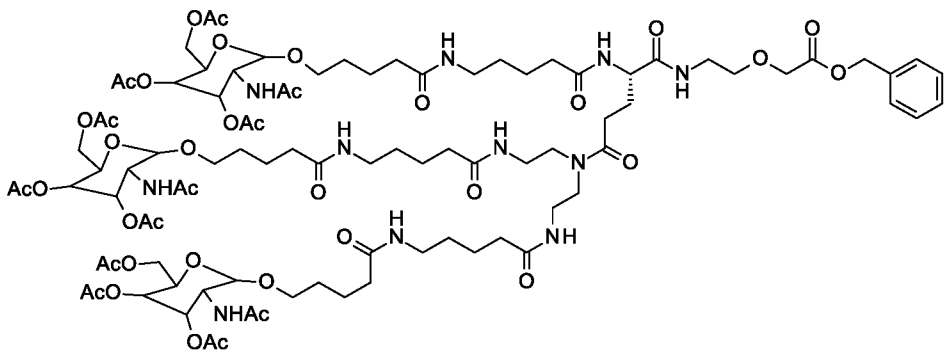


[00110] A solution of 4-[[1-[(2S)-2-[6-[[(2S)-2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]-5-[bis[2-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]hexanoylamino]-3-[bis(4-methoxyphenyl)-phenyl-methoxy]propanoyl]-4-piperidyl]methoxy]-4-oxo-butanoic acid (1.00 g, 0.61 mmol) in MeCN (6 mL) and DCM (1 mL) is transferred to a resin loading cartridge. To the vessel are added 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (386 mg, 0.97 mmol) and DIEA (0.25 mL, 0.48 mmol) and the cartridge is shaken at ambient

temperature for 5 minutes. After this time, 1000 Å LCAA controlled-pore glass resin (5.39 g, 90 µmol/g loading, purchased from ChemGenes) is added and the mixture is shaken at ambient temperature for 18 hours. After this time, the cartridge is drained by suction and the resin is washed by shaking with DCM (10 mL) for 10 minutes. The cartridge is drained and the washing and draining procedure is repeated with 10% MeOH/DCM (10 mL) and Et2O (10 mL). After draining, a solution of acetic anhydride (6.4 mL), pyridine (20 mL) and TEA (0.22 mL) is added and the cartridge is shaken for 2 hours. After this time, the cartridge is drained and the washing and draining procedure above is repeated using DCM (10 mL), 10% MeOH/DCM (10 mL) and diethyl ether (10 mL). After draining, the resin is dried under vacuum for 30 minutes. The resin loading is determined using a standard trityl assay. The resin loading was calculated to be 34.7 µmol/g.

Preparation 37

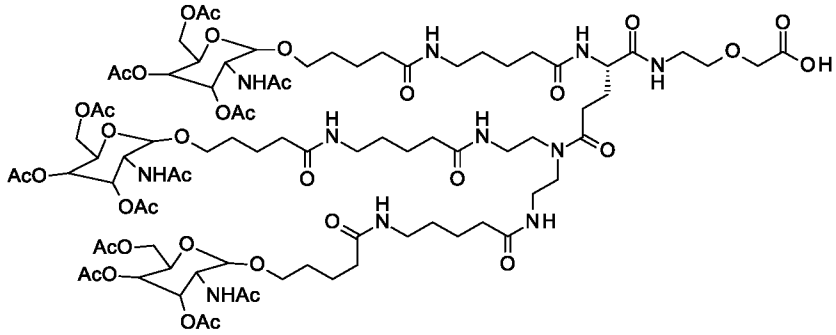
Benzyl 2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]acetate



[00111] The title compound is prepared from (2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoic acid and benzyl 2-(2-aminoethoxy)acetate hydrochloride in a manner essentially analogous to the method of preparation 10. ES/MS m/z 1005.2 (M+2H/2).

Preparation 38

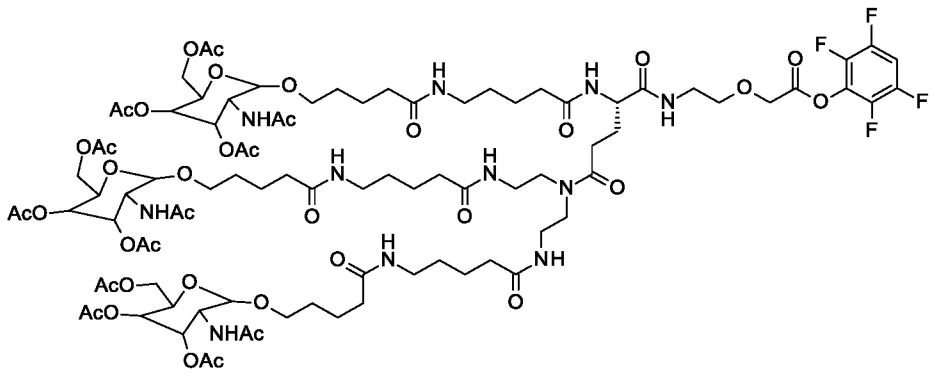
2-[2-[[(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]acetic acid



[00112] Benzyl 2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]acetate (0.120 mmol, 240 mg) is combined with 5% Pd/C (1.17 mmol, 124 mg) in MeOH (12.0 ml). The mixture is hydrogenated on a Parr shaker (ambient temperature, 10 psi) for 48 minutes, filtered through diatomaceous earth, and concentrated in vacuo to give the title compound as a gray solid (187 mg, 82%). ES/MS m/z 960.0 (M+2H/2).

Preparation 39

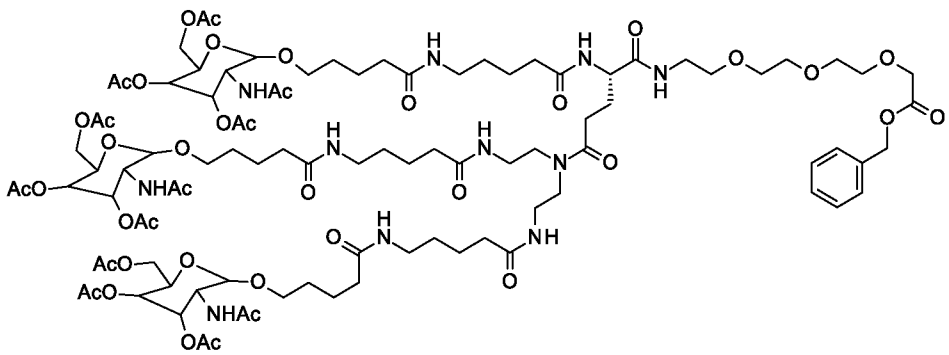
(2,3,5,6-Tetrafluorophenyl) 2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]acetate



[00113] To 2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]acetic acid (0.096 mmol, 184 mg) and DIEA (0.765 mmol, 140 µL) in DCM (3.0 ml) is added (2,3,5,6-tetrafluorophenyl) 2,2,2-trifluoroacetate (0.383 mmol, 100 mg) to the mixture dropwise. The mixture is stirred at ambient temperature for 16 hours. The reaction mixture is purified directly by silica gel flash chromatography eluting with 0% to 50% MeOH/DCM to give the title compound as a tan solid (197 mg, 99%). ES/MS m/z 1034.0 (M+2H/2).

Preparation 40

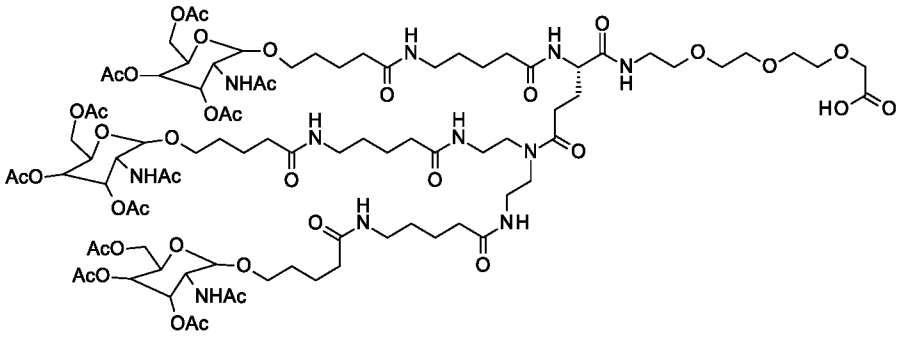
Benzyl 2-[2-[2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]acetate



[00114] The title compound is prepared from (2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoic acid and benzyl 2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]acetate hydrochloride in a manner essentially analogous to the method of preparation 10. ES/MS m/z 1049.0 (M+2H/2).

Preparation 41

2-[2-[2-[2-[[(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]acetic acid

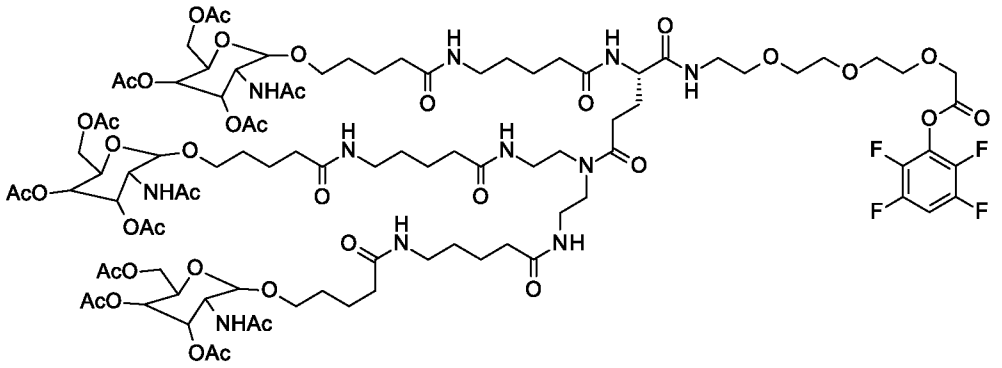


[00115] Benzyl 2-[2-[2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]acetate (0.118 mmol, 247 mg) is combined with 5% Pd/C (1.17 mmol, 124 mg) in MeOH (12.0 mL). The mixture is hydrogenated on a Parr shaker (ambient temperature, 10 psi) for 1 hour, filtered through diatomaceous earth, and concentrated in vacuo to give the title compound as a gray solid (227 mg, 96%). ES/MS m/z 1004.0 (M+2H/2).

Preparation 42

(2,3,5,6-Tetrafluorophenyl) 2-[2-[2-[2-[[(2S)-2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-

[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]acetate



[00116] To 2-[2-[2-[2-[[(2S)-2-[5-[5-[3-Acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]-5-[bis[2-[5-[5-[3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]pentanoylamino]ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]acetic acid (0.111 mmol, 222 mg) and DIEA (0.883 mmol, 154 µL) in DCM (3.0 ml) is added (2,3,5,6-tetrafluorophenyl) 2,2,2-trifluoroacetate (0.443 mmol, 116 mg) to the mixture dropwise. The mixture is stirred at ambient temperature for 16 hours. The reaction mixture is purified directly by silica gel flash chromatography eluting with 0% to 50% MeOH/DCM to give the title compound as a tan solid (174 mg, 73%). ES/MS m/z 1078.2 (M+2H/2).

Example 1: Conjugation Protocol

[00117] For the synthesis of GalNAc-conjugated sense strands, a sense strand with a 3' C6-NH2 functional group was first synthesized using standard phosphoramidite chemistry. A stock solution of GalNAc ligand-NHS ester (10 mmol/L in acetonitrile; 1 eq) was prepared. Borate buffer (10% v/v; 20x) was added to oligonucleotide C6-NH2 sense strand in an Eppendorf tube, then GalNAc ligand (5 eq) was added. The mixture was shaken at ambient temperature for 16 hours. After this time, the mixture was transferred to a 15 mL falcon tube, ammonium hydroxide (28 mass%) was added, and the mixture was shaken at ambient temperature for 2 hours. The ammonia was then removed in vacuo. The residue was purified by ion-exchange chromatography. Conditions: Solvent

A: 15% MeCN/20 mM NaH2PO4, Solvent B: 15%MeCN/20mM NaH2PO4, 1M NaBr; 35-55%B over 5 CV at 8 mL/min, column temperature 60 °C. The desired fractions were pooled and desalted by spin-filtration using an Eppendorf centrifuge or desalting column. After desalting, the material was recovered and OD and volume were measured to obtain concentration.

[00118] Alternatively, conjugation was to the 5' position of the sense strand through immobilizing the GalNAc ligand on microporous polystyrene resin or controlled pore glass and synthesizing using established solid phase oligonucleotide synthesis methods with 5'-CE ß-cyanoethyl) phosphoramidites.

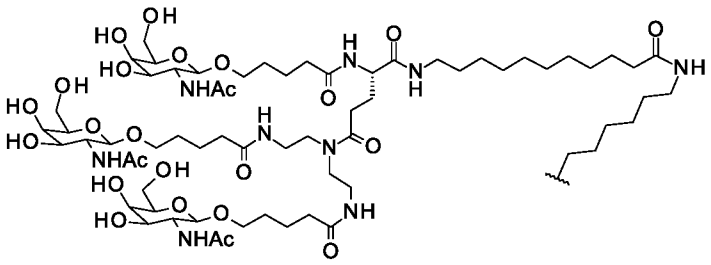
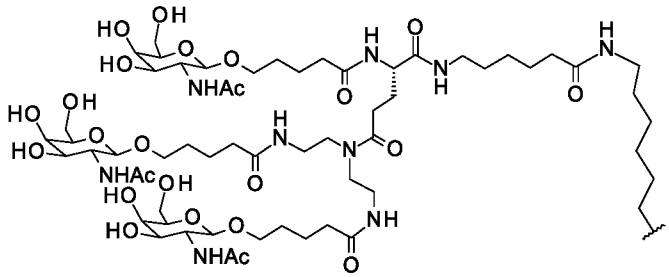
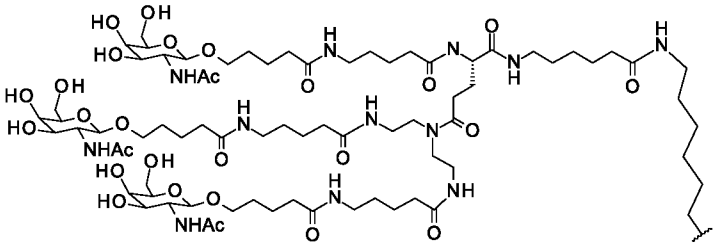
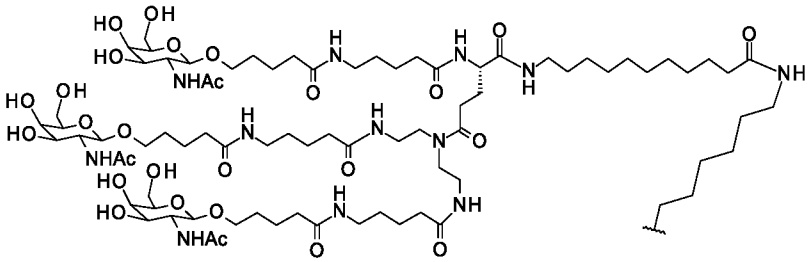
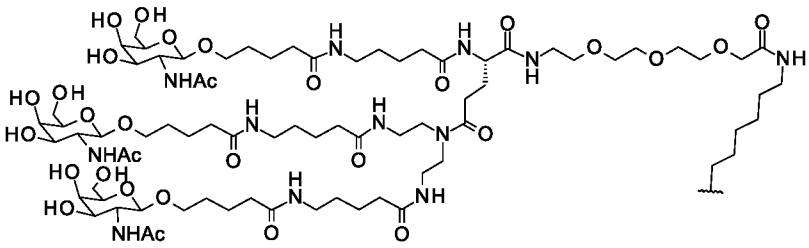
[00119] Alternatively, the GalNAc ligand was converted to a suitable phosphoramidite and delivered to the 5' position of the sense strand using standard phosphoramidite chemistry.

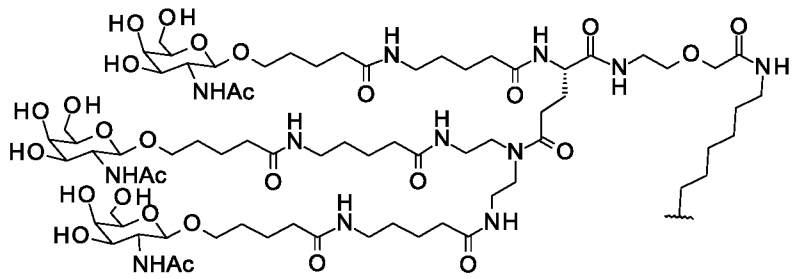
Example 2: Annealing

[00120] To generate the siRNA duplexes of a sense and antisense strand, the following procedures were performed. To a falcon tube containing oligonucleotide sense strand-GalNAc conjugate, the corresponding antisense oligonucleotide (1 eq) was added and vortexed for 10 seconds before spin-filtering through 100K MWCO Amicon filter unit to remove particulates. The filtrate was recovered and concentrated in vacuo on a Genevac evaporator. The residue was reconstituted in 1x PBS, filtered through 0.2 µ filter, and OD and volume were measured to obtain concentration.

[00121] An endotoxin test was performed using a Limulus amebocyte lysate on an Endosafe®-nexgen PTS instrument.

Table 1 – Exemplary molecules synthesized utilizing the aforementioned conjugation and annealing protocols.

Molecule Identifier	Ligand attached to 3' of sense strand
1	
2	
3	
4	
5	

6	

Example 3:

General procedure for oligo synthesis using GalNAc-functionalized CPG

[00122] Oligo synthesis was conducted on a MerMade™ 12 instrument using phosphoramidite chemistry. Sense strands were synthesized from the prefunctionalized GalNAc solid support and antisense strands were synthesized using standard support preloaded with the first nucleotide of the oligo sequence. Oligos were cleaved and deprotected using concentrated ammonium hydroxide solution (28% by mass) and purified by ion exchange chromatography using conditions described above. Desalting, annealing, and endotoxin testing were conducted.

[00123] The sequence of antisense oligonucleotides were designed using 15 to 50 nucleotides of the following FAS transcript (SEQ ID NO: 1), where T nucleotides were replaced by U nucleotides, and where one or more nucleotides and one or more internucleotide linkages were optionally further modified as described herein.

Homo sapiens FAS Cell Death Receptor (FAS) transcript,

SEQ ID NO: 1

```
   1 ctcttctccc gcgggttggt ggacccgctc agtacggagt tggggaagct ctttcacttc
  61 ggaggattgc tcaacaacca tgctgggcat ctggaccctc ctacctctgg ttcttacgtc
 121 tgttgctaga ttatcgtcca aaagtgttaa tgcccaagtg actgacatca actccaaggg
 181 attggaattg aggaagactg ttactacagt tgagactcag aacttggaag gcctgcatca
 241 tgatggccaa ttctgccata agccctgtcc tccaggtgaa aggaaagcta gggactgcac
 301 agtcaatggg gatgaaccag actgcgtgcc ctgccaagaa gggaaggagt acacagacaa
 361 agcccatttt tcttccaaat gcagaagatg tagattgtgt gatgaaggac atggcttaga
 421 agtggaaata aactgcaccc ggacccagaa taccaagtgc agatgtaaac caaacttttt
 481 ttgtaactct actgtatgtg aacactgtga cccttgcacc aaatgtgaac atggaatcat
 541 caaggaatgc acactcacca gcaacaccaa gtgcaaagag gaaggatcca gatctaactt
 601 ggggtggctt tgtcttcttc ttttgccaat tccactaatt gtttgggtga agagaaagga
 661 agtacagaaa acatgcagaa agcacagaaa ggaaaaccaa ggttctcatg aatctccaac
 721 tttaaatcct gaaacagtgg caataaattt atctgatgtt gacttgagta aatatatcac
 781 cactattgct ggagtcatga cactaagtca agttaaaggc tttgttcgaa agaatggtgt
 841 caatgaagcc aaaatagatg agatcaagaa tgacaatgtc caagacacag cagaacagaa
 901 agttcaactg cttcgtaatt ggcatcaact tcatggaaag aaagaagcgt atgacacatt
 961 gattaaagat ctcaaaaaag ccaatctttg tactcttgca gagaaaattc agactatcat
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1021 cctcaaggac attactagtg actcagaaaa ttcaaacttc agaaatgaaa tccaaagctt
1081 ggtctagagt gaaaaacaac aaattcagtt ctgagtatat gcaattagtg tttgaaaaga
1141 ttcttaatag ctggctgtaa atactgcttg gtttttttact gggtacattt tatcatttat
1201 tagcgctgaa gagccaacat atttgtagat ttttaatatc tcatgattct gcctccaagg
1261 atgtttaaaa tctagttggg aaaacaaact tcatcaagag taaatgcagt ggcatgctaa
1321 gtacccaaat aggagtgtat gcagaggatg aaagattaag attatgctct ggcatctaac
1381 atatgattct gtagtatgaa tgtaatcagt gtatgttagt acaaatgtct atccacaggc
1441 taaccccact ctatgaatca atagaagaag ctatgacctt ttgctgaaat atcagttact
1501 gaacaggcag gccactttgc ctctaaatta cctctgataa ttctagagat tttaccatat
1561 ttctaaactt tgtttataac tctgagaaga tcatatttat gtaaagtata tgtatttgag
1621 tgcagaattt aaataaggct ctacctcaaa gacctttgca cagtttattg gtgtcatatt
1681 atacaatatt tcaattgtga attcacatag aaaacattaa attataatgt ttgactatta
1741 tatatgtgta tgcattttac tggctcaaaa ctacctactt ctttctcagg catcaaaagc
1801 attttgagca ggagagtatt actagagctt tgccacctct ccatttttgc cttggtgctc
1861 atcttaatgg cctaatgcac ccccaaacat ggaaatatca ccaaaaaata cttaatagtc
1921 caccaaaagg caagactgcc cttagaaatt ctagcctggt ttggagatac taactgctct
1981 cagagaaagt agctttgtga catgtcatga acccatgttt gcaatcaaag atgataaaat
2041 agattcttat ttttccccca cccccgaaaa tgttcaataa tgtcccatgt aaaacctgct
2101 acaaatggca gcttatacat agcaatggta aaatcatcat ctggatttag gaattgctct
2161 tgtcataccc ccaagtttct aagatttaag attctcctta ctactatcct acgtttaaat
2221 atctttgaaa gtttgtatta aatgtgaatt ttaagaaata atatttatat ttctgtaaat
2281 gtaaactgtg aagatagtta taaactgaag cagatacctg gaaccaccta aagaacttcc
2341 atttatggag gatttttttg ccccttgtgt ttggaattat aaaatatagg taaaagtacg
2401 taattaaata atgtttttgg tatttctggt tttctctttt ttggtagggg cttgcttttt
2461 ggttttgtct tccttttctc taactgatgc taaatataac ttgtctttaa tgcttcttgg
2521 atcccttaga aggtacttcc tttttaacct taacccttttt agtagttaaa taattatttc
2581 cataggttgc tattgccaag aagacctctt ccaaacagca catgattatt cgtcaaacag
2641 tttcgtattc cagatactgg aatgtggata agaaagtata catttcaagg ggtaggtttt
2701 attattaaga aagccaaatg aggattttga aatattcttt cctgcatatt atccattcta
2761 gctacatgct ggccagtggg ccacctttct tttctgcaat ttaatgctag taatatattc
2821 tatttaaccc atgagtccca aagtattagc atttcaacat gtaagcatgt cggtaagata
2881 gttgtgcttt gcttagggtt ccctcctgtg ttatggtctg gaaagtgtct ttaggcagaa
2941 agtctgagtg atcacagggt tcactcatta atttctcttt tctgagccat catagtctgt
3001 gctgtctgct ctccagtttt ctatttctag acagaagtag ggcaagttag gtactagtta
3061 ttcttcatgg ccagaagtgc aagttctact ttgcaagaca agattaagtt agagaacacc
3121 ctattccact ttggtgaact cagagcaaga actttgagtt cctttgggag gaagacagtg
3181 gagaagtctt tgtacttggt gatgtggttt ttttcctcat ggcttcacct agtggcccca
3241 agcatgactt ctcccatgtc aatgagcaca gccacattcc cgagttgagg tgaccccacg
3301 gtccagaatc atcctcattc tggtgaacct ggttctcttt gtggtgggca tactgggtag
3361 gagaatcacc caaaggtcac ccatgagctg cagaaaaaaa ggctatttgc agaaggagct
3421 cacagatcac attgaaagca ttgcatattc aaacatcttg gtcttcttta ttggcatgcc
3481 cacagggtct tctgacctct gattagatca gacacttttt agatattgaa tcatcagttt
3541 ctgtacaact atctgaataa ggtatataat caatgaaatt tagaattttt ttctatgctt
3601 actcctgatt ggtaatttgt ttgggtttag aattctatac aaggccattt gtaattttcc
3661 tcagcacttt aaaaatatta aaccatgttt tcttaa
```

[00124] Exemplary antisense strand sequences of 18 nucleotides in length are shown in Table 2 below, which may be optionally further modified and synthesized and incorporated into the RNAi agents, as described herein.

Table 2 Antisense 18 mers of FAS RNAi agents

SEQ ID NO:	AntiSense 18mer 5' to 3'
SEQ ID NO: 2	UCUAAGCCAUGUCCUUCA
SEQ ID NO: 3	UACAAAAAAAGUUUGGUU
SEQ ID NO: 4	AUCACACAAUCUACAUCU
SEQ ID NO: 5	AUAUAUUUACUCAAGUCA
SEQ ID NO: 6	UGGACAUUGUCAUUCUUG
SEQ ID NO: 7	UAUAUUUACUCAAGUCAA
SEQ ID NO: 8	UUGAUCUCAUCUAUUUUG
SEQ ID NO: 9	CAAUCUACAUCUUCUGCA
SEQ ID NO: 10	ACCAUUCUUUCGAACAAA
SEQ ID NO: 11	GUGAUAUAUUUACUCAAG
SEQ ID NO: 12	UGUCAUUCUUGAUCUCAU
SEQ ID NO: 13	GUGGUGAUAUAUUUACUC
SEQ ID NO: 14	CAUUCUUGAUCUCAUCUA
SEQ ID NO: 15	CACCAUUCUUUCGAACAA
SEQ ID NO: 16	AAGCCAUGUCCUUCAUCA
SEQ ID NO: 17	UGAUAUAUUUACUCAAGU
SEQ ID NO: 18	UACGAAGCAGUUGAACUU
SEQ ID NO: 19	ACACAAUCUACAUCUUCU
SEQ ID NO: 20	CAAGGGUCACAGUGUUCA
SEQ ID NO: 21	CUUUAACUUGACUUAGUG
SEQ ID NO: 22	UACAUCUGCACUUGGUAU
SEQ ID NO: 23	GCUGUGUCUUGGACAUUG
SEQ ID NO: 24	UUCUAAGCCAUGUCCUUC
SEQ ID NO: 25	UACAUCUUCUGCAUUUGG
SEQ ID NO: 26	ACAUUGUCAUUCUUGAUC
SEQ ID NO: 27	UUGGUUUACAUCUGCACU
SEQ ID NO: 28	ACACCAUUCUUUCGAACA
SEQ ID NO: 29	CACACAAUCUACAUCUUC
SEQ ID NO: 30	UCUCUGCAAGAGUACAAA
SEQ ID NO: 31	UUGGUGCAAGGGUCACAG
SEQ ID NO: 32	CUACAUCUUCUGCAUUUG
SEQ ID NO: 33	UCUGCUGUGUCUUGGACA
SEQ ID NO: 34	GUACUCCUUCCCUUCUUG
SEQ ID NO: 35	UGGUGAUAUAUUUACUCA
SEQ ID NO: 36	GUCAUUCUUGAUCUCAUC
SEQ ID NO: 37	GAUAUAUUUACUCAAGUC
SEQ ID NO: 38	AUAGUGGUGAUAUAUUUA
SEQ ID NO: 39	GUUUACAUCUGCACUUGG
SEQ ID NO: 40	GUCACAGUGUUCACAUAC
SEQ ID NO: 41	GGACAUUGUCAUUCUUGA

SEQ ID NO: 42	UUAACUUGACUUAGUGUC
SEQ ID NO: 43	AUUCUUGAUCUCAUCUAU
SEQ ID NO: 44	GGGUCACAGUGUUCACAU
SEQ ID NO: 45	UUACAUCUGCACUUGGUA
SEQ ID NO: 46	CAUUGUCAUUCUUGAUCU
SEQ ID NO: 47	UUCUCUGCAAGAGUACAA
SEQ ID NO: 48	UCUCAUCUAUUUUGGCUU
SEQ ID NO: 49	UAGUAAUGUCCUUGAGGA
SEQ ID NO: 50	AGGGUCACAGUGUUCACA
SEQ ID NO: 51	AGUCACUUGGGCAUUAAC
SEQ ID NO: 52	CCAAUUACGAAGCAGUUG
SEQ ID NO: 53	CACAAUCUACAUCUUCUG
SEQ ID NO: 54	UAGUGGUGAUAUAUUUAC
SEQ ID NO: 55	UGGUGAGUGUGCAUUCCU
SEQ ID NO: 56	CCUAGCUUUCCUUUCACC
SEQ ID NO: 57	GUGUCUUGGACAUUGUCA
SEQ ID NO: 58	UGCUGGUGAGUGUGCAUU
SEQ ID NO: 59	CCUUUCACCUGGAGGACA
SEQ ID NO: 60	ACAAUCUACAUCUUCUGC
SEQ ID NO: 61	CCAUGUCCUUCAUCACAC
SEQ ID NO: 62	UUACGAAGCAGUUGAACU
SEQ ID NO: 63	CAGUCACUUGGGCAUUAA
SEQ ID NO: 64	UAUUUACUCAAGUCAACA
SEQ ID NO: 65	AUCUCAUCUAUUUUGGCU
SEQ ID NO: 66	CAAUUACGAAGCAGUUGA
SEQ ID NO: 67	AAUUUUCUCUGCAAGAGU
SEQ ID NO: 68	UCACUAGUAAUGUCCUUG
SEQ ID NO: 69	UGUCUGUGUACUCCUUCC
SEQ ID NO: 70	AACUUGACUUAGUGUCAU
SEQ ID NO: 71	CACUAGUAAUGUCCUUGA
SEQ ID NO: 72	CCAUUCUUUCGAACAAAG
SEQ ID NO: 73	UGGCAGGGCACGCAGUCU
SEQ ID NO: 74	UGAUCUCAUCUAUUUUGG
SEQ ID NO: 75	CUGUGUACUCCUUCCCUU
SEQ ID NO: 76	UGUCCUUCAUCACACAAU
SEQ ID NO: 77	AGCAAUCCUCCGAAGUGA
SEQ ID NO: 78	UGCAGUCCCUAGCUUUCC
SEQ ID NO: 79	UUCCACUUCUAAGCCAUG
SEQ ID NO: 80	AUUUUCUCUGCAAGAGUA
SEQ ID NO: 81	GGGCACGCAGUCUGGUUC
SEQ ID NO: 82	UAACUUGACUUAGUGUCA
SEQ ID NO: 83	GACACCAUUCUUUCGAAC
SEQ ID NO: 84	UGGUGCAAGGGUCACAGU

SEQ ID NO: 85	UCCGGGUGCAGUUUAUUU
SEQ ID NO: 86	UCACUUGGGCAUUAACAC
SEQ ID NO: 87	AAUUACGAAGCAGUUGAA
SEQ ID NO: 88	UUGAGCAAUCCUCCGAAG
SEQ ID NO: 89	CUCAUCUAUUUUGGCUUC
SEQ ID NO: 90	GUUGAGCAAUCCUCCGAA
SEQ ID NO: 91	CCCUAGCUUUCCUUUCAC
SEQ ID NO: 92	GUCUGUGUACUCCUUCCC
SEQ ID NO: 93	GGGUGCAGUUUAUUUCCA
SEQ ID NO: 94	UGUGUCUUGGACAUUGUC
SEQ ID NO: 95	AUGUCCUUCAUCACACAA
SEQ ID NO: 96	ACGCAGUCUGGUUCAUCC
SEQ ID NO: 97	UCAGUCACUUGGGCAUUA
SEQ ID NO: 98	GGCAGGGCACGCAGUCUG
SEQ ID NO: 99	GGUGCAAGGGUCACAGUG
SEQ ID NO: 100	UUUGUCUGUGUACUCCUU
SEQ ID NO: 101	CACGCAGUCUGGUUCAUC
SEQ ID NO: 102	CUUCUUGGCAGGGCACGC
SEQ ID NO: 103	UGCAAGGGUCACAGUGUU
SEQ ID NO: 104	CAUGGUUGUUGAGCAAUC
SEQ ID NO: 105	UUUAACUUGACUUAGUGU
SEQ ID NO: 106	AGUCCCUAGCUUUCCUUU
SEQ ID NO: 107	UGCUGUGUCUUGGACAUU
SEQ ID NO: 108	GUCCCUAGCUUUCCUUUC
SEQ ID NO: 109	UCCUCCGAAGUGAAAGAG
SEQ ID NO: 110	AUCUUCUGCAUUUGGAAG
SEQ ID NO: 111	UGGUUGUUGAGCAAUCCU
SEQ ID NO: 112	UACUCCUUCCCUUCUUGG

Table 3A – Exemplary full-length sense and antisense strands of FAS RNAi agents

Duplex	SEQ ID NO:	Sense Strand	SEQ ID NO:	Antisense Strand
D: 1	SEQ ID NO: 113	GAUGAAGGACAUGGCUUAGAA	SEQ ID NO: 224	UUCUAAGCCAUGUCCUUCAUCAC
D: 2	SEQ ID NO: 114	UAAACCAAACUUUUUUUGUAA	SEQ ID NO: 225	UUACAAAAAAAGUUUGGUUUACA
D: 3	SEQ ID NO: 115	GAAGAUGUAGAUUGUGUGAUA	SEQ ID NO: 226	UAUCACACAAUCUACAUCUUCUG
D: 4	SEQ ID NO: 116	GUUGACUUGAGUAAAUAUAUA	SEQ ID NO: 227	UAUAUAUUUACUCAAGUCAACAU

D: 5	SEQ ID NO: 117	AUCAAGAAUGACAAUGUCCAA	SEQ ID NO: 228	UUGGACAUUGUCAUUCUUGAUCU
D: 6	SEQ ID NO: 118	UGUUGACUUGAGUAAAUAUAA	SEQ ID NO: 229	UUAUAUUUACUCAAGUCAACAUC
D: 7	SEQ ID NO: 119	GCCAAAAUAGAUGAGAUCAAA	SEQ ID NO: 230	UUUGAUCUCAUCUAUUUUGGCUU
D: 8	SEQ ID NO: 120	AAUGCAGAAGAUGUAGAUUGA	SEQ ID NO: 231	UCAAUCUACAUCUUCUGCAUUUG
D: 9	SEQ ID NO: 121	GCUUUGUUCGAAAGAAUGGUA	SEQ ID NO: 232	UACCAUUCUUUCGAACAAAGCCU
D: 10	SEQ ID NO: 122	GACUUGAGUAAAUAUAUCACA	SEQ ID NO: 233	UGUGAUAUAUUUACUCAAGUCAA
D: 11	SEQ ID NO: 123	AGAUGAGAUCAAGAAUGACAA	SEQ ID NO: 234	UUGUCAUUCUUGAUCUCAUCUAU
D: 12	SEQ ID NO: 124	UUGAGUAAAUAUAUCACCACA	SEQ ID NO: 235	UGUGGUGAUAUAUUUACUCAAGU
D: 13	SEQ ID NO: 125	AAUAGAUGAGAUCAAGAAUGA	SEQ ID NO: 236	UCAUUCUUGAUCUCAUCUAUUUU
D: 14	SEQ ID NO: 126	CUUUGUUCGAAAGAAUGGUGA	SEQ ID NO: 237	UCACCAUUCUUUCGAACAAAGCC
D: 15	SEQ ID NO: 127	UGUGAUGAAGGACAUGGCUUA	SEQ ID NO: 238	UAAGCCAUGUCCUUCAUCACACA
D: 16	SEQ ID NO: 128	UGACUUGAGUAAAUAUAUCAA	SEQ ID NO: 239	UUGAUAUAUUUACUCAAGUCAAC
D: 17	SEQ ID NO: 129	GAAAGUUCAACUGCUUCGUAA	SEQ ID NO: 240	UUACGAAGCAGUUGAACUUUCUG
D: 18	SEQ ID NO: 130	GCAGAAGAUGUAGAUUGUGUA	SEQ ID NO: 241	UACACAAUCUACAUCUUCUGCAU
D: 19	SEQ ID NO: 131	UGUGAACACUGUGACCCUUGA	SEQ ID NO: 242	UCAAGGGUCACAGUGUUCACAUA
D: 20	SEQ ID NO: 132	GACACUAAGUCAAGUUAAAGA	SEQ ID NO: 243	UCUUUAACUUGACUUAGUGUCAU
D: 21	SEQ ID NO: 133	GAAUACCAAGUGCAGAUGUAA	SEQ ID NO: 244	UUACAUCUGCACUUGGUAUUCUG
D: 22	SEQ ID NO: 134	GACAAUGUCCAAGACACAGCA	SEQ ID NO: 245	UGCUGUGUCUUGGACAUUGUCAU

D: 23	SEQ ID NO: 135	AUGAAGGACAUGGCUUAGAAA	SEQ ID NO: 246	UUUCUAAGCCAUGUCCUUCAUCA
D: 24	SEQ ID NO: 136	UUCCAAAUGCAGAAGAUGUAA	SEQ ID NO: 247	UUACAUCUUCUGCAUUUGGAAGA
D: 25	SEQ ID NO: 137	GAGAUCAAGAAUGACAAUGUA	SEQ ID NO: 248	UACAUUGUCAUUCUUGAUCUCAU
D: 26	SEQ ID NO: 138	CAAGUGCAGAUGUAAACCAAA	SEQ ID NO: 249	UUUGGUUUACAUCUGCACUUGGU
D: 27	SEQ ID NO: 139	UUUGUUCGAAAGAAUGGUGUA	SEQ ID NO: 250	UACACCAUUCUUUCGAACAAAGC
D: 28	SEQ ID NO: 140	CAGAAGAUGUAGAUUGUGUGA	SEQ ID NO: 251	UCACACAAUCUACAUCUUCUGCA
D: 29	SEQ ID NO: 141	UCUUUGUACUCUUGCAGAGAA	SEQ ID NO: 252	UUCUCUGCAAGAGUACAAAGAUU
D: 30	SEQ ID NO: 142	CACUGUGACCCUUGCACCAAA	SEQ ID NO: 253	UUUGGUGCAAGGGUCACAGUGUU
D: 31	SEQ ID NO: 143	UCCAAAUGCAGAAGAUGUAGA	SEQ ID NO: 254	UCUACAUCUUCUGCAUUUGGAAG
D: 32	SEQ ID NO: 144	AAUGUCCAAGACACAGCAGAA	SEQ ID NO: 255	UUCUGCUGUGUCUUGGACAUUGU
D: 33	SEQ ID NO: 145	GCCAAGAAGGGAAGGAGUACA	SEQ ID NO: 256	UGUACUCCUUCCCUUCUUGGCAG
D: 34	SEQ ID NO: 146	CUUGAGUAAAUAUAUCACCAA	SEQ ID NO: 257	UUGGUGAUAUAUUUACUCAAGUC
D: 35	SEQ ID NO: 147	UAGAUGAGAUCAAGAAUGACA	SEQ ID NO: 258	UGUCAUUCUUGAUCUCAUCUAUU
D: 36	SEQ ID NO: 148	UUGACUUGAGUAAAUAUAUCA	SEQ ID NO: 259	UGAUAUAUUUACUCAAGUCAACA
D: 37	SEQ ID NO: 149	AGUAAAUAUAUCACCACUAUA	SEQ ID NO: 260	UAUAGUGGUGAUAUAUUUACUCA
D: 38	SEQ ID NO: 150	UACCAAGUGCAGAUGUAAACA	SEQ ID NO: 261	UGUUUACAUCUGCACUUGGUAUU
D: 39	SEQ ID NO: 151	CUGUAUGUGAACACUGUGACA	SEQ ID NO: 262	UGUCACAGUGUUCACAUACAGUA
D: 40	SEQ ID NO: 152	GAUCAAGAAUGACAAUGUCCA	SEQ ID NO: 263	UGGACAUUGUCAUUCUUGAUCUC

D: 41	SEQ ID NO: 153	AUGACACUAAGUCAAGUUAAA	SEQ ID NO: 264	UUUAACUUGACUUAGUGUCAUGA
D: 42	SEQ ID NO: 154	AAAUAGAUGAGAUCAAGAAUA	SEQ ID NO: 265	UAUUCUUGAUCUCAUCUAUUUUG
D: 43	SEQ ID NO: 155	GUAUGUGAACACUGUGACCCA	SEQ ID NO: 266	UGGGUCACAGUGUUCACAUACAG
D: 44	SEQ ID NO: 156	AAUACCAAGUGCAGAUGUAAA	SEQ ID NO: 267	UUUACAUCUGCACUUGGUAUUCU
D: 45	SEQ ID NO: 157	UGAGAUCAAGAAUGACAAUGA	SEQ ID NO: 268	UCAUUGUCAUUCUUGAUCUCAUC
D: 46	SEQ ID NO: 158	CUUUGUACUCUUGCAGAGAAA	SEQ ID NO: 269	UUUCUCUGCAAGAGUACAAAGAU
D: 47	SEQ ID NO: 159	UGAAGCCAAAAUAGAUGAGAA	SEQ ID NO: 270	UUCUCAUCUAUUUUGGCUUCAUU
D: 48	SEQ ID NO: 160	CAUCCUCAAGGACAUUACUAA	SEQ ID NO: 271	UUAGUAAUGUCCUUGAGGAUGAU
D: 49	SEQ ID NO: 161	UAUGUGAACACUGUGACCCUA	SEQ ID NO: 272	UAGGGUCACAGUGUUCACAUACA
D: 50	SEQ ID NO: 162	GUGUUAAUGCCCAAGUGACUA	SEQ ID NO: 273	UAGUCACUUGGGCAUUAACACUU
D: 51	SEQ ID NO: 163	UUCAACUGCUUCGUAAUUGGA	SEQ ID NO: 274	UCCAAUUACGAAGCAGUUGAACU
D: 52	SEQ ID NO: 164	UGCAGAAGAUGUAGAUUGUGA	SEQ ID NO: 275	UCACAAUCUACAUCUUCUGCAUU
D: 53	SEQ ID NO: 165	GAGUAAAUAUAUCACCACUAA	SEQ ID NO: 276	UUAGUGGUGAUAUAUUUACUCAA
D: 54	SEQ ID NO: 166	CAAGGAAUGCACACUCACCAA	SEQ ID NO: 277	UUGGUGAGUGUGCAUUCCUUGAU
D: 55	SEQ ID NO: 167	CAGGUGAAAGGAAAGCUAGGA	SEQ ID NO: 278	UCCUAGCUUUCCUUUCACCUGGA
D: 56	SEQ ID NO: 168	AAUGACAAUGUCCAAGACACA	SEQ ID NO: 279	UGUGUCUUGGACAUUGUCAUUCU
D: 57	SEQ ID NO: 169	GGAAUGCACACUCACCAGCAA	SEQ ID NO: 280	UUGCUGGUGAGUGUGCAUUCCUU
D: 58	SEQ ID NO: 170	CCUGUCCUCCAGGUGAAAGGA	SEQ ID NO: 281	UCCUUUCACCUGGAGGACAGGGC

D: 59	SEQ ID NO: 171	AUGCAGAAGAUGUAGAUUGUA	SEQ ID NO: 282	UACAAUCUACAUCUUCUGCAUUU
D: 60	SEQ ID NO: 172	UUGUGUGAUGAAGGACAUGGA	SEQ ID NO: 283	UCCAUGUCCUUCAUCACACAAUC
D: 61	SEQ ID NO: 173	AAAGUUCAACUGCUUCGUAAA	SEQ ID NO: 284	UUUACGAAGCAGUUGAACUUUCU
D: 62	SEQ ID NO: 174	UGUUAAUGCCCAAGUGACUGA	SEQ ID NO: 285	UCAGUCACUUGGGCAUUAACACU
D: 63	SEQ ID NO: 175	GAUGUUGACUUGAGUAAAUAA	SEQ ID NO: 286	UUAUUUACUCAAGUCAACAUCAG
D: 64	SEQ ID NO: 176	GAAGCCAAAAUAGAUGAGAUA	SEQ ID NO: 287	UAUCUCAUCUAUUUUGGCUUCAU
D: 65	SEQ ID NO: 177	GUUCAACUGCUUCGUAAUUGA	SEQ ID NO: 288	UCAAUUACGAAGCAGUUGAACUU
D: 66	SEQ ID NO: 178	GUACUCUUGCAGAGAAAAUUA	SEQ ID NO: 289	UAAUUUUCUCUGCAAGAGUACAA
D: 67	SEQ ID NO: 179	CUCAAGGACAUUACUAGUGAA	SEQ ID NO: 290	UUCACUAGUAAUGUCCUUGAGGA
D: 68	SEQ ID NO: 180	AGGGAAGGAGUACACAGACAA	SEQ ID NO: 291	UUGUCUGUGUACUCCUUCCCUUC
D: 69	SEQ ID NO: 181	UCAUGACACUAAGUCAAGUUA	SEQ ID NO: 292	UAACUUGACUUAGUGUCAUGACU
D: 70	SEQ ID NO: 182	CCUCAAGGACAUUACUAGUGA	SEQ ID NO: 293	UCACUAGUAAUGUCCUUGAGGAU
D: 71	SEQ ID NO: 183	GGCUUUGUUCGAAAGAAUGGA	SEQ ID NO: 294	UCCAUUCUUUCGAACAAAGCCUU
D: 72	SEQ ID NO: 184	CCAGACUGCGUGCCCUGCCAA	SEQ ID NO: 295	UUGGCAGGGCACGCAGUCUGGUU
D: 73	SEQ ID NO: 185	AGCCAAAAUAGAUGAGAUCAA	SEQ ID NO: 296	UUGAUCUCAUCUAUUUUGGCUUC
D: 74	SEQ ID NO: 186	AGAAGGGAAGGAGUACACAGA	SEQ ID NO: 297	UCUGUGUACUCCUUCCCUUCUUG
D: 75	SEQ ID NO: 187	AGAUUGUGUGAUGAAGGACAA	SEQ ID NO: 298	UUGUCCUUCAUCACACAAUCUAC
D: 76	SEQ ID NO: 188	UUUCACUUCGGAGGAUUGCUA	SEQ ID NO: 299	UAGCAAUCCUCCGAAGUGAAAGA

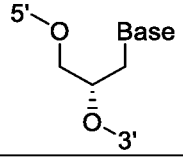
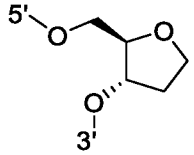
D: 77	SEQ ID NO: 189	AAGGAAAGCUAGGGACUGCAA	SEQ ID NO: 300	UUGCAGUCCCUAGCUUUCCUUUC
D: 78	SEQ ID NO: 190	GACAUGGCUUAGAAGUGGAAA	SEQ ID NO: 301	UUUCCACUUCUAAGCCAUGUCCU
D: 79	SEQ ID NO: 191	UGUACUCUUGCAGAGAAAAUA	SEQ ID NO: 302	UAUUUUCUCUGCAAGAGUACAAA
D: 80	SEQ ID NO: 192	AUGAACCAGACUGCGUGCCCA	SEQ ID NO: 303	UGGGCACGCAGUCUGGUUCAUCC
D: 81	SEQ ID NO: 193	CAUGACACUAAGUCAAGUUAA	SEQ ID NO: 304	UUAACUUGACUUAGUGUCAUGAC
D: 82	SEQ ID NO: 194	UUGUUCGAAAGAAUGGUGUCA	SEQ ID NO: 305	UGACACCAUUCUUUCGAACAAAG
D: 83	SEQ ID NO: 195	ACACUGUGACCCUUGCACCAA	SEQ ID NO: 306	UUGGUGCAAGGGUCACAGUGUUC
D: 84	SEQ ID NO: 196	GGAAAUAAACUGCACCCGGAA	SEQ ID NO: 307	UUCCGGGUGCAGUUUAUUUCCAC
D: 85	SEQ ID NO: 197	AAGUGUUAAUGCCCAAGUGAA	SEQ ID NO: 308	UUCACUUGGGCAUUAACACUUUU
D: 86	SEQ ID NO: 198	AGUUCAACUGCUUCGUAAUUA	SEQ ID NO: 309	UAAUUACGAAGCAGUUGAACUUU
D: 87	SEQ ID NO: 199	CACUUCGGAGGAUUGCUCAAA	SEQ ID NO: 310	UUUGAGCAAUCCUCCGAAGUGAA
D: 88	SEQ ID NO: 200	AUGAAGCCAAAAUAGAUGAGA	SEQ ID NO: 311	UCUCAUCUAUUUUGGCUUCAUUG
D: 89	SEQ ID NO: 201	ACUUCGGAGGAUUGCUCAACA	SEQ ID NO: 312	UGUUGAGCAAUCCUCCGAAGUGA
D: 90	SEQ ID NO: 202	AGGUGAAAGGAAAGCUAGGGA	SEQ ID NO: 313	UCCCUAGCUUUCCUUUCACCUGG
D: 91	SEQ ID NO: 203	AAGGGAAGGAGUACACAGACA	SEQ ID NO: 314	UGUCUGUGUACUCCUUCCCUUCU
D: 92	SEQ ID NO: 204	AGUGGAAAUAAACUGCACCCA	SEQ ID NO: 315	UGGGUGCAGUUUAUUUCCACUUC
D: 93	SEQ ID NO: 205	AUGACAAUGUCCAAGACACAA	SEQ ID NO: 316	UUGUGUCUUGGACAUUGUCAUUC
D: 94	SEQ ID NO: 206	GAUUGUGUGAUGAAGGACAUA	SEQ ID NO: 317	UAUGUCCUUCAUCACACAAUCUA

D: 95	SEQ ID NO: 207	GGGGAUGAACCAGACUGCGUA	SEQ ID NO: 318	UACGCAGUCUGGUUCAUCCCCAU
D: 96	SEQ ID NO: 208	GUUAAUGCCCAAGUGACUGAA	SEQ ID NO: 319	UUCAGUCACUUGGGCAUUAACAC
D: 97	SEQ ID NO: 209	ACCAGACUGCGUGCCCUGCCA	SEQ ID NO: 320	UGGCAGGGCACGCAGUCUGGUUC
D: 98	SEQ ID NO: 210	AACACUGUGACCCUUGCACCA	SEQ ID NO: 321	UGGUGCAAGGGUCACAGUGUUCA
D: 99	SEQ ID NO: 211	GGAAGGAGUACACAGACAAAA	SEQ ID NO: 322	UUUUGUCUGUGUACUCCUUCCCU
D: 100	SEQ ID NO: 212	GGGAUGAACCAGACUGCGUGA	SEQ ID NO: 323	UCACGCAGUCUGGUUCAUCCCCA
D: 101	SEQ ID NO: 213	CUGCGUGCCCUGCCAAGAAGA	SEQ ID NO: 324	UCUUCUUGGCAGGGCACGCAGUC
D: 102	SEQ ID NO: 214	UGAACACUGUGACCCUUGCAA	SEQ ID NO: 325	UUGCAAGGGUCACAGUGUUCACA
D: 103	SEQ ID NO: 215	AGGAUUGCUCAACAACCAUGA	SEQ ID NO: 326	UCAUGGUUGUUGAGCAAUCCUCC
D: 104	SEQ ID NO: 216	UGACACUAAGUCAAGUUAAAA	SEQ ID NO: 327	UUUUAACUUGACUUAGUGUCAUG
D: 105	SEQ ID NO: 217	UGAAAGGAAAGCUAGGGACUA	SEQ ID NO: 328	UAGUCCCUAGCUUUCCUUUCACC
D: 106	SEQ ID NO: 218	ACAAUGUCCAAGACACAGCAA	SEQ ID NO: 329	UUGCUGUGUCUUGGACAUUGUCA
D: 107	SEQ ID NO: 219	GUGAAAGGAAAGCUAGGGACA	SEQ ID NO: 330	UGUCCCUAGCUUUCCUUUCACCU
D: 108	SEQ ID NO: 220	AGCUCUUUCACUUCGGAGGAA	SEQ ID NO: 331	UUCCUCCGAAGUGAAAGAGCUUC
D: 109	SEQ ID NO: 221	UUCUUCCAAAUGCAGAAGAUA	SEQ ID NO: 332	UAUCUUCUGCAUUUGGAAGAAAA
D: 110	SEQ ID NO: 222	GGAGGAUUGCUCAACAACCAA	SEQ ID NO: 333	UUGGUUGUUGAGCAAUCCUCCGA
D: 111	SEQ ID NO: 223	UGCCAAGAAGGGAAGGAGUAA	SEQ ID NO: 334	UUACUCCUUCCCUUCUUGGCAGG
D: 112	SEQ ID NO: 335	GAAAGUUCA-(AP)-CUGCUUCGUAA	SEQ ID NO: 240	UUACGAAGCAGUUGAACUUUCUG

D: 113	SEQ ID NO: 336	GUUGACUUG-(AP)-GUAAAUAUAUA	SEQ ID NO: 227	UAUAUAUUUACUCAAGUCAACAU
D: 114	SEQ ID NO: 116	GUUGACUUGAGUAAAUAUAUA	SEQ ID NO: 337	UAUAU-GNA(A)-UUUACUCAAGUCAACAU
D: 115	SEQ ID NO: 116	GUUGACUUGAGUAAAUAUAUA	SEQ ID NO: 338	UAUAUA-GNA(U)-UUACUCAAGUCAACAU
D:236	SEQ ID NO: 576	AGAAAGUUCAACUGCUUCGUA	SEQ ID NO: 577	UACGAAGCAGUUGAACUUUCUGU

GNA indicates a glycol nucleic acid nucleotide (structure shown in Table 3B);
(AP) means an apurinic/apyrimidinic residue, also called an abasic residue (structure shown in Table 3B).

Table 3B: Structures of GNA and abasic residue

GNA	
Abasic	

EXAMPLE 4:
In vitro knockdown of hFAS in HepG2 cells

[00125] The RNAi agents in Tables 4A and 4B were tested in HepG2 cells. Reverse transfection was carried out by adding 24.7µl of Opti-MEM plus 0.3µl of Lipofectamine RNAiMAX per well to 25µl of each 4X human FAS-GalNAc siRNA to an individual well in a 96-well collagen I-coated plate. The mixture was incubated at room temperature for 20 minutes and then fifty µl of Growth Media containing HepG2 cells at 300,000 cells/ml were added to the human FAS-GalNAc siRNA/RNAiMAX mixture. Final concentration of the siRNAs as with the above was 500 nM for a single concentration screen. Cells were incubated for 24-48 hours before RNA isolation with Quick-RNA 96 Kit. RNA was then stored at -80 oC or subject to cDNA synthesis. Briefly, cDNA was synthesized from the purified RNA using Fast Advanced RT Master Mix (Invitrogen). A master mix of 5µl 2X Fast Advanced RT Buffer and 0.5µl 20X Fast

Advanced RT Enzyme Mix per reaction was prepared. 5.5µl master mix and 4.5µl RNA were mixed for a final volume of 10µl. cDNA was generated using a ProFlex PCR System (Life Technologies) through the following steps: 37oC for 30 minutes, 95 oC for 5 minutes, and 4 oC hold.

[00126] Two µl of cDNA were added to a master mix containing 2.5µl of H2O, 0.5µl 20X TaqMan Gene Expression Assay Buffer (Life Technologies) and 5µl 2X TaqMan Universal PCR Master Mix (Life Technologies). A QuantStudio 7 Flex Real-Time PCR System (Life Technologies) was used to complete the following PCR cycles: 50 oC for 2 minutes, 95 oC for 10 minutes, 40 cycles of 95 oC for 15 seconds and 60 oC for 1 minute. TaqMan Gene Expression Assays were performed. Data analysis uses the ddCt method.

[00127] Select siRNAs from each assay were used for determining an IC50, using 1:3 serial dilution to final concentrations of 200, 67, 22, 7.41, 2.47, 0.82, and 0.27 nM FAS-GalNAc RNAi agent for concentration response curves. IC50 values were calculated using a 4-parameter fit model using XLFit.

[00128] Data is shown in Table 5.

EXAMPLE 5:
In vivo knockdown in hFAS-AAV treated mice with the FAS RNAi agents herein

[00129] Mice were administered AAV vector for expressing human FAS (1x1011 GC/mouse) via retroorbital injection after anesthetization via isoflurane. 100ul of AAV (in PBS) is injected into the venous sinus and mice were monitored for recovery in cage. Two weeks following AAV administration, mice were administered a set of siRNA agents of Table 4A and 4B, as indicated in Table 6A and 6B, subcutaneously, except that all siRNA agents were lacking the phosphate addition on the 5' end of the antisense strand for administration to mice.

[00130] Mice were sacrificed, and serum and livers (in RNAlater Stabilization Solution, Ambion) were collected. Total liver RNA was isolated, purified, and subject to QRT-PCR as described above.

[00131] Results showed the gene expression of human FAS target gene normalized to mouse Rplp0 (Life Technologies, part#: Mm01974474_gH), and represented as the

relative knockdown of human FAS mRNA expression compared to vehicle-treated control animals. Knockdown results at 2 weeks post treatment at 5 mg/kg (mpk), or 10 weeks at 1 mg/kg, 3 mg/kg and 5 mg/kg doses are shown for the RNAi agents indicated in Tables 6A and 6B.

[00132] Some RNAi agents tested for gene expression knockdown in vivo were further tested for protein knock-down per Example 6.

Table 4A – FAS-GalNAc RNAi agents, modified sense and antisense strands

Duplex	SEQ ID NO:	Modified Sequence
D: 112	SEQ ID NO: 339	mG*mA*mUmGmAmAfGmGfAfCfAmUmGmGmCmUmUmAmG*mA*mAP
	SEQ ID NO: 340	PmU*fU*mCmUmAfAmGmCmCmAmUmGmUfCmCfUmUmCmAmUmC*mA*mC
D: 113	SEQ ID NO: 341	mU*mA*mAmAmCmCfAmAfAfCfUmUmUmUmUmUmGmU*mA*mAP
	SEQ ID NO: 342	PmU*fU*mAmCmAfAmAmAmAmAmGmUfUmUfGmGmUmUmUmA*mC*mA
D: 114	SEQ ID NO: 343	mG*mA*mAmGmAmUfGmUfAfGfAmUmUmGmUmGmUmGmA*mU*mAP
	SEQ ID NO: 344	PmU*fA*mUmCmAfCmAmCmAmAmUmCmUfAmCfAmUmCmUmUmC*mU*mG
D: 115	SEQ ID NO: 345	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 346	PmU*fA*mUmAmUfAmUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 116	SEQ ID NO: 347	mA*mU*mCmAmAmGfAmAfUfGfAmCmAmAmUmGmUmCmC*mA*mAP
	SEQ ID NO: 348	PmU*fU*mGmGmAfCmAmUmUmGmUmCmAfUmUfCmUmUmGmAmU*mC*mU
D: 117	SEQ ID NO: 349	mU*mG*mUmUmGmAfCmUfUfGfAmGmUmAmAmAmUmAmU*mA*mAP
	SEQ ID NO: 350	PmU*fU*mAmUmAfUmUmUmAmCmUmCmAfAmGfUmCmAmAmCmA*mU*mC
D: 118	SEQ ID NO: 351	mG*mC*mCmAmAmAfAmUfAfGfAmUmGmAmGmAmUmCmA*mA*mAP
	SEQ ID NO: 352	PmU*fU*mUmGmAfUmCmUmCmAmUmCmUfAmUfUmUmUmGmGmC*mU*mU
D: 119	SEQ ID NO: 353	mA*mA*mUmGmCmAfGmAfAfGfAmUmGmUmAmGmAmUmU*mG*mAP
	SEQ ID NO: 354	PmU*fC*mAmAmUfCmUmAmCmAmUmCmUfUmCfUmGmCmAmUmU*mU*mG
D: 120	SEQ ID NO: 355	mG*mC*mUmUmUmGfUmUfCfGfAmAmAmGmAmAmUmGmG*mU*mAP
	SEQ ID NO: 356	PmU*fA*mCmCmAfUmUmCmUmUmUmCmGfAmAfCmAmAmAmGmC*mC*mU
D: 121	SEQ ID NO: 357	mG*mA*mCmUmUmGfAmGfUfAfAmAmUmAmUmAmUmCmA*mC*mAP
	SEQ ID NO: 358	PmU*fG*mUmGmAfUmAmUmAmUmUmUmAfCmUfCmAmAmGmUmC*mA*mA
D: 122	SEQ ID NO: 359	mA*mG*mAmUmGmAfGmAfUfCfAmAmGmAmAmUmGmAmC*mA*mAP
	SEQ ID NO: 360	PmU*fU*mGmUmCfAmUmUmCmUmUmGmAfUmCfUmCmAmUmCmU*mA*mU
D: 123	SEQ ID NO: 361	mU*mU*mGmAmGmUfAmAfAfUfAmUmAmUmCmAmCmCmA*mC*mAP
	SEQ ID NO: 362	PmU*fG*mUmGmGfUmGmAmUmAmUmAmUfUmUfAmCmUmCmAmA*mG*mU
D: 124	SEQ ID NO: 363	mA*mA*mUmAmGmAfUmGfAfGfAmUmCmAmAmGmAmAmU*mG*mAP
	SEQ ID NO: 364	PmU*fC*mAmUmUfCmUmUmGmAmUmCmUfCmAfUmCmUmAmUmU*mU*mU
D: 125	SEQ ID NO: 365	mC*mU*mUmUmGmUfUmCfGfAfAmAmGmAmAmUmGmGmU*mG*mAP
	SEQ ID NO: 366	PmU*fC*mAmCmCfAmUmUmCmUmUmUmCfGmAfAmCmAmAmAmG*mC*mC
D: 126	SEQ ID NO: 367	mU*mG*mUmGmAmUfGmAfAfGfGmAmCmAmUmGmGmCmU*mU*mAP
	SEQ ID NO: 368	PmU*fA*mAmGmCfCmAmUmGmUmCmCmUfUmCfAmUmCmAmCmA*mC*mA

D: 127	SEQ ID NO: 369	mU*mG*mAmCmUmUfGmAfGfUfAmAmAmUmAmUmAmUmC*mA*mAP
	SEQ ID NO: 370	PmU*fU*mGmAmUfAmUmAmUmUmUmAmCfUmCfAmAmGmUmCmA*mA*mC
D: 128	SEQ ID NO: 371	mG*mA*mAmAmGmUfUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 372	PmU*fU*mAmCmGfAmAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 129	SEQ ID NO: 373	mG*mC*mAmGmAmAfGmAfUfGfUmAmGmAmUmUmGmUmG*mU*mAP
	SEQ ID NO: 374	PmU*fA*mCmAmCfAmAmUmCmUmAmCmAfUmCfUmUmCmUmGmC*mA*mU
D: 130	SEQ ID NO: 375	mU*mG*mUmGmAmAfCmAfCfUfGmUmGmAmCmCmCmUmU*mG*mAP
	SEQ ID NO: 376	PmU*fC*mAmAmGfGmGmUmCmAmCmAmGfUmGfUmUmCmAmCmA*mU*mA
D: 131	SEQ ID NO: 377	mG*mA*mCmAmCmUfAmAfGfUfCmAmAmGmUmUmAmAmA*mG*mAP
	SEQ ID NO: 378	PmU*fC*mUmUmUfAmAmCmUmUmGmAmCfUmUfAmGmUmGmUmC*mA*mU
D: 132	SEQ ID NO: 379	mG*mA*mAmUmAmCfCmAfAfGfUmGmCmAmGmAmUmGmU*mA*mAP
	SEQ ID NO: 380	PmU*fU*mAmCmAfUmCmUmGmCmAmCmUfUmGfGmUmAmUmUmC*mU*mG
D: 133	SEQ ID NO: 381	mG*mA*mCmAmAmUfGmUfCfCfAmAmGmAmCmAmCmAmG*mC*mAP
	SEQ ID NO: 382	PmU*fG*mCmUmGfUmGmUmCmUmUmGmGfAmCfAmUmUmGmUmC*mA*mU
D: 134	SEQ ID NO: 383	mA*mU*mGmAmAmGfGmAfCfAfUmGmGmCmUmUmAmGmA*mA*mAP
	SEQ ID NO: 384	PmU*fU*mUmCmUfAmAmGmCmCmAmUmGfUmCfCmUmUmCmAmU*mC*mA
D: 135	SEQ ID NO: 385	mU*mU*mCmCmAmAfAmUfGfCfAmGmAmAmGmAmUmGmU*mA*mAP
	SEQ ID NO: 386	PmU*fU*mAmCmAfUmCmUmUmCmUmGmCfAmUfUmUmGmGmAmA*mG*mA
D: 136	SEQ ID NO: 387	mG*mA*mGmAmUmCfAmAfGfAfAmUmGmAmCmAmAmUmG*mU*mAP
	SEQ ID NO: 388	PmU*fA*mCmAmUfUmGmUmCmAmUmUmCfUmUfGmAmUmCmUmC*mA*mU
D: 137	SEQ ID NO: 389	mC*mA*mAmGmUmGfCmAfGfAfUmGmUmAmAmAmCmCmA*mA*mAP
	SEQ ID NO: 390	PmU*fU*mUmGmGfUmUmUmAmCmAmUmCfUmGfCmAmCmUmUmG*mG*mU
D: 138	SEQ ID NO: 391	mU*mU*mUmGmUmUfCmGfAfAfAmGmAmAmUmGmGmUmG*mU*mAP
	SEQ ID NO: 392	PmU*fA*mCmAmCfCmAmUmUmCmUmUmUfCmGfAmAmCmAmAmA*mG*mC
D: 139	SEQ ID NO: 393	mC*mA*mGmAmAmGfAmUfGfUfAmGmAmUmUmGmUmGmU*mG*mAP
	SEQ ID NO: 394	PmU*fC*mAmCmAfCmAmAmUmCmUmAmCfAmUfCmUmUmCmUmG*mC*mA
D: 140	SEQ ID NO: 395	mU*mC*mUmUmUmGfUmAfCfUfCmUmUmGmCmAmGmAmG*mA*mAP
	SEQ ID NO: 396	PmU*fU*mCmUmCfUmGmCmAmAmGmAmGfUmAfCmAmAmAmGmA*mU*mU
D: 141	SEQ ID NO: 397	mC*mA*mCmUmGmUfGmAfCfCfCfCmUmUmGmCmAmCmCmA*mA*mAP
	SEQ ID NO: 398	PmU*fU*mUmGmGfUmGmCmAmAmGmGmGfUmCfAmCmAmGmUmG*mU*mU
D: 142	SEQ ID NO: 399	mU*mC*mCmAmAmAfUmGfCfAfGmAmAmGmAmUmGmUmA*mG*mAP
	SEQ ID NO: 400	PmU*fC*mUmAmCfAmUmCmUmUmCmUmGfCmAfUmUmUmGmGmA*mA*mG
D: 143	SEQ ID NO: 401	mA*mA*mUmGmUmCfCmAfAfGfAmCmAmCmAmGmCmAmG*mA*mAP
	SEQ ID NO: 402	PmU*fU*mCmUmGfCmUmGmUmGmUmCmUfUmGfGmAmCmAmUmU*mG*mU
D: 144	SEQ ID NO: 403	mG*mC*mCmAmAmGfAmAfGfGfGmAmAmGmGmAmGmUmA*mC*mAP
	SEQ ID NO: 404	PmU*fG*mUmAmCfUmCmCmUmUmCmCmCfUmUfCmUmUmGmGmC*mA*mG
D: 145	SEQ ID NO: 405	mC*mU*mUmGmAmGfUmAfAfAfUmAmUmAmUmCmAmCmC*mA*mAP
	SEQ ID NO: 406	PmU*fU*mGmGmUfGmAmUmAmUmAmUmUfUmAfCmUmCmAmAmG*mU*mC
D: 146	SEQ ID NO: 407	mU*mA*mGmAmUmGfAmGfAfUfCmAmAmGmAmAmUmGmA*mC*mAP
	SEQ ID NO: 408	PmU*fG*mUmCmAfUmUmCmUmUmGmAmUfCmUfCmAmUmCmUmA*mU*mU
D: 147	SEQ ID NO: 409	mU*mU*mGmAmCmUfUmGfAfGfUmAmAmAmUmAmUmAmU*mC*mAP
	SEQ ID NO: 410	PmU*fG*mAmUmAfUmAmUmUmUmAmCmUfCmAfAmGmUmCmAmA*mC*mA

D: 148	SEQ ID NO: 411	mA*mG*mUmAmAmAfUmAfUfAfUmCmAmCmCmAmCmUmA*mU*mAP
	SEQ ID NO: 412	PmU*fA*mUmAmGfUmGmGmUmGmAmUmAfUmAfUmUmUmAmCmU*mC*mA
D: 149	SEQ ID NO: 413	mU*mA*mCmCmAmAfGmUfGfCfAmGmAmUmGmUmAmAmA*mC*mAP
	SEQ ID NO: 414	PmU*fG*mUmUmUfAmCmAmUmCmUmGmCfAmCfUmUmGmGmUmA*mU*mU
D: 150	SEQ ID NO: 415	mC*mU*mGmUmAmUfGmUfGfAfAmCmAmCmUmGmUmGmA*mC*mAP
	SEQ ID NO: 416	PmU*fG*mUmCmAfCmAmGmUmGmUmUmCfAmCfAmUmAmCmAmG*mU*mA
D: 151	SEQ ID NO: 417	mG*mA*mUmCmAmAfGmAfAfUfGmAmCmAmAmUmGmUmC*mC*mAP
	SEQ ID NO: 418	PmU*fG*mGmAmCfAmUmUmGmUmCmAmUfUmCfUmUmGmAmUmC*mU*mC
D: 152	SEQ ID NO: 419	mA*mU*mGmAmCmAfCmUfAfAfGmUmCmAmAmGmUmUmA*mA*mAP
	SEQ ID NO: 420	PmU*fU*mUmAmAfCmUmUmGmAmCmUmUfAmGfUmGmUmCmAmU*mG*mA
D: 153	SEQ ID NO: 421	mA*mA*mAmUmAmGfAmUfGfAfGmAmUmCmAmAmGmAmA*mU*mAP
	SEQ ID NO: 422	PmU*fA*mUmUmCfUmUmGmAmUmCmUmCfAmUfCmUmAmUmUmU*mU*mG
D: 154	SEQ ID NO: 423	mG*mU*mAmUmGmUfGmAfAfCfAmCmUmGmUmGmAmCmC*mC*mAP
	SEQ ID NO: 424	PmU*fG*mGmGmUfCmAmCmAmGmUmGmUfUmCfAmCmAmUmAmC*mA*mG
D: 155	SEQ ID NO: 425	mA*mA*mUmAmCmCfAmAfGfUfGmCmAmGmAmUmGmUmA*mA*mAP
	SEQ ID NO: 426	PmU*fU*mUmAmCfAmUmCmUmGmCmAmCfUmUfGmGmUmAmUmU*mC*mU
D: 156	SEQ ID NO: 427	mU*mG*mAmGmAmUfCmAfAfGfAmAmUmGmAmCmAmAmU*mG*mAP
	SEQ ID NO: 428	PmU*fC*mAmUmUfGmUmCmAmUmUmCmUfUmGfAmUmCmUmCmA*mU*mC
D: 157	SEQ ID NO: 429	mC*mU*mUmUmGmUfAmCfUfCfUmUmGmCmAmGmAmGmA*mA*mAP
	SEQ ID NO: 430	PmU*fU*mUmCmUfCmUmGmCmAmAmGmAfGmUfAmCmAmAmAmG*mA*mU
D: 158	SEQ ID NO: 431	mU*mG*mAmAmGmCfCmAfAfAfAmUmAmGmAmUmGmAmG*mA*mAP
	SEQ ID NO: 432	PmU*fU*mCmUmCfAmUmCmUmAmUmUmUfUmGfGmCmUmUmCmA*mU*mU
D: 159	SEQ ID NO: 433	mC*mA*mUmCmCmUfCmAfAfGfGmAmCmAmUmUmAmCmU*mA*mAP
	SEQ ID NO: 434	PmU*fU*mAmGmUfAmAmUmGmUmCmCmUfUmGfAmGmGmAmUmG*mA*mU
D: 160	SEQ ID NO: 435	mU*mA*mUmGmUmGfAmAfCfAfCmUmGmUmGmAmCmCmC*mU*mAP
	SEQ ID NO: 436	PmU*fA*mGmGmGfUmCmAmCmAmGmUmGfUmUfCmAmCmAmUmA*mC*mA
D: 161	SEQ ID NO: 437	mG*mU*mGmUmUmAfAmUfGfCfCmCmAmAmGmUmGmAmC*mU*mAP
	SEQ ID NO: 438	PmU*fA*mGmUmCfAmCmUmUmGmGmGmCfAmUfUmAmAmCmAmC*mU*mU
D: 162	SEQ ID NO: 439	mU*mU*mCmAmAmCfUmGfCfUfUmCmGmUmAmAmUmUmG*mG*mAP
	SEQ ID NO: 440	PmU*fC*mCmAmAfUmUmAmCmGmAmAmGfCmAfGmUmUmGmAmA*mC*mU
D: 163	SEQ ID NO: 441	mU*mG*mCmAmGmAfAmGfAfUfGmUmAmGmAmUmUmGmU*mG*mAP
	SEQ ID NO: 442	PmU*fC*mAmCmAfAmUmCmUmAmCmAmUfCmUfUmCmUmGmCmA*mU*mU
D: 164	SEQ ID NO: 443	mG*mA*mGmUmAmAfAmUfAfUfAmUmCmAmCmCmAmCmU*mA*mAP
	SEQ ID NO: 444	PmU*fU*mAmGmUfGmGmUmGmAmUmAmUfAmUfUmUmAmCmUmC*mA*mA
D: 165	SEQ ID NO: 445	mC*mA*mAmGmGmAfAmUfGfCfAmCmAmCmUmCmAmCmC*mA*mAP
	SEQ ID NO: 446	PmU*fU*mGmGmUfGmAmGmUmGmUmGmCfAmUfUmCmCmUmUmG*mA*mU
D: 166	SEQ ID NO: 447	mC*mA*mGmGmUmGfAmAfAfGfGmAmAmAmGmCmUmAmG*mG*mAP
	SEQ ID NO: 448	PmU*fC*mCmUmAfGmCmUmUmUmCmCmUfUmUfCmAmCmCmUmG*mG*mA
D: 167	SEQ ID NO: 449	mA*mA*mUmGmAmCfAmAfUfGfUmCmCmAmAmGmAmCmA*mC*mAP
	SEQ ID NO: 450	PmU*fG*mUmGmUfCmUmUmGmGmAmCmAfUmUfGmUmCmAmUmU*mC*mU
D: 168	SEQ ID NO: 451	mG*mG*mAmAmUmGfCmAfCfAfCmUmCmAmCmCmAmGmC*mA*mAP
	SEQ ID NO: 452	PmU*fU*mGmCmUfGmGmUmGmAmGmUmGfUmGfCmAmUmUmCmC*mU*mU

D: 169	SEQ ID NO: 453	mC*mC*mUmGmUmCfCmUfCfCfAmGmGmUmGmAmAmAmG*mG*mAP
	SEQ ID NO: 454	PmU*fC*mCmUmUfUmCmAmCmCmUmGmGfAmGfGmAmCmAmGmG*mG*mC
D: 170	SEQ ID NO: 455	mA*mU*mGmCmAmGfAmAfGfAfUmGmUmAmGmAmUmUmG*mU*mAP
	SEQ ID NO: 456	PmU*fA*mCmAmAfUmCmUmAmCmAmUmCfUmUfCmUmGmCmAmU*mU*mU
D: 171	SEQ ID NO: 457	mU*mU*mGmUmGmUfGmAfUfGfAmAmGmGmAmCmAmUmG*mG*mAP
	SEQ ID NO: 458	PmU*fC*mCmAmUfGmUmCmCmUmUmCmAfUmCfAmCmAmCmAmA*mU*mC
D: 172	SEQ ID NO: 459	mA*mA*mAmGmUmUfCmAfAfCfUmGmCmUmUmCmGmUmA*mA*mAP
	SEQ ID NO: 460	PmU*fU*mUmAmCfGmAmAmGmCmAmGmUfUmGfAmAmCmUmUmU*mC*mU
D: 173	SEQ ID NO: 461	mU*mG*mUmUmAmAfUmGfCfCfCmAmAmGmUmGmAmCmU*mG*mAP
	SEQ ID NO: 462	PmU*fC*mAmGmUfCmAmCmUmUmGmGmGfCmAfUmUmAmAmCmA*mC*mU
D: 174	SEQ ID NO: 463	mG*mA*mUmGmUmUfGmAfCfUfUmGmAmGmUmAmAmAmU*mA*mAP
	SEQ ID NO: 464	PmU*fU*mAmUmUfUmAmCmUmCmAmAmGfUmCfAmAmCmAmUmC*mA*mG
D: 175	SEQ ID NO: 465	mG*mA*mAmGmCmCfAmAfAfAfUmAmGmAmUmGmAmGmA*mU*mAP
	SEQ ID NO: 466	PmU*fA*mUmCmUfCmAmUmCmUmAmUmUfUmUfGmGmCmUmUmC*mA*mU
D: 176	SEQ ID NO: 467	mG*mU*mUmCmAmAfCmUfGfCfUmUmCmGmUmAmAmUmU*mG*mAP
	SEQ ID NO: 468	PmU*fC*mAmAmUfUmAmCmGmAmAmGmCfAmGfUmUmGmAmAmC*mU*mU
D: 177	SEQ ID NO: 469	mG*mU*mAmCmUmCfUmUfGfCfAmGmAmGmAmAmAmAmU*mU*mAP
	SEQ ID NO: 470	PmU*fA*mAmUmUfUmUmCmUmCmUmGmCfAmAfGmAmGmUmAmC*mA*mA
D: 178	SEQ ID NO: 471	mC*mU*mCmAmAmGfGmAfCfAfUmUmAmCmUmAmGmUmG*mA*mAP
	SEQ ID NO: 472	PmU*fU*mCmAmCfUmAmGmUmAmAmUmGfUmCfCmUmUmGmAmG*mG*mA
D: 179	SEQ ID NO: 473	mA*mG*mGmGmAmAfGmGfAfGfUmAmCmAmCmAmGmAmC*mA*mAP
	SEQ ID NO: 474	PmU*fU*mGmUmCfUmGmUmGmUmAmCmUfCmCfUmUmCmCmCmU*mU*mC
D: 180	SEQ ID NO: 475	mU*mC*mAmUmGmAfCmAfCfUfAmAmGmUmCmAmAmGmU*mU*mAP
	SEQ ID NO: 476	PmU*fA*mAmCmUfUmGmAmCmUmUmAmGfUmGfUmCmAmUmGmA*mC*mU
D: 181	SEQ ID NO: 477	mC*mC*mUmCmAmAfGmGfAfCfAmUmUmAmCmUmAmGmU*mG*mAP
	SEQ ID NO: 478	PmU*fC*mAmCmUfAmGmUmAmAmUmGmUfCmCfUmUmGmAmGmG*mA*mU
D: 182	SEQ ID NO: 479	mG*mG*mCmUmUmUfGmUfUfCfGmAmAmAmGmAmAmUmG*mG*mAP
	SEQ ID NO: 480	PmU*fC*mCmAmUfUmCmUmUmUmCmGmAfAmCfAmAmAmGmCmC*mU*mU
D: 183	SEQ ID NO: 481	mC*mC*mAmGmAmCfUmGfCfGfUmGmCmCmCmUmGmCmC*mA*mAP
	SEQ ID NO: 482	PmU*fU*mGmGmCfAmGmGmGmCmAmCmGfCmAfGmUmCmUmGmG*mU*mU
D: 184	SEQ ID NO: 483	mA*mG*mCmCmAmAfAmAfUfAfGmAmUmGmAmGmAmUmC*mA*mAP
	SEQ ID NO: 484	PmU*fU*mGmAmUfCmUmCmAmUmCmUmAfUmUfUmUmGmGmCmU*mU*mC
D: 185	SEQ ID NO: 485	mA*mG*mAmAmGmGfGmAfAfGfGmAmGmUmAmCmAmCmA*mG*mAP
	SEQ ID NO: 486	PmU*fC*mUmGmUfGmUmAmCmUmCmCmUfUmCfCmCmUmUmCmU*mU*mG
D: 186	SEQ ID NO: 487	mA*mG*mAmUmUmGfUmGfUfGfAmUmGmAmAmGmGmAmC*mA*mAP
	SEQ ID NO: 488	PmU*fU*mGmUmCfCmUmUmCmAmUmCmAfCmAfCmAmAmUmCmU*mA*mC
D: 187	SEQ ID NO: 489	mU*mU*mUmCmAmCfUmUfCfGfGmAmGmGmAmUmUmGmC*mU*mAP
	SEQ ID NO: 490	PmU*fA*mGmCmAfAmUmCmCmUmCmCmGfAmAfGmUmGmAmAmA*mG*mA
D: 188	SEQ ID NO: 491	mA*mA*mGmGmAmAfAmGfCfUfAmGmGmGmAmCmUmGmC*mA*mAP
	SEQ ID NO: 492	PmU*fU*mGmCmAfGmUmCmCmCmUmAmGfCmUfUmUmCmCmUmU*mU*mC
D: 189	SEQ ID NO: 493	mG*mA*mCmAmUmGfGmCfUfUfAmGmAmAmGmUmGmGmA*mA*mAP
	SEQ ID NO: 494	PmU*fU*mUmCmCfAmCmUmUmCmUmAmAfGmCfCmAmUmGmUmC*mC*mU

D: 190	SEQ ID NO: 495	mU*mG*mUmAmCmUfCmUfUfGfCmAmGmAmGmAmAmAmA*mU*mAP
	SEQ ID NO: 496	PmU*fA*mUmUmUfUmCmUmCmUmGmCmAfAmGfAmGmUmAmCmA*mA*mA
D: 191	SEQ ID NO: 497	mA*mU*mGmAmAmCfCmAfGfAfCmUmGmCmGmUmGmCmC*mC*mAP
	SEQ ID NO: 498	PmU*fG*mGmGmCfAmCmGmCmAmGmUmCfUmGfGmUmUmCmAmU*mC*mC
D: 192	SEQ ID NO: 499	mC*mA*mUmGmAmCfAmCfUfAfAmGmUmCmAmAmGmUmU*mA*mAP
	SEQ ID NO: 500	PmU*fU*mAmAmCfUmUmGmAmCmUmUmAfGmUfGmUmCmAmUmG*mA*mC
D: 193	SEQ ID NO: 501	mU*mU*mGmUmUmCfGmAfAfAfGmAmAmUmGmGmUmGmU*mC*mAP
	SEQ ID NO: 502	PmU*fG*mAmCmAfCmCmAmUmUmCmUmUfUmCfGmAmAmCmAmA*mA*mG
D: 194	SEQ ID NO: 503	mA*mC*mAmCmUmGfUmGfAfCfCmCmUmUmGmCmAmCmC*mA*mAP
	SEQ ID NO: 504	PmU*fU*mGmGmUfGmCmAmAmGmGmGmUfCmAfCmAmGmUmGmU*mU*mC
D: 195	SEQ ID NO: 505	mG*mG*mAmAmAmUfAmAfAfCfUmGmCmAmCmCmCmGmG*mA*mAP
	SEQ ID NO: 506	PmU*fU*mCmCmGfGmGmUmGmCmAmGmUfUmUfAmUmUmUmCmC*mA*mC
D: 196	SEQ ID NO: 507	mA*mA*mGmUmGmUfUmAfAfUfGmCmCmCmAmAmGmUmG*mA*mAP
	SEQ ID NO: 508	PmU*fU*mCmAmCfUmUmGmGmGmCmAmUfUmAfAmCmAmCmUmU*mU*mU
D: 197	SEQ ID NO: 509	mA*mG*mUmUmCmAfAmCfUfGfCmUmUmCmGmUmAmAmU*mU*mAP
	SEQ ID NO: 510	PmU*fA*mAmUmUfAmCmGmAmAmGmCmAfGmUfUmGmAmAmCmU*mU*mU
D: 198	SEQ ID NO: 511	mC*mA*mCmUmUmCfGmGfAfGfGmAmUmUmGmCmUmCmA*mA*mAP
	SEQ ID NO: 512	PmU*fU*mUmGmAfGmCmAmAmUmCmCmUfCmCfGmAmAmGmUmG*mA*mA
D: 199	SEQ ID NO: 513	mA*mU*mGmAmAmGfCmCfAfAfAmAmUmAmGmAmUmGmA*mG*mAP
	SEQ ID NO: 514	PmU*fC*mUmCmAfUmCmUmAmUmUmUmUfGmGfCmUmUmCmAmU*mU*mG
D: 200	SEQ ID NO: 515	mA*mC*mUmUmCmGfGmAfGfGfAmUmUmGmCmUmCmAmA*mC*mAP
	SEQ ID NO: 516	PmU*fG*mUmUmGfAmGmCmAmAmUmCmCfUmCfCmGmAmAmGmU*mG*mA
D: 201	SEQ ID NO: 517	mA*mG*mGmUmGmAfAmAfGfGfAmAmAmGmCmUmAmGmG*mG*mAP
	SEQ ID NO: 518	PmU*fC*mCmCmUfAmGmCmUmUmUmCmCfUmUfUmCmAmCmCmU*mG*mG
D: 202	SEQ ID NO: 519	mA*mA*mGmGmGmAfAmGfGfAfGmUmAmCmAmCmAmGmA*mC*mAP
	SEQ ID NO: 520	PmU*fG*mUmCmUfGmUmGmUmAmCmUmCfCmUfUmCmCmCmUmU*mC*mU
D: 203	SEQ ID NO: 521	mA*mG*mUmGmGmAfAmAfUfAfAmAmCmUmGmCmAmCmC*mC*mAP
	SEQ ID NO: 522	PmU*fG*mGmGmUfGmCmAmGmUmUmUmAfUmUfUmCmCmAmCmU*mU*mC
D: 204	SEQ ID NO: 523	mA*mU*mGmAmCmAfAmUfGfUfCmCmAmAmGmAmCmAmC*mA*mAP
	SEQ ID NO: 524	PmU*fU*mGmUmGfUmCmUmUmGmGmAmCfAmUfUmGmUmCmAmU*mU*mC
D: 205	SEQ ID NO: 525	mG*mA*mUmUmGmUfGmUfGfAfUmGmAmAmGmGmAmCmA*mU*mAP
	SEQ ID NO: 526	PmU*fA*mUmGmUfCmCmUmUmCmAmUmCfAmCfAmCmAmAmUmC*mU*mA
D: 206	SEQ ID NO: 527	mG*mG*mGmGmAmUfGmAfAfCfCmAmGmAmCmUmGmCmG*mU*mAP
	SEQ ID NO: 528	PmU*fA*mCmGmCfAmGmUmCmUmGmGmUfUmCfAmUmCmCmCmC*mA*mU
D: 207	SEQ ID NO: 529	mG*mU*mUmAmAmUfGmCfCfCfAmAmGmUmGmAmCmUmG*mA*mAP
	SEQ ID NO: 530	PmU*fU*mCmAmGfUmCmAmCmUmUmGmGfGmCfAmUmUmAmAmC*mA*mC
D: 208	SEQ ID NO: 531	mA*mC*mCmAmGmAfCmUfGfCfGmUmGmCmCmCmUmGmC*mC*mAP
	SEQ ID NO: 532	PmU*fG*mGmCmAfGmGmGmCmAmCmGmCfAmGfUmCmUmGmGmU*mU*mC
D: 209	SEQ ID NO: 533	mA*mA*mCmAmCmUfGmUfGfAfCmCmCmUmUmGmCmAmC*mC*mAP
	SEQ ID NO: 534	PmU*fG*mGmUmGfCmAmAmGmGmGmUmCfAmCfAmGmUmGmUmU*mC*mA
D: 210	SEQ ID NO: 535	mG*mG*mAmAmGmGfAmGfUfAfCmAmCmAmGmAmCmAmA*mA*mAP
	SEQ ID NO: 536	PmU*fU*mUmUmGfUmCmUmGmUmGmUmAfCmUfCmCmUmUmCmC*mC*mU

D: 211	SEQ ID NO: 537	mG*mG*mGmAmUmGfAmAfCfCfAmGmAmCmUmGmCmGmU*mG*mAP
	SEQ ID NO: 538	PmU*fC*mAmCmGfCmAmGmUmCmUmGmGfUmUfCmAmUmCmCmC*mC*mA
D: 212	SEQ ID NO: 539	mC*mU*mGmCmGmUfGmCfCfCfUmGmCmCmAmAmGmAmA*mG*mAP
	SEQ ID NO: 540	PmU*fC*mUmUmCfUmUmGmGmCmAmGmGfGmCfAmCmGmCmAmG*mU*mC
D: 213	SEQ ID NO: 541	mU*mG*mAmAmCmAfCmUfGfUfGmAmCmCmCmUmUmGmC*mA*mAP
	SEQ ID NO: 542	PmU*fU*mGmCmAfAmGmGmGmUmCmAmCfAmGfUmGmUmUmCmA*mC*mA
D: 214	SEQ ID NO: 543	mA*mG*mGmAmUmUfGmCfUfCfAmAmCmAmAmCmCmAmU*mG*mAP
	SEQ ID NO: 544	PmU*fC*mAmUmGfGmUmUmGmUmUmGmAfGmCfAmAmUmCmCmU*mC*mC
D: 215	SEQ ID NO: 545	mU*mG*mAmCmAmCfUmAfAfGfUmCmAmAmGmUmUmAmA*mA*mAP
	SEQ ID NO: 546	PmU*fU*mUmUmAfAmCmUmUmGmAmCmUfUmAfGmUmGmUmCmA*mU*mG
D: 216	SEQ ID NO: 547	mU*mG*mAmAmAmGfGmAfAfAfGmCmUmAmGmGmGmAmC*mU*mAP
	SEQ ID NO: 548	PmU*fA*mGmUmCfCmCmUmAmGmCmUmUfUmCfCmUmUmUmCmA*mC*mC
D: 217	SEQ ID NO: 549	mA*mC*mAmAmUmGfUmCfCfAfAmGmAmCmAmCmAmGmC*mA*mAP
	SEQ ID NO: 550	PmU*fU*mGmCmUfGmUmGmUmCmUmUmGfGmAfCmAmUmUmGmU*mC*mA
D: 218	SEQ ID NO: 551	mG*mU*mGmAmAmAfGmGfAfAfAmGmCmUmAmGmGmGmA*mC*mAP
	SEQ ID NO: 552	PmU*fG*mUmCmCfCmUmAmGmCmUmUmUfCmCfUmUmUmCmAmC*mC*mU
D: 219	SEQ ID NO: 553	mA*mG*mCmUmCmUfUmUfCfAfCmUmUmCmGmGmAmGmG*mA*mAP
	SEQ ID NO: 554	PmU*fU*mCmCmUfCmCmGmAmAmGmUmGfAmAfAmGmAmGmCmU*mU*mC
D: 220	SEQ ID NO: 555	mU*mU*mCmUmUmCfCmAfAfAfUmGmCmAmGmAmAmGmA*mU*mAP
	SEQ ID NO: 556	PmU*fA*mUmCmUfUmCmUmGmCmAmUmUfUmGfGmAmAmGmAmA*mA*mA
D: 221	SEQ ID NO: 557	mG*mG*mAmGmGmAfUmUfGfCfUmCmAmAmCmAmAmCmC*mA*mAP
	SEQ ID NO: 558	PmU*fU*mGmGmUfUmGmUmUmGmAmGmCfAmAfUmCmCmUmCmC*mG*mA
D: 222	SEQ ID NO: 559	mU*mG*mCmCmAmAfGmAfAfGfGmGmAmAmGmGmAmGmU*mA*mAP
	SEQ ID NO: 560	PmU*fU*mAmCmUfCmCmUmUmCmCmCmUfUmCfUmUmGmGmCmA*mG*mG
D: 223	SEQ ID NO: 561	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 562	mU*fU*mAmCmGmAmAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 224	SEQ ID NO: 561	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 563	mU*fU*mAmCfGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 225	SEQ ID NO: 561	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 571	mU*fU*fAmCmGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 226	SEQ ID NO: 564	mG*mA*mAmAmGmUmUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 563	mU*fU*mAmCfGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 227	SEQ ID NO: 564	mG*mA*mAmAmGmUmUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mAP
	SEQ ID NO: 571	mU*fU*fAmCmGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 228	SEQ ID NO: 565	mG*mU*mUmGmAmCfUmUfG-(AP)-fGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 566	mU*fA*mUmAfUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 229	SEQ ID NO: 565	mG*mU*mUmGmAmCfUmUfG-(AP)-fGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 567	mU*fA*fUmAmUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 230	SEQ ID NO: 568	mG*mU*mUmGmAmCmUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 566	mU*fA*mUmAfUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 231	SEQ ID NO: 568	mG*mU*mUmGmAmCmUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 567	mU*fA*fUmAmUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 232	SEQ ID NO: 345	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 569	mU*fA*mUmAfU-GNA(A)-fUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU

D: 233	SEQ ID NO: 345	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mAP
	SEQ ID NO: 570	mU*fA*mUmAmUfA-GNA(U)-mUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 237	SEQ ID NO: 578	mA*mG*mAmAmAmGfUmUfCfAfAmCmUmGmCmUmUmCmG*mU*mAP
	SEQ ID NO: 579	mU*fA*mCmGmAfAmGmCmAmGmUmUmGfAmAfCmUmUmUmCmU*mG*mU
D: 238	SEQ ID NO: 580	mC*mU*mGmUmAmUmGmUfGfAfAmCmAmCmUmGmUmGmA*mC*mAP
	SEQ ID NO: 581	mU*fG*fUmCmAmCfAmGmUmGmUmUmCfAmCfAmUmAmCmAmG*mU*mA
D: 239	SEQ ID NO: 582	mU*mG*mAmCmUmUmGmAfGfUfAmAmAmUmAmUmAmUmC*mA*mAP
	SEQ ID NO: 583	mU*fU*fGmAmUmAfUmAmUmUmUmAmCfUmCfAmAmGmUmCmA*mA*mC
D: 240	SEQ ID NO: 584	mG*mU*mAmUmGmUmGmAfAfCfAmCmUmGmUmGmAmCmC*mC*mAP
	SEQ ID NO: 585	mU*fG*fGmGmUmCfAmCmAmGmUmGmUfUmCfAmCmAmUmAmC*mA*mG

P indicates a 5' phosphate;
m indicates 2'O-methyl modified ribose on the listed nucleotide;
f indicates 2'F modified ribose on the listed nucleotide;
* indicates a phosphorothioate bond (in place of a phosphodiester bond);
GNA indicates a glycol nucleic acid nucleotide;
(AP) means an apurinic/apyrimidinic residue, also called an abasic residue.

Table 4B– FAS-GalNAc RNAi agents, modified sense and antisense strands

Duplex	**SEQ ID NO:**	**Modified Sequence**
D: 241	SEQ ID NO: 586	mG*mA*mUmGmAmAfGmGfAfCfAmUmGmGmCmUmUmAmG*mA*mA
	SEQ ID NO: 587	mU*fU*mCmUmAfAmGmCmCmAmUmGmUfCmCfUmUmCmAmUmC*mA*mC
D: 242	SEQ ID NO: 588	mU*mA*mAmAmCmCfAmAfAfCfUmUmUmUmUmUmUmGmU*mA*mA
	SEQ ID NO: 589	mU*fU*mAmCmAfAmAmAmAmAmAmGmUfUmUfGmGmUmUmUmA*mC*mA
D: 243	SEQ ID NO: 590	mG*mA*mAmGmAmUfGmUfAfGfAmUmUmGmUmGmUmGmA*mU*mA
	SEQ ID NO: 591	mU*fA*mUmCmAfCmAmCmAmAmUmCmUfAmCfAmUmCmUmUmC*mU*mG
D: 244	SEQ ID NO: 592	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 593	mU*fA*mUmAmUfAmUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 245	SEQ ID NO: 594	mA*mU*mCmAmAmGfAmAfUfGfAmCmAmAmUmGmUmCmC*mA*mA
	SEQ ID NO: 595	mU*fU*mGmGmAfCmAmUmUmGmUmCmAfUmUfCmUmUmGmAmU*mC*mU
D: 246	SEQ ID NO: 596	mU*mG*mUmUmGmAfCmUfUfGfAmGmUmAmAmAmUmAmU*mA*mA
	SEQ ID NO: 597	mU*fU*mAmUmAfUmUmUmAmCmUmCmAfAmGfUmCmAmAmCmA*mU*mC
D: 247	SEQ ID NO: 598	mG*mC*mCmAmAmAfAmUfAfGfAmUmGmAmGmAmUmCmA*mA*mA
	SEQ ID NO: 599	mU*fU*mUmGmAfUmCmUmCmAmUmCmUfAmUfUmUmUmGmGmC*mU*mU
	SEQ ID NO: 600	mA*mA*mUmGmCmAfGmAfAfGfAmUmGmUmAmGmAmUmU*mG*mA

D: 248	SEQ ID NO: 601	mU*fC*mAmAmUfCmUmAmCmAmUmCmUfUmCfUmGmCmAmUmU*mU*mG
D: 249	SEQ ID NO: 602	mG*mC*mUmUmUmGfUmUfCfGfAmAmAmGmAmAmUmGmG*mU*mA
	SEQ ID NO: 603	mU*fA*mCmCmAfUmUmCmUmUmUmCmGfAmAfCmAmAmAmGmC*mC*mU
D: 250	SEQ ID NO: 604	mG*mA*mCmUmUmGfAmGfUfAfAmAmUmAmUmAmUmCmA*mC*mA
	SEQ ID NO: 605	mU*fG*mUmGmAfUmAmUmAmUmUmUmAfCmUfCmAmAmGmUmC*mA*mA
D: 251	SEQ ID NO: 606	mA*mG*mAmUmGmAfGmAfUfCfAmAmGmAmAmUmGmAmC*mA*mA
	SEQ ID NO: 607	mU*fU*mGmUmCfAmUmUmCmUmUmGmAfUmCfUmCmAmUmCmU*mA*mU
D: 252	SEQ ID NO: 608	mU*mU*mGmAmGmUfAmAfAfUfAmUmAmUmCmAmCmCmA*mC*mA
	SEQ ID NO: 609	mU*fG*mUmGmGfUmGmAmUmAmUmAmUfUmUfAmCmUmCmAmA*mG*mU
D: 253	SEQ ID NO: 610	mA*mA*mUmAmGmAfUmGfAfGfAmUmCmAmAmGmAmAmU*mG*mA
	SEQ ID NO: 611	mU*fC*mAmUmUfCmUmUmGmAmUmCmUfCmAfUmCmUmAmUmU*mU*mU
D: 254	SEQ ID NO: 612	mC*mU*mUmUmGmUfUmCfGfAfAmAmGmAmAmUmGmGmU*mG*mA
	SEQ ID NO: 613	mU*fC*mAmCmCfAmUmUmCmUmUmUmCfGmAfAmCmAmAmAmG*mC*mC
D: 255	SEQ ID NO: 614	mU*mG*mUmGmAmUfGmAfAfGfGfmAmCmAmUmGmGmCmU*mU*mA
	SEQ ID NO: 615	mU*fA*mAmGmCfCmAmUmGmUmCmCmUfUmCfAmUmCmAmCmA*mC*mA
D: 256	SEQ ID NO: 616	mU*mG*mAmCmUmUfGmAfGfUfAmAmAmUmAmUmAmUmC*mA*mA
	SEQ ID NO: 617	mU*fU*mGmAmUfAmUmAmUmUmUmAmCfUmCfAmAmGmUmCmA*mA*mC
D: 257	SEQ ID NO: 618	mG*mA*mAmAmGmUfUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 619	mU*fU*mAmCmGfAmAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 258	SEQ ID NO: 620	mG*mC*mAmGmAmAfGmAfUfGfUmAmGmAmUmUmGmUmG*mU*mA
	SEQ ID NO: 621	mU*fA*mCmAmCfAmAmUmCmUmAmCmAfUmCfUmUmCmUmGmC*mA*mU
D: 259	SEQ ID NO: 622	mU*mG*mUmGmAmAfCmAfCfUfGmUmGmAmCmCmCmUmU*mG*mA
	SEQ ID NO: 623	mU*fC*mAmAmGfGmGmUmCmAmCmAmGfUmGfUmUmCmAmCmA*mU*mA
D: 260	SEQ ID NO: 624	mG*mA*mCmAmCmUfAmAfGfUfCmAmAmGmUmUmAmAmA*mG*mA
	SEQ ID NO: 625	mU*fC*mUmUmUfAmAmCmUmUmGmAmCfUmUfAmGmUmGmUmC*mA*mU
D: 261	SEQ ID NO: 626	mG*mA*mAmUmAmCfCmAfAfGfUmGmCmAmGmAmUmGmU*mA*mA
	SEQ ID NO: 627	mU*fU*mAmCmAfUmCmUmGmCmAmCmUfUmGfGmUmAmUmUmC*mU*mG
D: 262	SEQ ID NO: 628	mG*mA*mCmAmAmUfGmUfCfCfAmAmGmAmCmAmCmAmG*mC*mA
	SEQ ID NO: 629	mU*fG*mCmUmGfUmGmUmCmUmUmGmGfAmCfAmUmUmGmUmC*mA*mU
D: 263	SEQ ID NO: 630	mA*mU*mGmAmAmGfGmAfCfAfUmGmGmCmUmUmAmGmA*mA*mA
	SEQ ID NO: 631	mU*fU*mUmCmUfAmAmGmCmCmAmUmGfUmCfCmUmUmCmAmU*mC*mA
D: 264	SEQ ID NO: 632	mU*mU*mCmCmAmAfAmUfGfCfAmGmAmAmGmAmUmGmU*mA*mA
	SEQ ID NO: 633	mU*fU*mAmCmAfUmCmUmUmCmUmGmCfAmUfUmUmGmGmAmA*mG*mA
D: 265	SEQ ID NO: 634	mG*mA*mGmAmUmCfAmAfGfAfAmUmGmAmCmAmAmUmG*mU*mA
	SEQ ID NO: 635	mU*fA*mCmAmUfUmGmUmCmAmUmUmCfUmUfGmAmUmCmUmC*mA*mU
D: 266	SEQ ID NO: 636	mC*mA*mAmGmUmGfCmAfGfAfUmGmUmAmAmAmCmCmA*mA*mA
	SEQ ID NO: 637	mU*fU*mUmGmGfUmUmUmAmCmAmUmCfUmGfCmAmCmUmUmG*mG*mU
D: 267	SEQ ID NO: 638	mU*mU*mUmGmUmUfCmGfAfAfAmGmAmAmUmGmGmUmG*mU*mA
	SEQ ID NO: 639	mU*fA*mCmAmCfCmAmUmUmCmUmUmUfCmGfAmAmCmAmAmA*mG*mC
D: 268	SEQ ID NO: 640	mC*mA*mGmAmAmGfAmUfGfUfAmGmAmUmUmGmUmGmU*mG*mA
	SEQ ID NO: 641	mU*fC*mAmCmAfCmAmAmUmCmUmAmCfAmUfCmUmUmCmUmG*mC*mA

D: 269	SEQ ID NO: 642	mU*mC*mUmUmUmGfUmAfCfUfCmUmUmGmCmAmGmAmG*mA*mA
	SEQ ID NO: 643	mU*fU*mCmUmCfUmGmCmAmAmGmAmGfUmAfCmAmAmAmGmA*mU*mU
D: 270	SEQ ID NO: 644	mC*mA*mCmUmGmUfGmAfCfCfCmUmUmGmCmAmCmCmA*mA*mA
	SEQ ID NO: 645	mU*fU*mUmGmGfUmGmCmAmAmGmGmGfUmCfAmCmAmGmUmG*mU*mU
D: 271	SEQ ID NO: 646	mU*mC*mCmAmAmAfUmGfCfAfGmAmAmGmAmUmGmUmA*mG*mA
	SEQ ID NO: 647	mU*fC*mUmAmCfAmUmCmUmUmCmUmGfCmAfUmUmUmGmGmA*mA*mG
D: 272	SEQ ID NO: 648	mA*mA*mUmGmUmCfCmAfAfGfAmCmAmCmAmGmCmAmG*mA*mA
	SEQ ID NO: 649	mU*fU*mCmUmGfCmUmGmUmGmUmCmUfUmGfGmAmCmAmUmU*mG*mU
D: 273	SEQ ID NO: 650	mG*mC*mCmAmAmGfAmAfGfGfGmAmAmGmGmAmGmUmA*mC*mA
	SEQ ID NO: 651	mU*fG*mUmAmCfUmCmCmUmUmCmCmCfUmUfCmUmUmGmGmC*mA*mG
D: 274	SEQ ID NO: 652	mC*mU*mUmGmAmGfUmAfAfAfUmAmUmAmUmCmAmCmC*mA*mA
	SEQ ID NO: 653	mU*fU*mGmGmUfGmAmUmAmUmAmUmUfUmAfCmUmCmAmAmG*mU*mC
D: 275	SEQ ID NO: 654	mU*mA*mGmAmUmGfAmGfAfUfCmAmAmGmAmAmUmGmA*mC*mA
	SEQ ID NO: 655	mU*fG*mUmCmAfUmUmCmUmUmGmAmUfCmUfCmAmUmCmUmA*mU*mU
D: 276	SEQ ID NO: 656	mU*mU*mGmAmCmUfUmGfAfGfUmAmAmAmUmAmUmAmU*mC*mA
	SEQ ID NO: 657	mU*fG*mAmUmAfUmAmUmUmUmAmCmUfCmAfAmGmUmCmAmA*mC*mA
D: 277	SEQ ID NO: 658	mA*mG*mUmAmAmAfUmAfUfAfUmCmAmCmCmAmCmUmA*mU*mA
	SEQ ID NO: 659	mU*fA*mUmAmGfUmGmGmUmGmAmUmAfUmAfUmUmUmAmCmU*mC*mA
D: 278	SEQ ID NO: 660	mU*mA*mCmCmAmAfGmUfGfCfAmGmAmUmGmUmAmAmA*mC*mA
	SEQ ID NO: 661	mU*fG*mUmUmUfAmCmAmUmCmUmGmCfAmCfUmUmGmGmUmA*mU*mU
D: 279	SEQ ID NO: 662	mC*mU*mGmUmAmUfGmUfGfAfAmCmAmCmUmGmUmGmA*mC*mA
	SEQ ID NO: 663	mU*fG*mUmCmAfCmAmGmUmGmUmUmCfAmCfAmUmAmCmAmG*mU*mA
D: 280	SEQ ID NO: 664	mG*mA*mUmCmAmAfGmAfAfUfGmAmCmAmAmUmGmUmC*mC*mA
	SEQ ID NO: 665	mU*fG*mGmAmCfAmUmUmGmUmCmAmUfUmCfUmUmGmAmUmC*mU*mC
D: 281	SEQ ID NO: 666	mA*mU*mGmAmCmAfCmUfAfAfGmUmCmAmAmGmUmUmA*mA*mA
	SEQ ID NO: 667	mU*fU*mUmAmAfCmUmUmGmAmCmUmUfAmGfUmGmUmCmAmU*mG*mA
D: 282	SEQ ID NO: 668	mA*mA*mAmUmAmGfAmUfGfAfGmAmUmCmAmAmGmAmA*mU*mA
	SEQ ID NO: 669	mU*fA*mUmUmCfUmUmGmAmUmCmUmCfAmUfCmUmAmUmUmU*mU*mG
D: 283	SEQ ID NO: 670	mG*mU*mAmUmGmUfGmAfAfCfAmCmUmGmUmGmAmCmC*mC*mA
	SEQ ID NO: 671	mU*fG*mGmGmUfCmAmCmAmGmUmGmUfUmCfAmCmAmUmAmC*mA*mG
D: 284	SEQ ID NO: 672	mA*mA*mUmAmCmCfAmAfGfUfGmCmAmGmAmUmGmUmA*mA*mA
	SEQ ID NO: 673	mU*fU*mUmAmCfAmUmCmUmGmCmAmCfUmUfGmGmUmAmUmU*mC*mU
D: 285	SEQ ID NO: 674	mU*mG*mAmGmAmUfCmAfAfGfAmAmUmGmAmCmAmAmU*mG*mA
	SEQ ID NO: 675	mU*fC*mAmUmUfGmUmCmAmUmUmCmUfUmGfAmUmCmUmCmA*mU*mC
D: 286	SEQ ID NO: 676	mC*mU*mUmUmGmUfAmCfUfCfUmUmGmCmAmGmAmGmA*mA*mA
	SEQ ID NO: 677	mU*fU*mUmCmUfCmUmGmCmAmAmGmAfGmUfAmCmAmAmAmG*mA*mU
D: 287	SEQ ID NO: 678	mU*mG*mAmAmGmCfCmAfAfAfAmUmAmGmAmUmGmAmG*mA*mA
	SEQ ID NO: 679	mU*fU*mCmUmCfAmUmCmUmAmUmUmUfUmGfGmCmUmUmCmA*mU*mU
D: 288	SEQ ID NO: 680	mC*mA*mUmCmCmUfCmAfAfGfGmAmCmAmUmUmAmCmU*mA*mA
	SEQ ID NO: 681	mU*fU*mAmGmUfAmAmUmGmUmCmCmUfUmGfAmGmGmAmUmG*mA*mU
D: 289	SEQ ID NO: 682	mU*mA*mUmGmUmGfAmAfCfAfCmUmGmUmGmAmCmCmC*mU*mA
	SEQ ID NO: 683	mU*fA*mGmGmGfUmCmAmCmAmGmUmGfUmUfCmAmCmAmUmA*mC*mA

D: 290	SEQ ID NO: 684	mG*mU*mGmUmUmAfAmUfGfCfCmCmAmAmGmUmGmAmC*mU*mA
	SEQ ID NO: 685	mU*fA*mGmUmCfAmCmUmUmGmGmGmCfAmUfUmAmAmCmAmC*mU*mU
D: 291	SEQ ID NO: 686	mU*mU*mCmAmAmCfUmGfCfUfUmCmGmUmAmAmUmUmG*mG*mA
	SEQ ID NO: 687	mU*fC*mCmAmAfUmUmAmCmGmAmAmGfCmAfGmUmUmGmAmA*mC*mU
D: 292	SEQ ID NO: 688	mU*mG*mCmAmGmAfAmGfAfUfGmUmAmGmAmUmUmGmU*mG*mA
	SEQ ID NO: 689	mU*fC*mAmCmAfAmUmCmUmAmCmAmUfCmUfUmCmUmGmCmA*mU*mU
D: 293	SEQ ID NO: 690	mG*mA*mGmUmAmAfAmUfAfUfAmUmCmAmCmCmAmCmU*mA*mA
	SEQ ID NO: 691	mU*fU*mAmGmUfGmGmUmGmAmUmAmUfAmUfUmUmAmCmUmC*mA*mA
D: 294	SEQ ID NO: 692	mC*mA*mAmGmGmAfAmUfGfCfAmCmAmCmUmCmAmCmC*mA*mA
	SEQ ID NO: 693	mU*fU*mGmGmUfGmAmGmUmGmUmGmCfAmUfUmCmCmUmUmG*mA*mU
D: 295	SEQ ID NO: 694	mC*mA*mGmGmUmGfAmAfAfGfGmAmAmAmGmCmUmAmG*mG*mA
	SEQ ID NO: 695	mU*fC*mCmUmAfGmCmUmUmUmCmCmUfUmUfCmAmCmCmUmG*mG*mA
D: 296	SEQ ID NO: 696	mA*mA*mUmGmAmCfAmAfUfGfUmCmCmAmAmGmAmCmA*mC*mA
	SEQ ID NO: 697	mU*fG*mUmGmUfCmUmUmGmGmAmCmAfUmUfGmUmCmAmUmU*mC*mU
D: 297	SEQ ID NO: 698	mG*mG*mAmAmUmGfCmAfCfAfCmUmCmAmCmCmAmGmC*mA*mA
	SEQ ID NO: 699	mU*fU*mGmCmUfGmGmUmGmAmGmUmGfUmGfCmAmUmUmCmC*mU*mU
D: 298	SEQ ID NO: 700	mC*mC*mUmGmUmCfCmUfCfCfAmGmGmUmGmAmAmAmG*mG*mA
	SEQ ID NO: 701	mU*fC*mCmUmUfUmCmAmCmCmUmGmGfAmGfGmAmCmAmGmG*mG*mC
D: 299	SEQ ID NO: 702	mA*mU*mGmCmAmGfAmAfGfAfUmGmUmAmGmAmUmUmG*mU*mA
	SEQ ID NO: 703	mU*fA*mCmAmAfUmCmUmAmCmAmUmCfUmUfCmUmGmCmAmU*mU*mU
D: 300	SEQ ID NO: 704	mU*mU*mGmUmGmUfGmAfUfGfAmAmGmGmAmCmAmUmG*mG*mA
	SEQ ID NO: 705	mU*fC*mCmAmUfGmUmCmCmUmUmCmAfUmCfAmCmAmCmAmA*mU*mC
D: 301	SEQ ID NO: 706	mA*mA*mAmGmUmUfCmAfAfCfUmGmCmUmUmCmGmUmA*mA*mA
	SEQ ID NO: 707	mU*fU*mUmAmCfGmAmAmGmCmAmGmUfUmGfAmAmCmUmUmU*mC*mU
D: 302	SEQ ID NO: 708	mU*mG*mUmUmAmAfUmGfCfCfCmAmAmGmUmGmAmCmU*mG*mA
	SEQ ID NO: 709	mU*fC*mAmGmUfCmAmCmUmUmGmGmGfCmAfUmUmAmAmCmA*mC*mU
D: 303	SEQ ID NO: 710	mG*mA*mUmGmUmUfGmAfCfUfUmGmAmGmUmAmAmAmU*mA*mA
	SEQ ID NO: 711	mU*fU*mAmUmUfUmAmCmUmCmAmAmGfUmCfAmAmCmAmUmC*mA*mG
D: 304	SEQ ID NO: 712	mG*mA*mAmGmCmCfAmAfAfAfUmAmGmAmUmGmAmGmA*mU*mA
	SEQ ID NO: 713	mU*fA*mUmCmUfCmAmUmCmUmAmUmUfUmUfGmGmCmUmUmC*mA*mU
D: 305	SEQ ID NO: 714	mG*mU*mUmCmAmAfCmUfGfCfUmUmCmGmUmAmAmUmU*mG*mA
	SEQ ID NO: 715	mU*fC*mAmAmUfUmAmCmGmAmAmGmCfAmGfUmUmGmAmAmC*mU*mU
D: 306	SEQ ID NO: 716	mG*mU*mAmCmUmCfUmUfGfCfAmGmAmGmAmAmAmAmU*mU*mA
	SEQ ID NO: 717	mU*fA*mAmUmUfUmUmCmUmCmUmGmCfAmAfGmAmGmUmAmC*mA*mA
D: 307	SEQ ID NO: 718	mC*mU*mCmAmAmGfGmAfCfAfUmUmAmCmUmAmGmUmG*mA*mA
	SEQ ID NO: 719	mU*fU*mCmAmCfUmAmGmUmAmAmUmGfUmCfCmUmUmGmAmG*mG*mA
D: 308	SEQ ID NO: 720	mA*mG*mGmGmAmAfGmGfAfGfUmAmCmAmCmAmGmAmC*mA*mA
	SEQ ID NO: 721	mU*fU*mGmUmCfUmGmUmGmUmAmCmUfCmCfUmUmCmCmCmU*mU*mC
D: 309	SEQ ID NO: 722	mU*mC*mAmUmGmAfCmAfCfUfAmAmGmUmCmAmAmGmU*mU*mA
	SEQ ID NO: 723	mU*fA*mAmCmUfUmGmAmCmUmUmAmGfUmGfUmCmAmUmGmA*mC*mU
D: 310	SEQ ID NO: 724	mC*mC*mUmCmAmAfGmGfAfCfAmUmUmAmCmUmAmGmU*mG*mA
	SEQ ID NO: 725	mU*fC*mAmCmUfAmGmUmAmAmUmGmUfCmCfUmUmGmAmGmG*mA*mU

D: 311	SEQ ID NO: 726	mG*mG*mCmUmUmUfGmUfUfCfGmAmAmAmGmAmAmUmG*mG*mA
	SEQ ID NO: 727	mU*fC*mCmAmUfUmCmUmUmUmCmGmAfAmCfAmAmAmGmCmC*mU*mU
D: 312	SEQ ID NO: 728	mC*mC*mAmGmAmCfUmGfCfGfUmGmCmCmCmUmGmCmC*mA*mA
	SEQ ID NO: 729	mU*fU*mGmGmCfAmGmGmGmCmAmCmGfCmAfGmUmCmUmGmG*mU*mU
D: 313	SEQ ID NO: 730	mA*mG*mCmCmAmAfAmAfUfAfGmAmUmGmAmGmAmUmC*mA*mA
	SEQ ID NO: 731	mU*fU*mGmAmUfCmUmCmAmUmCmUmAfUmUfUmUmGmGmCmU*mU*mC
D: 314	SEQ ID NO: 732	mA*mG*mAmAmGmGfGmAfAfGfGmAmGmUmAmCmAmCmA*mG*mA
	SEQ ID NO: 733	mU*fC*mUmGmUfGmUmAmCmUmCmCmUfUmCfCmCmUmUmCmU*mU*mG
D: 315	SEQ ID NO: 734	mA*mG*mAmUmUmGfUmGfUfGfAmUmGmAmAmGmGmAmC*mA*mA
	SEQ ID NO: 735	mU*fU*mGmUmCfCmUmUmCmAmUmCmAfCmAfCmAmAmUmCmU*mA*mC
D: 316	SEQ ID NO: 736	mU*mU*mUmCmAmCfUmUfCfGfGmAmGmGmAmUmUmGmC*mU*mA
	SEQ ID NO: 737	mU*fA*mGmCmAfAmUmCmCmUmCmCmGfAmAfGmUmGmAmAmA*mG*mA
D: 317	SEQ ID NO: 738	mA*mA*mGmGmAmAfAmGfCfUfAmGmGmGmAmCmUmGmC*mA*mA
	SEQ ID NO: 739	mU*fU*mGmCmAfGmUmCmCmCmUmAmGfCmUfUmUmCmCmUmU*mU*mC
D: 318	SEQ ID NO: 740	mG*mA*mCmAmUmGfGmCfUfUfAmGmAmAmGmUmGmGmA*mA*mA
	SEQ ID NO: 741	mU*fU*mUmCmCfAmCmUmUmCmUmAmAfGmCfCmAmUmGmUmC*mC*mU
D: 319	SEQ ID NO: 742	mU*mG*mUmAmCmUfCmUfUfGfCmAmGmAmGmAmAmAmA*mU*mA
	SEQ ID NO: 743	mU*fA*mUmUmUfUmCmUmCmUmGmCmAfAmGfAmGmUmAmCmA*mA*mA
D: 320	SEQ ID NO: 744	mA*mU*mGmAmAmCfCmAfGfAfCmUmGmCmGmUmGmCmC*mC*mA
	SEQ ID NO: 745	mU*fG*mGmGmCfAmCmGmCmAmGmUmCfUmGfGmUmUmCmAmU*mC*mC
D: 321	SEQ ID NO: 746	mC*mA*mUmGmAmCfAmCfUfAfAmGmUmCmAmAmGmUmU*mA*mA
	SEQ ID NO: 747	mU*fU*mAmAmCfUmUmGmAmCmUmUmAfGmUfGmUmCmAmUmG*mA*mC
D: 322	SEQ ID NO: 748	mU*mU*mGmUmUmCfGmAfAfAfGmAmAmUmGmGmUmGmU*mC*mA
	SEQ ID NO: 749	mU*fG*mAmCmAfCmCmAmUmUmCmUmUfUmCfGmAmAmCmAmA*mA*mG
D: 323	SEQ ID NO: 750	mA*mC*mAmCmUmGfUmGfAfCfCmCmUmUmGmCmAmCmC*mA*mA
	SEQ ID NO: 751	mU*fU*mGmGmUfGmCmAmAmGmGmGmUfCmAfCmAmGmUmGmU*mU*mC
D: 324	SEQ ID NO: 752	mG*mG*mAmAmAmUfAmAfAfCfUmGmCmAmCmCmCmGmG*mA*mA
	SEQ ID NO: 753	mU*fU*mCmCmGfGmGmUmGmCmAmGmUfUmUfAmUmUmUmCmC*mA*mC
D: 325	SEQ ID NO: 754	mA*mA*mGmUmGmUfUmAfAfUfGmCmCmCmAmAmGmUmG*mA*mA
	SEQ ID NO: 755	mU*fU*mCmAmCfUmUmGmGmGmCmAmUfUmAfAmCmAmCmUmU*mU*mU
D: 326	SEQ ID NO: 756	mA*mG*mUmUmCmAfAmCfUfGfCmUmUmCmGmUmAmAmU*mU*mA
	SEQ ID NO: 757	mU*fA*mAmUmUfAmCmGmAmAmGmCmAfGmUfUmGmAmAmCmU*mU*mU
D: 327	SEQ ID NO: 758	mC*mA*mCmUmUmCfGmGfAfGfGmAmUmUmGmCmUmCmA*mA*mA
	SEQ ID NO: 759	mU*fU*mUmGmAfGmCmAmAmUmCmCmUfCmCfGmAmAmGmUmG*mA*mA
D: 328	SEQ ID NO: 760	mA*mU*mGmAmAmGfCmCfAfAfAmAmUmAmGmAmUmGmA*mG*mA
	SEQ ID NO: 761	mU*fC*mUmCmAfUmCmUmAmUmUmUmUfGmGfCmUmUmCmAmU*mU*mG
D: 329	SEQ ID NO: 762	mA*mC*mUmUmCmGfGmAfGfGfAmUmUmGmCmUmCmAmA*mC*mA
	SEQ ID NO: 763	mU*fG*mUmUmGfAmGmCmAmAmUmCmCfUmCfCmGmAmAmGmU*mG*mA
D: 330	SEQ ID NO: 764	mA*mG*mGmUmGmAfAmAfGfGfAmAmAmGmCmUmAmGmG*mG*mA
	SEQ ID NO: 765	mU*fC*mCmCmUfAmGmCmUmUmUmCmCfUmUfUmCmAmCmCmU*mG*mG
D: 331	SEQ ID NO: 766	mA*mA*mGmGmGmAfAmGfGfAfGmUmAmCmAmCmAmGmA*mC*mA
	SEQ ID NO: 767	mU*fG*mUmCmUfGmUmGmUmAmCmUmCfCmUfUmCmCmCmUmU*mC*mU

D: 332	SEQ ID NO: 768	mA*mG*mUmGmGmAfAmAfUfAfAmAmCmUmGmCmAmCmC*mC*mA
	SEQ ID NO: 769	mU*fG*mGmGmUfGmCmAmGmUmUmUmAfUmUfUmCmCmAmCmU*mU*mC
D: 333	SEQ ID NO: 770	mA*mU*mGmAmCmAfAmUfGfUfCmCmAmAmGmAmCmAmC*mA*mA
	SEQ ID NO: 771	mU*fU*mGmUmGfUmCmUmUmGmGmAmCfAmUfUmGmUmCmAmU*mU*mC
D: 334	SEQ ID NO: 772	mG*mA*mUmUmGmUfGmUfGfAfUmGmAmAmGmGmAmCmA*mU*mA
	SEQ ID NO: 773	mU*fA*mUmGmUfCmCmUmUmCmAmUmCfAmCfAmCmAmAmUmC*mU*mA
D: 335	SEQ ID NO: 774	mG*mG*mGmGmAmUfGmAfAfCfCmAmGmAmCmUmGmCmG*mU*mA
	SEQ ID NO: 775	mU*fA*mCmGmCfAmGmUmCmUmGmGmUfUmCfAmUmCmCmCmC*mA*mU
D: 336	SEQ ID NO: 776	mG*mU*mUmAmAmUfGmCfCfCfAmAmGmUmGmAmCmUmG*mA*mA
	SEQ ID NO: 777	mU*fU*mCmAmGfUmCmAmCmUmUmGmGfGmCfAmUmUmAmAmC*mA*mC
D: 337	SEQ ID NO: 778	mA*mC*mCmAmGmAfCmUfGfCfGmUmGmCmCmCmUmGmC*mC*mA
	SEQ ID NO: 779	mU*fG*mGmCmAfGmGmGmCmAmCmGmCfAmGfUmCmUmGmGmU*mU*mC
D: 338	SEQ ID NO: 780	mA*mA*mCmAmCmUfGmUfGfAfCmCmCmUmUmGmCmAmC*mC*mA
	SEQ ID NO: 781	mU*fG*mGmUmGfCmAmAmGmGmGmUmCfAmCfAmGmUmGmUmU*mC*mA
D: 339	SEQ ID NO: 782	mG*mG*mAmAmGmGfAmGfUfAfCmAmCmAmGmAmCmAmA*mA*mA
	SEQ ID NO: 783	mU*fU*mUmUmGfUmCmUmGmUmGmUmAfCmUfCmCmUmUmCmC*mC*mU
D: 340	SEQ ID NO: 784	mG*mG*mGmAmUmGfAmAfCfCfAmGmAmCmUmGmCmGmU*mG*mA
	SEQ ID NO: 785	mU*fC*mAmCmGfCmAmGmUmCmUmGmGfUmUfCmAmUmCmCmC*mC*mA
D: 341	SEQ ID NO: 786	mC*mU*mGmCmGmUfGmCfCfCfUmGmCmCmAmAmGmAmA*mG*mA
	SEQ ID NO: 787	mU*fC*mUmUmCfUmUmGmGmCmAmGmGfGmCfAmCmGmCmAmG*mU*mC
D: 342	SEQ ID NO: 788	mU*mG*mAmAmCmAfCmUfGfUfGmAmCmCmCmUmUmGmC*mA*mA
	SEQ ID NO: 789	mU*fU*mGmCmAfAmGmGmGmUmCmAmCfAmGfUmGmUmUmCmA*mC*mA
D: 343	SEQ ID NO: 790	mA*mG*mGmAmUmUfGmCfUfCfAmAmCmAmAmCmCmAmU*mG*mA
	SEQ ID NO: 791	mU*fC*mAmUmGfGmUmUmGmUmUmGmAfGmCfAmAmUmCmCmU*mC*mC
D: 344	SEQ ID NO: 792	mU*mG*mAmCmAmCfUmAfAfGfUmCmAmAmGmUmUmAmA*mA*mA
	SEQ ID NO: 793	mU*fU*mUmUmAfAmCmUmUmGmAmCmUfUmAfGmUmGmUmCmA*mU*mG
D: 345	SEQ ID NO: 794	mU*mG*mAmAmAmGfGmAfAfAfGmCmUmAmGmGmGmAmC*mU*mA
	SEQ ID NO: 795	mU*fA*mGmUmCfCmCmUmAmGmCmUmUfUmCfCmUmUmUmCmA*mC*mC
D: 346	SEQ ID NO: 796	mA*mC*mAmAmUmGfUmCfCfAfAmGmAmCmAmCmAmGmC*mA*mA
	SEQ ID NO: 797	mU*fU*mGmCmUfGmUmGmUmCmUmUmGfGmAfCmAmUmUmGmU*mC*mA
D: 347	SEQ ID NO: 798	mG*mU*mGmAmAmAfGmGfAfAfAmGmCmUmAmGmGmGmA*mC*mA
	SEQ ID NO: 799	mU*fG*mUmCmCfCmUmAmGmCmUmUmUfCmCfUmUmUmCmAmC*mC*mU
D: 348	SEQ ID NO: 800	mA*mG*mCmUmCmUfUmUfCfAfCmUmUmCmGmGmAmGmG*mA*mA
	SEQ ID NO: 801	mU*fU*mCmCmUfCmCmGmAmAmGmUmGfAmAfAmGmAmGmCmU*mU*mC
D: 349	SEQ ID NO: 802	mU*mU*mCmUmUmCfCmAfAfAfUmGmCmAmGmAmAmGmA*mU*mA
	SEQ ID NO: 803	mU*fA*mUmCmUfUmCmUmGmCmAmUmUfUmGfGmAmAmGmAmA*mA*mA
D: 350	SEQ ID NO: 804	mG*mG*mAmGmGmAfUmUfGfCfUmCmAmAmCmAmAmCmC*mA*mA
	SEQ ID NO: 805	mU*fU*mGmGmUfUmGmUmUmGmAmGmCfAmAfUmCmCmUmCmC*mG*mA
D: 351	SEQ ID NO: 806	mU*mG*mCmCmAmAfGmAfAfGfGmGmAmAmGmGmAmGmU*mA*mA
	SEQ ID NO: 807	mU*fU*mAmCmUfCmCmUmUmCmCmCmUfUmCfUmUmGmGmCmA*mG*mG
D: 352	SEQ ID NO: 808	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 562	mU*fU*mAmCmGmAmAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG

D: 353	SEQ ID NO: 808	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 563	mU*fU*mAmCfGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 354	SEQ ID NO: 808	mG*mA*mAmAmGmUfUmCfA-(AP)-fCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 571	mU*fU*fAmCmGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 355	SEQ ID NO: 809	mG*mA*mAmAmGmUmUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 563	mU*fU*mAmCfGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 356	SEQ ID NO: 809	mG*mA*mAmAmGmUmUmCfAfAfCmUmGmCmUmUmCmGmU*mA*mA
	SEQ ID NO: 571	mU*fU*fAmCmGmAfAmGmCmAmGmUmUfGmAfAmCmUmUmUmC*mU*mG
D: 357	SEQ ID NO: 810	mG*mU*mUmGmAmCfUmUfG-(AP)-fGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 566	mU*fA*mUmAfUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 358	SEQ ID NO: 810	mG*mU*mUmGmAmCfUmUfG-(AP)-fGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 567	mU*fA*fUmAmUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 359	SEQ ID NO: 811	mG*mU*mUmGmAmCmUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 566	mU*fA*mUmAfUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 360	SEQ ID NO: 811	mG*mU*mUmGmAmCmUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 567	mU*fA*fUmAmUmAfUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 361	SEQ ID NO: 592	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 569	mU*fA*mUmAfU-GNA(A)- fUmUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 362	SEQ ID NO: 592	mG*mU*mUmGmAmCfUmUfGfAfGmUmAmAmAmUmAmUmA*mU*mA
	SEQ ID NO: 570	mU*fA*mUmAmUfA-GNA(U)- mUmUmAmCmUmCfAmAfGmUmCmAmAmC*mA*mU
D: 363	SEQ ID NO: 812	mA*mG*mAmAmAmGfUmUfCfAfAmCmUmGmCmUmUmCmG*mU*mA
	SEQ ID NO: 813	mU*fA*mCmGmAfAmGmCmAmGmUmUmGfAmAfCmUmUmUmCmU*mG*mU
D: 364	SEQ ID NO: 814	mC*mU*mGmUmAmUmGmUfGfAfAmCmAmCmUmGmUmGmA*mC*mA
	SEQ ID NO: 815	mU*fG*fUmCmAmCfAmGmUmGmUmUmCfAmCfAmUmAmCmAmG*mU*mA
D: 365	SEQ ID NO: 816	mU*mG*mAmCmUmUmGmAfGfUfAmAmAmUmAmUmAmUmC*mA*mA
	SEQ ID NO: 817	mU*fU*fGmAmUmAfUmAmUmUmUmAmCfUmCfAmAmGmUmCmA*mA*mC
D: 366	SEQ ID NO: 818	mG*mU*mAmUmGmUmGmAfAfCfAmCmUmGmUmGmAmCmC*mC*mA
	SEQ ID NO: 819	mU*fG*fGmGmUmCfAmCmAmGmUmGmUfUmCfAmCmAmUmAmC*mA*mG

m indicates 2'O-methyl modified ribose on the listed nucleotide;
f indicates 2'F modified ribose on the listed nucleotide;
* indicates a phosphorothioate bond (in place of a phosphodiester bond);
GNA indicates a glycol nucleic acid nucleotide;
(AP) means an apurinic/apyrimidinic residue, also called an abasic residue.

Table 5: Percent Inhibition of human FAS expression in HepG2 cells

	NV-hFAS HepG2 qPCR siRNA Knockdown (% inhibition) Single point Summary		NV-hFAS HepG2 qPCR siRNA Knockdown Concentration Response Curve Summary	
Duplex	% Inh	Conc. (nM)	Rel IC50 (nM)	% pct Rel Max
D: 112	95.1	500	0.372	93.1
D: 113	93.5	500	0.425	92
D: 114	92.8	500	0.847	92.8
D: 115	92.8	500	0.551	92.4
D: 116	92.6	500	0.517	92.7
D: 117	92.1	500	0.274	93.9
D: 118	91.9	500	0.854	90.4
D: 119	91.5	500	0.311	91.9
D: 120	91	500	0.486	74.8
D: 121	89.6	500	0.441	91.3
D: 122	89.5	500	0.4	84.2
D: 123	89.5	500	0.969	88.2
D: 124	89.1	500	0.49	93.4
D: 125	88.6	500	0.908	82.9
D: 126	88.1	500		
D: 127	87.9	500	0.311	89.1
D: 128	87.8	500	0.274	89.6
D: 129	87.4	500	0.78	78.5
D: 130	87.1	500	0.766	86.8
D: 131	87	500	1.35	86.4
D: 132	87	500	0.488	77.9
D: 133	87	500	0.856	90.7
D: 134	86.9	500		
D: 135	86.5	500	0.642	85.8
D: 136	86.3	500	0.55	82.4
D: 137	85.4	500	0.511	89.6
D: 138	85.3	500	0.702	70.4
D: 139	85.1	500	0.401	88.1
D: 140	85.1	500	1.12	89.8
D: 141	84.8	500	0.537	91.1
D: 142	84.4	500	0.989	84.2
D: 143	84.2	500	0.733	77.9
D: 144	84.1	500		
D: 145	84	500	0.76	86.9
D: 146	83.6	500	0.426	90.4
D: 147	83.2	500	0.446	88.2

D: 148	83	500	0.933	80.3
D: 149	82.6	500	0.855	86.7
D: 150	82.4	500	0.705	88.7
D: 151	81.8	500	0.849	84.5
D: 152	81.7	500	0.611	86.8
D: 153	81.4	500	0.505	85
D: 154	80.8	500	0.519	83.4
D: 155	80.7	500		
D: 156	80.6	500	1.2	82.8
D: 157	80.4	500	2.32	77.6
D: 158	80.1	500		
D: 159	80.1	500	0.388	79.4
D: 160	80	500		
D: 161	79.9	500	0.38	83.2
D: 162	79.7	500	1.01	78.7
D: 163	79.3	500		
D: 164	78.8	500	0.913	76
D: 165	78.6	500		
D: 166	78	500		
D: 167	77.9	500	1.63	67.3
D: 168	76.8	500		
D: 169	76.6	500		
D: 170	76.5	500		
D: 171	76.4	500		
D: 172	76.4	500		
D: 173	75.7	500	1.35	80.3
D: 174	75.2	500		
D: 175	74.9	500		
D: 176	74.9	500		
D: 177	74.9	500		
D: 178	74.3	500		
D: 179	73.9	500		
D: 180	72.3	500		
D: 181	72.2	500		
D: 182	71.9	500		
D: 183	71.8	500		
D: 184	71.8	500		
D: 185	71.2	500		
D: 186	71.1	500		
D: 187	71.1	500		
D: 188	70.8	500		
D: 189	70.7	500		
D: 190	70.5	500		

D: 191	70	500		
D: 192	70	500		
D: 193	69.3	500		
D: 194	68.4	500		
D: 195	67.1	500		
D: 196	66.5	500		
D: 197	66	500		
D: 198	65.5	500		
D: 199	65	500		
D: 200	64.5	500		
D: 201	64.5	500		
D: 202	63.6	500		
D: 203	63.5	500		
D: 204	63.5	500		
D: 205	63.4	500		
D: 206	61.1	500		
D: 207	61	500		
D: 208	60.7	500		
D: 209	59.8	500		
D: 210	59.3	500		
D: 211	58.4	500		
D: 212	58.4	500		
D: 213	57.8	500		
D: 214	55.8	500		
D: 215	55.1	500		
D: 216	54	500		
D: 217	53.6	500		
D: 218	52.5	500		
D: 219	52	500		
D: 220	51.4	500		
D: 221	50.1	500		
D: 222	50	500		
D: 223			0.281 (0.192, n=2)	57.3 (7.22, n=2)
D: 224			0.417 (0.0001, n=2)	80.9 (1.16, n=2)
D: 225			0.375 (0.0255, n=2)	77.7 (2.09, n=2)
D: 226			0.121 (0.0048, n=2)	82.9 (2.92, n=2)
D: 227			0.0774 (0.0472, n=2)	80.4 (1.43, n=2)
D: 228			0.469	90.6
D: 229			0.325	88.5

D: 230			0.231	94.2
D: 231			0.0324	91.4
D: 232			0.0895	90.2
D: 233			0.448	81.9
D: 238			0.095	94
D: 239			0.129	93.9
D: 227			0.031	80
D: 231			0.045	93.9
D: 240			0.535	95.2

Table 6A: In vivo FAS mRNA Knockdown (%KD) and remaining FAS protein in RNAi agent treated mice expressing hFAS

	In vivo RNAi treatment in AAV-hFAS Mice					
		Run 1	Run 2	Run 3	Run 4	
Duplex*	10 wk, 1 mpk, %huFas mRNA KD	10 wk, 3 mpk, %huFas mRNA KD	10 wk, 3 mpk, %huFas mRNA KD	3 wk, 3 mpk, %huFas mRNA KD	3 wk, 3 mpk, %huFas mRNA KD	2 wk, 5 mpk, %huFas protein remaining
D: 117						66
D: 112						34
D: 124						54
D: 116	2.4	20.2				67
D: 114	17.8	26.3				68
D: 115	6.3	51	51			83
D: 113	17	11.5				70
D: 119						61
D: 133						40
D: 121						64
D: 141	20.8	29.3				67
D: 137						50
D: 146						42
D: 128	10.9	44.2		71.3	52.8	91
D: 140						54
D: 118						70
D: 150	44.7	35.1				70
D: 127	26.2	52				78
D: 147						56
D: 139						39
D: 223			24.6			
D:224			25.3			
D: 225			48.4			
D: 226			39.1			

D:227			47.3			
D: 228			1.7			
D: 229			21.7			
D: 230			42.2			
D: 231			49.4			
D: 232			16.1			
D: 233			5.8			
D: 172				16		
D: 162					11.7	
D: 151				36.7		
D: 130					38.2	
D: 154					60.8	
D: 158					26.3	

*for all duplexes/RNAi agents tested via administration to mice, the antisense strand does not have an extra phosphate addition as shown in Table 4A.

Table 6B. In vivo FAS mRNA Knockdown (%KD) in RNAi agent treated mice expressing hFAS

Duplex	2 weeks			6 weeks		
	1 mpk, %huFas KD	3 mpk, %huFas KD	10 mpk, %huFas KD	1 mpk, %huFas KD	3 mpk, %huFas KD	10 mpk, %huFas KD
D: 238	50.3	71.6	95	44.7	60.2	85.4
D: 239	41.3	62.5	89.5	13	59.6	66.3
D: 227	54.2	81.5	94.6	22.1	60.9	91.8
D: 231	46.4	80.2	93.5	4	50.4	80.9

Example 6:
Protein determination in vivo in AAV-Fas expressing mice treated with RNAi agents as shown in Table 6A and 6B.

[00133] Liver samples from the above RNAi agent treated mice were snap frozen and stored at -80C. While frozen, the lysing matrix D tubes containing ~1/3 liver were transferred to wet ice and XY lite containing 2X Halt buffer was added to each sample at 700ul/tube. Samples were homogenized using Fast Prep 96 at 1800 rpm for 60 seconds and cooled on ice for 5 minutes. The process was then repeated for another 30 second round of homogenization and then spun down at 20,000 rcf for 5 minutes at 4C. Samples

were centrifuged at 20,000 rcf for 10min at 4C in Eppendorf tubes to remove cell debris. Protein quantitation was performed on supernatants using the following procedure.

[00134] All samples were equilibrated to 2.0 mg/ml. Supernatants (in XY buffer) were aliquoted into 2 96-well plates at 100ul sample per well and stored at -80C and subject to protein quantitation. Prepare BSA standard was prepared at 2 mg/ml and diluted in lysis buffer to create standards. Samples were diluted 1:50 by adding 2ul lysate to 98ul XY lite and HALT in a 96 well plate (Corning #3790) and mixed by pipetting. Next 3 mLs Biorad Reagent A and60 ul of Biorad Reagent S were combined to make Reagent C. 25 ul of Reagent C was added to each well in 96 well plate (Corning #3596). Next, 5uLof standards or diluted sample was added to each well of the 96 well plate containing reagent C and performed in duplicate. Absorbance was read at 750nm on SpectraMax in 77/3/350.

[00135] The quantified supernatants were then subject to an Elisa using the Human FAS DuoSet ELISA protocol. Capture antibody was diluted to the working concentration (1.0ug/ml) in PBS. Immediately, 100ul was added per well and incubated overnight at room temperature. The next day, plate wells were decanted and washed 3 times with 300uL/well wash buffer. Plates were blocked by adding 300ul/well Reagent diluent to each well and incubated at room temperature for 1 hour. The wells were decanted and washed 3 times with 300ul/well 1X wash buffer and blotted dry after the final wash. Standard curve with control FAS protein were made by diluting in reagent diluent to final concentrations of 4000, 2000, 1000, 500, 250, 125, 62.5, or 0 pg/ml. Thawed liver lysates or standards were added per well and the plate was sealed and incubated for 2 hours at room temperature with gentle shaking. Samples were added at 100ul/well at 0.1ug/ul for 10ug total protein/well diluted in reagent diluent. After incubation, assay plate was decanted and washed 3 times with 300ul/well 1X wash buffer and blotted dry. 100ul/well of the detection antibody diluted in reagent diluent was added. The plate was sealed and incubated for 2 hours at room temperature. The detection antibody was diluted to working concentration of 50 ng/ml with reagent diluent. After incubation, assay plate was decanted and washed 3 times with 300ul/well 1X wash buffer and blotted dry. 100ul/well of the working dilution (1:200) of Strep-HRP diluted in reagent diluent was added to the plate. The plate was covered and incubated for 20 minutes at room temperature, protecting the plate from direct light. After incubation, the assay plate was decanted,

washed 3 times and blotted dry. Next 100ul/well of Substrate Solution was added and the plate incubated for 20 minutes at room temperature, protecting from direct light. Stop Solution (50 uL) was added to each well and gently mixed. The OD of each well was measured within 30 minutes of adding stop solution using a SpectraMax in 77/3/350 at 450nm with correction of 540nm (OD@450nm – OD@540nm).

[00136] Results are shown in Tables 6A and 6B.

Example 7: Additional RNAi agent tested for knockdown

[00137] The additional FAS-GalNAc RNAi agent D-235 shown in Table 7 below is tested in vitro in HepG2 cells as described above and shows about 50% or greater knockdown as compared to a vehicle control. The RNAi agent is tested in AAV-hFAS treated mice, for mRNA knockdown and protein knockdown as described above.

Table 7: Additional Sequences

Duplex No:	SEQ ID NO:	Sequence – Sense Strand (top) 5' to 3' Antisense strand (bottom) 5' to 3'
D: 234	SEQ ID NO: 572	AGAAAGUUCAACUGCUUCGUA
	SEQ ID NO: 573	UACGAAGCAGUUGAACUUUCUGU
D: 235	SEQ ID NO: 574	mA*mG*mAmAmAmGfUmUfCfAfAmCmUmGmCmUmUmCmG*mU*mA
	SEQ ID NO: 575	mU*fA*mCmGmAfAmGmCmAmGmUmUmGfAmAfCmUmUmUmCmU*mG*mU

RNAi agents to mouse FAS mRNA were also generated and tested (see Table 8).

Table 8: RNAi agent to mouse FAS mRNA

Duplex	**SEQ ID NO:**	**Sequence – Sense Strand (top) 5' to 3' Antisense strand (bottom) 5' to 3'**
D: 367	SEQ ID NO: 820	GCCGAAUGUCGCAGAACCUUA
	SEQ ID NO: 821	UAAGGUUCUGCGACAUUCGGCUU
D: 368	SEQ ID NO: 822	CCGAAUGUCGCAGAACCUUAA
	SEQ ID NO: 823	UUAAGGUUCUGCGACAUUCGGCU

D: 369	SEQ ID NO: 824	AUGUCGCAGAACCUUAGAUAA
	SEQ ID NO: 825	UUAUCUAAGGUUCUGCGACAUUC
D: 370	SEQ ID NO: 826	mG*mC*mCmGmAmAmUmGfUfCfGmCmAmGmAmAmCmCmU*mU*mA
	SEQ ID NO: 827	mU*fA*fAmGmGmUfUmCmUmGmCmGmAfCmAfUmUmCmGmGmC*mU*mU
D: 371	SEQ ID NO: 828	mC*mC*mGmAmAmUmGmUfCfGfCmAmGmAmAmCmCmUmU*mA*mA
	SEQ ID NO: 829	mU*fU*fAmAmGmGfUmUmCmUmGmCmGfAmCfAmUmUmCmGmG*mC*mU
D: 372	SEQ ID NO: 830	mA*mU*mGmUmCmGmCmAfGfAfAmCmCmUmUmAmGmAmU*mA*mA
	SEQ ID NO: 831	mU*fU*fAmUmCmUfAmAmGmGmUmUmCfUmGfCmGmAmCmAmU*mU*mC

Example 8. Characterization of FAS RNAi agent in Cynomolgus Monkey

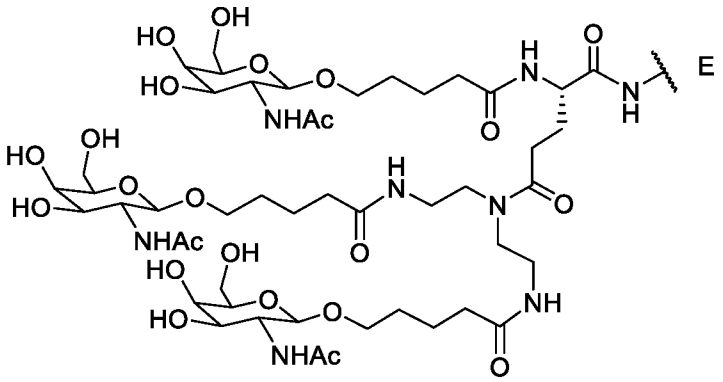
[00138] In vivo testing of selected FAS RNAi agents in Cynomolgus monkey (Macaca fascicularis) was conducted to assess their efficacy in silencing the target gene in liver. Cynomolgus monkeys (n=3/group) were given a single subcutaneous administration of the FAS RNAi agent (3mg/kg, 0.5ml/kg, in sterile 1x PBS (pH 7.2)) or sterile 1x PBS (pH 7.2) (0.5ml/kg). Following administration of FAS RNAi agent, incisional wedge biopsies of the liver were collected at 28 days post-administration. cDNA was prepared from RNA isolated from monkey liver samples, and qPCR was performed to determine FAS mRNA knockdown. Table 9 shows the mRNA knockdown of FAS expression in liver at 28 days after the administration of the FAS RNAi agent compared to PBS control group.

Table 9: FAS mRNA knockdown in cynomolgus monkey.

Duplex	28 days %huFas mRNA KD (3mg/kg)
D: 238	47
D: 227	72
D: 240	51

What is claimed is:

1. An RNAi agent for reducing FAS gene expression, wherein the RNAi agent comprises a delivery moiety of Formula I conjugated to R, wherein R is a double stranded RNA (dsRNA) comprising an antisense strand and a sense strand:



Formula I,

wherein R is conjugated to connection point E of Formula I, optionally via a linker, wherein the sense strand and the antisense strand form a duplex region, and wherein the antisense strand comprises a region of complementarity to a FAS mRNA target sequence of SEQ ID NO: 1, and wherein the sense and antisense strand each optionally comprise one or more modified nucleotides and one or more modified internucleotide linkages.

2. The RNAi agent of claim 1, wherein Formula I is conjugated to the sense strand, optionally via a linker.

3. The RNAi agent of claim 2, wherein Formula I is conjugated to the 3' terminal nucleotide of the sense strand, optionally via a linker.

4. The RNAi agent of any one of claims 1 to 3, wherein the antisense strand is 15 to 50 nucleotides in length.

5. The RNAi agent of any one of Claims 1 to 4, wherein the sense strand is 15 to 50 nucleotides in length.

6. The RNAi agent of any one of claims 1 to 5, wherein the antisense strand is between 18 and 23 nucleotides in length.

7. The RNAi agent of any one of claims 1 to 6, wherein the sense strand is between 18 and 21 nucleotides in length.

8. The RNAi agent of any one of claims 1 to 7, wherein the antisense strand is 23 nucleotides in length and the sense strand is 21 nucleotides in length.

9. The RNAi agent of any one of Claims 1 to 8, wherein the region of complementarity is at least 18 nucleotides in length.

10. The RNAi agent of any one of claims 1 to 9, wherein the antisense strand comprises a sequence selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 112.

11. The RNAi agent of any one of claims 1 to 10, wherein the antisense strand comprises at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 224 to 334, 337, 338, 573, and 577.

12. The RNAi agent of any one of claims 1 to 11, wherein the antisense strand has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 224 -334, 337, 338, 573, and 577, or a sequence having at least 90% sequence identity thereto.

13. The RNAi agent of any one of claims 1 to 12, wherein the sense strand is selected from the group consisting of SEQ ID NOs: 113 to 223, 335, 336, 572, and 576, or a sequence having at least 90% sequence identity thereto.

14. The RNAi agent of any one of claims 1 to 13, wherein the duplex region between the sense strand and the antisense strand comprises 0, 1, 2, or 3 mismatches between the sense strand and the antisense strand.

15. The RNAi agent of any one of claims 1 to 14, wherein the sense strand comprises a first nucleic acid sequence and the antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:
    a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 129, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 240;
    b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 116, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 227;
    c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 151, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 262;
    d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 128, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 239; and
    e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 155, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 266.

16. The RNAi agent of any one of claims 1 to 15, wherein the sense strand comprises a first nucleic acid sequence and the antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:
    a. the first nucleic acid sequence comprises SEQ ID NO: 129, and the second nucleic acid sequence comprises SEQ ID NO: 240;
    b. the first nucleic acid sequence comprises SEQ ID NO: 116, and the second nucleic acid sequence comprises SEQ ID NO: 227;
    c. the first nucleic acid sequence comprises SEQ ID NO: 151, and the second nucleic acid sequence comprises SEQ ID NO: 262;
    d. the first nucleic acid sequence comprises SEQ ID NO: 128, and the second nucleic acid sequence comprises SEQ ID NO: 239; and

e. the first nucleic acid sequence comprises SEQ ID NO: 155, and the second nucleic acid sequence comprises SEQ ID NO: 266.

17. The RNAi agent of any one of claims 1 to 16, wherein the sense strand or the antisense strand each independently comprise one or more modified nucleotides.

18. The RNAi agent of any one of claims 1 to 17, wherein the sense strand or the antisense strand each independently comprise one or more modified nucleotides, and the modified nucleotides are independently 2' fluoro modified nucleotide residues, 2'-O-methyl modified nucleotides, or glycol nucleic acid (GNA) nucleotides.

19. The RNAi agent of any one of claims 1 to 18, wherein the sense strand comprises one or more modified nucleotide residues, and wherein at least one modified nucleotide residue is a GNA nucleotide that is present in an internal position of the sense strand.

20. The RNAi agent of any one of claims 1 to 19, wherein each nucleotide of the sense strand and each nucleotide of the antisense strand is a modified nucleotide.

21. The RNAi agent of any one of claims 1 to 20, wherein the antisense strand is 23 nucleotides in length and wherein each nucleotide of the antisense strand is a modified nucleotide, and 2' fluoro modified nucleotides are present at

a. Positions 2, 3, 7, 14, and 16 from the 5' end of the antisense strand; or
b. Positions 2, 5, 7, 14, and 16 from the 5' end of the antisense strand; or
c. Positions 2, 3, 8, 14, and 16 from the 5' end of the antisense strand; or
d. Positions 2, 5, 8, 14, and 16 from the 5' end of the antisense strand; or
e. Positions 2, 6, 14, and 16 from the 5' end of the antisense strand.

22. The RNAi agent of any one of claims 1 to 21, wherein the sense strand and antisense strand each independently comprise one or more modified internucleotide linkages, and wherein each modified internucleotide linkage is a phosphorothioate linkage.

23. The RNAi agent of claim 1 to 22, wherein the sense strand and antisense strand each independently comprise four phosphorothioate linkages.

24. The RNAi agent of any one of claims 1 to 23, wherein the 5' terminal nucleotide of the antisense strand comprises a phosphate group or a phosphate analog.

25. The RNAi agent of any one of claims 1 to 24, wherein the antisense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 563, 566, 567, 569, 570, 571, 575, 579, 581, 583, 585, or a sequence having at least 90% sequence identity thereto, wherein the 5' terminal nucleotide of the antisense strand comprises a vinyl phosphonate, a phosphate, or a hydroxyl group.

26. The RNAi agent of any one of claims 1 to 25, wherein the sense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 564, 565, 568, 574, 578, 580, 582, 584 or a sequence having at least 90% sequence identity thereto, wherein the 5' terminal nucleotide of the antisense strand comprises a vinyl phosphonate, a phosphate, or a hydroxyl group.

27. The RNAi agent of any one of claims 1 to 24, wherein the antisense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 813, 815, 817, 819, or a sequence having at least 90% sequence identity thereto.

28. The RNAi agent of any one of claims 1 to 24 or 27, wherein the sense strand comprises a sequence selected from the group consisting of SEQ ID NOs: 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 809, 810, 811, 812, 814, 816, 818, or a sequence having at least 90% sequence identity thereto.

29. The RNAi agent of any one of claims 1 to 28, wherein the sense strand comprises a first nucleic acid sequence and the antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:
    a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 339, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 340;
    b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 341, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 342;

c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 343, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 344;
d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO:345, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 346;
e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 347, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 348;
f. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 349, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 350;
g. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 353, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 354;
h. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 363 and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 364; and
i. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 381, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 382.

30. The RNAi agent of any one of claims 1 to 28, wherein the sense strand comprises a first nucleic acid sequence and the antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:
a. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 564 or 809, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 571;
b. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 568 or 811, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 567;

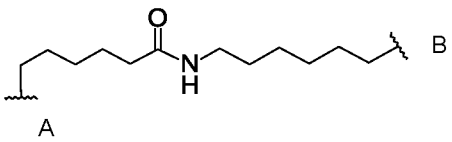
c. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 580 or 814, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 582 or 816, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 584 or 818, and the second nucleic acid sequence has at least 95% sequence identity to SEQ ID NO: 585 or 819.

31. The RNAi agent of any one of claims 1 to 28, wherein the sense strand comprises a first nucleic acid sequence and the antisense strand comprises a second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are selected from the group consisting of:
a. the first nucleic acid sequence comprises SEQ ID NO: 564 or 809, and the second nucleic acid sequence comprises SEQ ID NO: 571;
b. the first nucleic acid sequence comprises SEQ ID NO: 568 or 811, and the second nucleic acid sequence comprises SEQ ID NO: 567;
c. the first nucleic acid sequence comprises SEQ ID NO: 580 or 814, and the second nucleic acid sequence comprises SEQ ID NO: 581 or 815;
d. the first nucleic acid sequence comprises SEQ ID NO: 582 or 816, and the second nucleic acid sequence comprises SEQ ID NO: 583 or 817; and
e. the first nucleic acid sequence comprises SEQ ID NO: 584 or 818, and the second nucleic acid sequence comprises SEQ ID NO: 585 or 819.

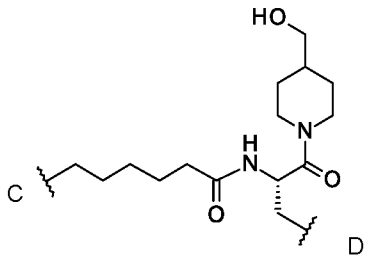
32. The RNAi agent of any one of claims 29-31, wherein the 5' terminal nucleotide of the antisense strand contains a vinyl phosphonate, a phosphate group, or an OH group.

33. The RNAi agent of any one of the claims 1 to 32, wherein R is conjugated to Formula I via a linker.

34. The RNAi agent of claims 1 to 33, wherein R is conjugated to Formula I via a linker, and wherein linker comprises a linker of Formula II having connection points A and B or the linker comprises Formula III having connection points C and D, and wherein:



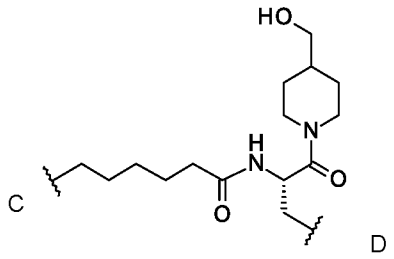
Formula II;



Formula III;

a. Formula I is conjugated to Formula II at connection point A and Formula II is conjugated to a phosphate group or a phosphorothioate group at connection point B, and the phosphate group or phosphorothioate group is further conjugated to R; or
b. Formula I is conjugated to Formula III at connection point C and Formula III is conjugated to a phosphate group or phosphorothioate group at connection point D, and the phosphate group or phosphorothioate group is further conjugated to R.

35. The RNAi agent of any one of claims 1 to 34, wherein R is conjugated to Formula I via a linker, and wherein the linker is a linker comprising Formula III having connection points C and D:



Formula III;

and wherein Formula I is conjugated to Formula III at connection point C and Formula III is conjugated to a phosphate group or a phosphorothioate group at connection point D, and the phosphate group or the phosphorothioate group is further conjugated to R.

36. The RNAi agent of any one of claims 1 to 35, wherein the RNAi agent decreases expression of the FAS gene in a liver cell, as compared to a control.

37. The RNAi agent of any one of claims 1 to 35, for use in therapy.

38. The RNAi agent of any one of claims 1 to 35, for use in the treatment of autoimmune hepatitis (AIH).

39. A pharmaceutical composition comprising the RNAi agent of any one of claims 1 to 35, and one or more pharmaceutically acceptable excipients.

40. The use of the RNAi agent of any one of claims 1 to 35, in the manufacture of a medicament for the treatment of autoimmune hepatitis (AIH).

41. A method of treating autoimmune hepatitis (AIH) in a patient in need thereof, comprising administering to the patient the RNAi agent of any one of claims 1 to 35, or a pharmaceutical composition thereof.

42. A method of decreasing FAS expression in a cell, comprising contacting the cell with the RNAi agent of any one of claims 1 to 35.

43. The method of claim 42, wherein the method further comprises incubating the cell for a time sufficient for decreasing the level of FAS mRNA by at least 50% as compared to an untreated or control treated cell.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/084990

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAVARI FERYAL ET AL: "Evaluation of the therapeutic potential effect of Fas receptor gene knockdown in experimental model of non-alcoholic steatohepatitis", FREE RADICAL RESEARCH, vol. 53, no. 5, 4 May 2019 (2019-05-04), pages 486-496, XP093141073, GB ISSN: 1071-5762, DOI: 10.1080/10715762.2019.1608982 ----- -/--	1-43

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2024	02/05/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Piret, Bernard

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Form PCT/ISA/210 (second sheet) (April 2005)

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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No.

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

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

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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