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APOLIPOPROTEIN A-I AGONISTS AND THEIR USE TO TREAT DYSLIPIDEMIC DISORDERS

1. INTRODUCTION

The invention relates to apolipoprotein A-I (ApoA-I) agonist compositions for treating disorders associated with dyslipoproteinemia, including hypercholesterolemia, cardiovascular disease, atherosclerosis, restenosis, and other disorders such as septic shock.

2. BACKGROUND OF THE INVENTION

Circulating cholesterol is carried by plasma lipoproteins -- particles of complex lipid and protein composition that transport lipids in the blood. Low density lipoproteins (LDL), and high density lipoproteins (HDL) are the major cholesterol carriers. LDL are believed to be responsible for the delivery of cholesterol from the liver (where it is synthesized or obtained from dietary sources) to extrahepatic tissues in the body. The term "reverse cholesterol transport" describes the transport of cholesterol from extrahepatic tissues to the liver where it is catabolized and eliminated. It is believed that plasma HDL particles play a major role in the reverse transport process, acting as scavengers of tissue cholesterol.

The evidence linking elevated serum cholesterol to coronary heart disease is overwhelming. For example, atherosclerosis is a slowly progressive disease characterized by the accumulation of cholesterol within the arterial wall. Compelling evidence supports the concept that lipids deposited in atherosclerotic lesions are derived primarily from plasma LDL; thus, LDLs have popularly become known as the "bad" cholesterol. In contrast, HDL serum levels correlate inversely with coronary heart disease -- indeed, high serum levels of HDL are regarded as a negative risk factor. It is hypothesized that high levels of plasma HDL are not only protective against coronary artery disease, but may actually induce regression of atherosclerotic plaques (e.g., see

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Badimon et al., 1992, Circulation 86 (Suppl. III):86-94). Thus, HDL have popularly become known as the "good" cholesterol.

2.1. CHOLESTEROL TRANSPORT

The fat-transport system can be divided into two pathways: an exogenous one for cholesterol and triglycerides absorbed from the intestine, and an endogenous one for cholesterol and triglycerides entering the bloodstream from the liver and other non-hepatic tissue.

In the exogenous pathway, dietary fats are packaged into lipoprotein particles called chylomicrons which enter the bloodstream and deliver their triglycerides to adipose tissue (for storage) and to muscle (for oxidation to supply energy). The remnant of the chylomicron, containing cholesteryl esters, is removed from the circulation by a specific receptor found only on liver cells. This cholesterol then becomes available again for cellular metabolism or for recycling to extrahepatic tissues as plasma lipoproteins.

In the endogenous pathway, the liver secretes a large, very-low-density lipoprotein particle (VLDL) into the bloodstream. The core of VLDLs consists mostly of triglycerides synthesized in the liver, with a smaller amount of cholesteryl esters (either synthesized in the liver or recycled from chylomicrons). Two predominant proteins are displayed on the surface of VLDLs, apoprotein B-100 and apoprotein E. When a VLDL reaches the capillaries of adipose tissue or of muscle, its triglycerides are extracted resulting in a new kind of particle, decreased in size and enriched in cholesteryl esters but retaining its two apoproteins, called intermediate-density lipoprotein (IDL).

In human beings, about half of the IDL particles are removed from the circulation quickly (within two to six hours of their formation), because they bind tightly to liver cells which extract their cholesterol to make new VLDL and bile acids. The IDL particles which are not taken up by the liver

remain in the circulation longer. In time, the apoprotein E dissociates from the circulating particles, converting them to LDL having apoprotein B-100 as their sole protein.

Primarily, the liver takes up and degrades most of the cholesterol to bile acids, which are the end products of cholesterol metabolism. The uptake of cholesterol containing particles is mediated by LDL receptors, which are present in high concentrations on hepatocytes. The LDL receptor binds both apoprotein E and apoprotein B-100, and is responsible for binding and removing both IDLs and LDLs from the circulation. However, the affinity of apoprotein E for the LDL receptor is greater than that of apoprotein B-100. As a result, the LDL particles have a much longer circulating life span than IDL particles -- LDLs circulate for an average of two and a half days before binding to the LDL receptors in the liver and other tissues. High serum levels of LDL (the "bad" cholesterol) are positively associated with coronary heart disease. For example, in atherosclerosis, cholesterol derived from circulating LDLs accumulates in the walls of arteries leading to the formation of bulky plaques that inhibit the flow of blood until a clot eventually forms, obstructing the artery causing a heart attack or stroke.

Ultimately, the amount of intracellular cholesterol liberated from the LDLs controls cellular cholesterol metabolism. The accumulation of cellular cholesterol derived from VLDLs and LDLs controls three processes: first, it reduces cellular cholesterol synthesis by turning off the synthesis of HMGCoA reductase -- a key enzyme in the cholesterol biosynthetic pathway. Second, the incoming LDL-derived cholesterol promotes storage of cholesterol by activating ACAT -- the cellular enzyme which converts cholesterol into cholesteryl esters that are deposited in storage droplets. Third, the accumulation of cholesterol within the cell drives a feedback mechanism that inhibits cellular synthesis of new LDL receptors. Cells, therefore, adjust their complement of LDL receptors so that enough

cholesterol is brought in to meet their metabolic needs, without overloading. (For a review, see Brown & Goldstein, In, The Pharmacological Basis Of Therapeutics, 8th Ed., Goodman & Gilman, Pergamon Press, NY, 1990, Ch. 36, pp. 874-896).

2.2. REVERSE CHOLESTEROL TRANSPORT

In sum, peripheral (non-hepatic) cells obtain their cholesterol from a combination of local synthesis and the uptake of preformed sterol from VLDLs and LDLs. In contrast, reverse cholesterol transport (RCT) is the pathway by which peripheral cell cholesterol can be returned to the liver for recycling to extrahepatic tissues, or excretion into the intestine in bile, either in modified or in oxidized form as bile acids. The RCT pathway represents the only means of eliminating cholesterol from most extrahepatic tissues, and is crucial to maintenance of the structure and function of most cells in the body.

The RCT consists mainly of three steps: (a) cholesterol efflux, the initial removal of cholesterol from various pools of peripheral cells; (b) cholesterol esterification by the action of lecithin:cholesterol acyltransferase (LCAT), preventing a re-entry of effluxed cholesterol into cells; and (c) uptake/delivery of HDL cholesteryl ester to liver cells. The RCT pathway is mediated by HDLs. HDL is a generic term for lipoprotein particles which are characterized by their high density. The main lipidic constituents of HDL complexes are various phospholipids, cholesterol (ester) and triglycerides. The most prominent apolipoprotein components are A-I and A-II which determine the functional characteristics of HDL; furthermore minor amounts of apolipoprotein C-I, C-II, C-III, D, E, J, etc. have been observed. HDL can exist in a wide variety of different sizes and different mixtures of the above-mentioned constituents depending on the status of remodeling during the metabolic RCT cascade.

The key enzyme involved in the RCT pathway is LCAT. LCAT is produced mainly in the liver and circulates in plasma associated with the HDL fraction. LCAT converts cell derived cholesterol to cholesteryl esters which are sequestered in HDL destined for removal. Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) contribute to further remodeling the circulating HDL population. CETP can move cholesteryl esters made by LCAT to other lipoproteins, particularly ApoB-containing lipoproteins, such as VLDL and LDL. PLTP supplies lecithin to HDL. HDL triglycerides can be catabolized by the extracellular hepatic triglyceride lipase, and lipoprotein cholesterol is removed by the liver via several mechanisms.

Each HDL particle contains at least one copy (and usually two to four copies) of ApoA-I. ApoA-I is synthesized by the liver and small intestine as preproapolipoprotein which is secreted as a proprotein that is rapidly cleaved to generate a mature polypeptide having 243 amino acid residues. ApoA-I consists mainly of 6 to 8 different 22 amino acid repeats spaced by a linker moiety which is often proline, and in some cases consists of a stretch made up of several residues. ApoA-I forms three types of stable complexes with lipids: small, lipid-poor complexes referred to as pre-beta-1 HDL; flattened discoidal particles containing polar lipids (phospholipid and cholesterol) referred to as pre-beta-2 HDL; and spherical particles containing both polar and nonpolar lipids, referred to as spherical or mature HDL (HDL_3 and HDL_2). Most HDL in the circulating population contain both ApoA-I and ApoA-II (the second major HDL protein) and are referred to herein as the AI/AII-HDL fraction of HDL. However, the fraction of HDL containing only ApoA-I (referred to herein as the AI-HDL fraction) appear to be more effective in RCT. Certain epidemiologic studies support the hypothesis that the AI-HDL fraction is anti-atherogenic. (Parra et al., 1992, Arterioscler. Thromb. 12:701-707; Decossin et al., 1997, Eur. J. Clin. Invest. 27:299-307).

Although the mechanism for cholesterol transfer from the cell surface (i.e., cholesterol efflux) is unknown, it is believed that the lipid-poor complex, pre-beta-1 HDL is the preferred acceptor for cholesterol transferred from peripheral tissue involved in RCT. (See Davidson et al., 1994, J. Biol. Chem. 269:22975-22982; Bielicki et al., 1992, J. Lipid Res. 33:1699-1709; Rothblat et al., 1992, J. Lipid Res. 33:1091-1097; and Kawano et al., 1993, Biochemistry 32:5025-5028; Kawano et al., 1997, Biochemistry 36:9816-9825). During this process of cholesterol recruitment from the cell surface, pre-beta-1 HDL is rapidly converted to pre-beta-2 HDL. PLTP may increase the rate of pre-beta-2 disc formation, but data indicating a role for PLTP in RCT is lacking. LCAT reacts preferentially with discoidal and spherical HDL, transferring the 2-acyl group of lecithin or other phospholipids to the free hydroxyl residue of cholesterol to generate cholesteryl esters (retained in the HDL) and lysolecithin. The LCAT reaction requires ApoA-I as activator; i.e., ApoA-I is the natural cofactor for LCAT. The conversion of cholesterol to its ester sequestered in the HDL prevents re-entry of cholesterol into the cell, the result being that cholesteryl esters are destined for removal. Cholesteryl esters in the mature HDL particles in the AI-HDL fraction (i.e., containing ApoA-I and no ApoA-II) are removed by the liver and processed into bile more effectively than those derived from HDL containing both ApoA-I and ApoA-II (the AI/AII-HDL fraction). This may be due, in part, to the more effective binding of AI-HDL to the hepatocyte membrane. The existence of an HDL receptor has been hypothesized, and recently a scavenger receptor, SR-BI, was identified as an HDL receptor (Acton et al., 1996, Science 271:518-520; Xu et al., 1997, Lipid Res. 38:1289-1298). The SR-BI is expressed most abundantly in steroidogenic tissues (e.g., the adrenals), and in the liver (Landshulz et al., 1996, J. Clin. Invest. 98:984-995; Rigotti et al., 1996, J. Biol. Chem. 271:33545-33549).

CETP does not appear to play a major role in RCT, and instead is involved in the metabolism of VLDL-and LDL-derived lipids. However, changes in CETP activity or its acceptors, VLDL and LDL, play a role in "remodeling" the HDL population. For example, in the absence of CETP, the HDLs become enlarged particles which are not cleared. (For reviews on RCT and HDLs, see Fielding & Fielding, 1995, J. Lipid Res. 36:211-228; Barrans et al., 1996, Biochem. Biophys. Acta. 1300:73-85; Hirano et al., 1997, Arterioscler. Thromb. Vasc. Biol. 17(6):1053-1059).

2.3. CURRENT TREATMENTS FOR DYSLIPOPROTEINEMIAS

A number of treatments are currently available for lowering serum cholesterol and triglycerides (see, e.g., Brown & Goldstein, supra). However, each has its own drawbacks and limitations in terms of efficacy, side-effects and qualifying patient population.

Bile-acid-binding resins are a class of drugs that interrupt the recycling of bile acids from the intestine to the liver; e.g., cholestyramine (Questran Light®, Bristol-Myers Squibb), and colestipol hydrochloride (Colestid®, The Upjohn Company). When taken orally, these positively-charged resins bind to the negatively charged bile acids in the intestine. Because the resins cannot be absorbed from the intestine, they are excreted carrying the bile acids with them. The use of such resins, however, at best only lowers serum cholesterol levels by about 20%, and is associated with gastrointestinal side-effects, including constipation and certain vitamin deficiencies. Moreover, since the resins bind other drugs, other oral medications must be taken at least one hour before or four to six hours subsequent to ingestion of the resin; thus, complicating heart patient's drug regimens.

The statins are cholesterol lowering agents that block cholesterol synthesis by inhibiting HMGCoA reductase -- the key enzyme involved in the cholesterol biosynthetic pathway. The statins, e.g., lovastatin (Mevacor®, Merck &

Co., Inc.), and pravastatin (Pravachol®, Bristol-Myers Squibb Co.) are sometimes used in combination with bile-acid-binding resins. The statins significantly reduce serum cholesterol and LDL-serum levels, and slow progression of coronary atherosclerosis. However, serum HDL cholesterol levels are only moderately increased. The mechanism of the LDL lowering effect may involve both reduction of VLDL concentration and induction of cellular expression of LDL-receptor, leading to reduced production and/or increased catabolism of LDLs. Side effects, including liver and kidney dysfunction are associated with the use of these drugs (Physicians Desk Reference, Medical Economics Co., Inc., Montvale, N.J., 1997). Recently, the FDA has approved atorvastatin (an HMGCoA reductase inhibitor developed by Parke-Davis) (Warner Lambert) for the market to treat rare but urgent cases of familial hypercholesterolemia (1995, Scrip 20(19):10).

Niacin, or nicotinic acid, is a water soluble vitamin B-complex used as a dietary supplement and antihyperlipidemic agent. Niacin diminishes production of VLDL and is effective at lowering LDL. In some cases, it is used in combination with bile-acid binding resins. Niacin can increase HDL when used at adequate doses, however, its usefulness is limited by serious side effects when used at such high doses.

Fibrates are a class of lipid-lowering drugs used to treat various forms of hyperlipidemia (i.e., elevated serum triglycerides) which may also be associated with hypercholesterolemia. Fibrates appear to reduce the VLDL fraction and modestly increase HDL -- however the effects of these drugs on serum cholesterol is variable. In the United States, fibrates have been approved for use as antilipidemic drugs, but have not received approval as hypercholesterolemia agents. For example, clofibrate (Atromid-S®, Wyeth-Ayerst Laboratories) is an antilipidemic agent which acts (via an unknown mechanism) to lower serum triglycerides by reducing the VLDL fraction. Although serum cholesterol may be reduced

in certain patient subpopulations, the biochemical response to the drug is variable, and is not always possible to predict which patients will obtain favorable results. Atromid-S® has not been shown to be effective for prevention of coronary heart disease. The chemically and pharmacologically related drug, gemfibrozil (Lopid®, Parke-Davis) is a lipid regulating agent which moderately decreases serum triglycerides and VLDL cholesterol, and moderately increases HDL cholesterol -- the HDL_2 and HDL_3 subfractions as well as both ApoA-I and A-II (i.e., the AI/AII-HDL fraction). However, the lipid response is heterogeneous, especially among different patient populations. Moreover, while prevention of coronary heart disease was observed in male patients between 40-55 without history or symptoms of existing coronary heart disease, it is not clear to what extent these findings can be extrapolated to other patient populations (e.g., women, older and younger males). Indeed, no efficacy was observed in patients with established coronary heart disease. Serious side-effects are associated with the use of fibrates including toxicity such as malignancy, (especially gastrointestinal cancer), gallbladder disease and an increased incidence in non-coronary mortality. These drugs are not indicated for the treatment of patients with high LDL or low HDL as their only lipid abnormality (Physician's Desk Reference, 1997, Medical Economics Co., Inc. Montvale, N.J.).

Oral estrogen replacement therapy may be considered for moderate hypercholesterolemia in post-menopausal women. However, increases in HDL may be accompanied with an increase in triglycerides. Estrogen treatment is, of course, limited to a specific patient population (postmenopausal women) and is associated with serious side effects including induction of malignant neoplasms, gall bladder disease, thromboembolic disease, hepatic adenoma, elevated blood pressure, glucose intolerance, and hypercalcemia.

Thus, there is a need to develop safer drugs that are efficacious in lowering serum cholesterol, increasing HDL

serum levels, preventing coronary heart disease, and/or treating existing disease, especially atherosclerosis.

2.4. ApoA-I AS A TARGET

None of the currently available drugs for lowering cholesterol safely elevate HDL levels and stimulate RCT -- most appear to operate on the cholesterol transport pathway, modulating dietary intake, recycling, synthesis of cholesterol, and the VLDL population.

While it is desirable to find drugs that stimulate cholesterol efflux and removal, several potential targets in the RCT exist -- e.g., LCAT, HDL and its various components (ApoA-I, ApoA-II and phospholipids), PLTP, and CETP -- and it is not known which target would be most effective at achieving desirable lipoprotein profiles and protective effects. Perturbation of any single component in the RCT pathway ultimately affects the composition of circulating lipoprotein populations, and the efficiency of RCT.

Several lines of evidence based on data obtained in vivo implicate the HDL and its major protein component, ApoA-I, in the prevention of atherosclerotic lesions, and potentially, the regression of plaques -- making these attractive targets for therapeutic intervention. First, an inverse correlation exists between serum ApoA-I (HDL) concentration and atherogenesis in man (Gordon & Rifkind, 1989, N. Eng. J. Med. 321:1311-1316; Gordon et al., 1989, Circulation 79:8-15). Indeed, specific subpopulations of HDL have been associated with a reduced risk for atherosclerosis in humans (Miller, 1987, Amer. Heart 113:589-597; Cheung et al., 1991, Lipid Res. 32:383-394); Fruchart & Ailhaud, 1992, Clin. Chem. 38:79).

Second, animal studies support the protective role of ApoA-I (HDL). Treatment of cholesterol fed rabbits with ApoA-I or HDL reduced the development and progression of plaque (fatty streaks) in cholesterol-fed rabbits. (Koizumi et al., 1988, J. Lipid Res. 29:1405-1415; Badimon et al.,

1989, Lab. Invest. 60:455-461; Badimon et al., 1990, J. Clin. Invest. 85:1234-1241). However, the efficacy varied depending upon the source of HDL (Beitz et al., 1992, Prostaglandins, Leukotrienes and Essential Fatty Acids 47:149-152; Mezdour et al., 1995, Atherosclerosis 113:237-246).

Third, direct evidence for the role of ApoA-I was obtained from experiments involving transgenic animals. The expression of the human gene for ApoA-I transferred to mice genetically predisposed to diet-induced atherosclerosis protected against the development of aortic lesions (Rubin et al., 1991, Nature 353:265-267). The ApoA-I transgene was also shown to suppress atherosclerosis in ApoE-deficient mice and in Apo(a) transgenic mice (Paszty et al., 1994, J. Clin. Invest. 94:899-903; Plump et al., 1994, Proc. Natl. Acad. Sci. USA 91:9607-9611; Liu et al., 1994, J. Lipid Res. 35:2263-2266). Similar results were observed in transgenic rabbits expressing human ApoA-I (Duverger, 1996, Circulation 94:713-717; Duverger et al., 1996, Arterioscler. Thromb. Vasc. Biol. 16:1424-1429), and in transgenic rats where elevated levels of human ApoA-I protected against atherosclerosis and inhibited restenosis following balloon angioplasty (Burkey et al., 1992, Circulation, Supplement I, 86:I-472, Abstract No. 1876; Burkey et al., 1995, J. Lipid Res. 36:1463-1473).

The AI-HDL appear to be more efficient at RCT than the AI/AII-HDL fraction. Studies with mice transgenic for human ApoA-I or Apo-I and ApoA-II (AI/AII) showed that the protein composition of HDL significantly affects its role -- AI-HDL is more anti-atherogenic than AI/AII-HDL (Schultz et al., 1993, Nature 365:762-764). Parallel studies involving transgenic mice expressing the human LCAT gene demonstrate that moderate increases in LCAT activity significantly change lipoprotein cholesterol levels, and that LCAT has a significant preference for HDL containing ApoA-I (Francone et al., 1995, J. Clinic. Invest. 96:1440-1448; Berard et al., 1997, Nature Medicine 3(7):744-749). While these data support a significant role for ApoA-I in activating LCAT and

stimulating RCT, additional studies demonstrate a more complicated scenario: a major component of HDL that modulates efflux of cell cholesterol is the phospholipids (Fournier et al., 1996, J. Lipid Res. 37:1704-1711).

In view of the potential role of HDL, i.e., both ApoA-I and its associated phospholipid, in the protection against atherosclerotic disease, human clinical trials utilizing recombinantly produced ApoA-I were commenced, discontinued and apparently re-commenced by UCB Belgium (Pharmaprojects, Oct. 27, 1995; IMS R&D Focus, June 30, 1997; Drug Status Update, 1997, Atherosclerosis 2(6):261-265); see also M. Eriksson at Congress, "The Role of HDL in Disease Prevention," Nov. 7-9, 1996, Fort Worth; Lacko & Miller, 1997, J. Lip. Res. 38:1267-1273; and WO94/13819) and were commenced and discontinued by Bio-Tech (Pharmaprojects, April 7, 1989). Trials were also attempted using ApoA-I to treat septic shock (Opal, "Reconstituted HDL as a Treatment Strategy for Sepsis," IBC's 7th International Conference on Sepsis, April 28-30, 1997, Washington, D.C.; Gouni et al., 1993, J. Lipid Res. 94:139-146; Levine, WO96/04916). However, there are many pitfalls associated with the production and use of ApoA-I, making it less than ideal as a drug; e.g., ApoA-I is a large protein that is difficult and expensive to produce; significant manufacturing and reproducibility problems must be overcome with respect to stability during storage, delivery of an active product and half-life in vivo.

In view of these drawbacks, attempts have been made to prepare peptides that mimic ApoA-I. Since the key activities of ApoA-I have been attributed to the presence of multiple repeats of a unique secondary structural feature in the protein -- a class A amphipathic α-helix (Segrest, 1974, FEBS Lett. 38:247-253), most efforts to design peptides which mimic the activity of ApoA-I have focused on designing peptides which form class A-type amphipathic α-helices.

Class A-type amphipathic α-helices are unique in that positively charged amino acid residues are clustered at

the hydrophobic-hydrophilic interface and negatively charged amino acid residues are clustered at the center of the hydrophilic face. Furthermore, class A α-helical peptides have a hydrophobic angle of less than 180° (Segrest et al., 1990, PROTEINS: Structure, Function and Genetics 8:103-117). The initial de novo strategies to design ApoA-I mimics were not based upon the primary sequences of naturally occurring apolipoproteins, but rather upon incorporating these unique Class A helix features into the sequences of the peptide analogues, as well as some of the properties of the ApoA-I domains (see, e.g., Davidson et al., 1996, Proc. Natl. Acad. Sci. USA 93:13605-13610; Rogers et al., 1997, Biochemistry 36:288-300; Lins et al., 1993, Biochim. Biophys. Acta biomembranes 1151:137-142; Ji and Jonas, 1995, J. Biol. Chem. 270:11290-11297; Collet et al., 1997, Journal of Lipid Research, 38:634-644; Sparrow and Gotto, 1980, Ann. N.Y. Acad. Sci. 348:187-211; Sparrow and Gotto, 1982, CRC Crit. Rev. Biochem. 13:87-107; Sorci-Thomas et al., 1993, J. Biol. Chem. 268:21403-21409; Wang et al., 1996, Biochim. Biophys. Acta 174-184; Minnich et al., 1992, J. Biol. Chem. 267:16553-16560; Holvoet et al., 1995, Biochemistry 34:13334-13342; Sorci-Thomas et al., 1997, J. Biol. Chem. 272(11):7278-7284; and Frank et al., 1997, Biochemistry 36:1798-1806).

In one study, Fukushima et al. synthesized a 22-residue peptide composed entirely of Glu, Lys and Leu residues arranged periodically so as to form an amphipathic α-helix with equal hydrophilic and hydrophobic faces ("ELK peptide") (Fukushima et al., 1979, J. Amer. Chem. Soc. 101(13):3703-3704; Fukushima et al., 1980, J. Biol. Chem. 255:10651-10657). The ELK peptide shares 41% sequence homology with the 198-219 fragment of ApoA-I. As studied by quantitative ultrafiltration, gel permeation chromatography and circular dichroism, this ELK peptide was shown to effectively associate with phospholipids and mimic some of the physical and chemical properties of ApoA-I (Kaiser et al., 1983, Proc. Natl. Acad. Sci. USA 80:1137-1140; Kaiser et al., 1984, Science 223:249-

255; Fukushima et al., 1980, supra; Nakagawa et al., 1985, J. Am. Chem. Soc. 107:7087-7092). Yokoyama et al. concluded from such studies that the crucial factor for LCAT activation is simply the presence of a large enough amphipathic structure (Yokoyama et al., 1980, J. Biol. Chem. 255(15):7333-7339). A dimer of this 22-residue peptide was later found to more closely mimic ApoA-I than the monomer; based on these results, it was suggested that the 44-mer, which is punctuated in the middle by a helix breaker (either Gly or Pro), represented the minimal functional domain in ApoA-I (Nakagawa et al., 1985, supra).

Another study involved model amphipathic peptides called "LAP peptides" (Pownall et al., 1980, Proc. Natl. Acad. Sci. USA 77(6):3154-3158; Sparrow et al., 1981, *In:* Peptides: Synthesis-Structure-Function, Roch and Gross, Eds., Pierce Chem. Co., Rockford, IL, 253-256). Based on lipid binding studies with fragments of native apolipoproteins, several LAP peptides were designed, named LAP-16, LAP-20 and LAP-24 (containing 16, 20 and 24 amino acid residues, respectively). These model amphipathic peptides share no sequence homology with the apolipoproteins and were designed to have hydrophilic faces organized in a manner unlike the class A-type amphipathic helical domains associated with apolipoproteins (Segrest et al., 1992, J. Lipid Res. 33:141-166). From these studies, the authors concluded that a minimal length of 20 residues is necessary to confer lipid-binding properties to model amphipathic peptides.

Studies with mutants of LAP20 containing a proline residue at different positions in the sequence indicated that a direct relationship exists between lipid binding and LCAT activation, but that the helical potential of a peptide alone does not lead to LCAT activation (Ponsin et al., 1986 J. Biol. Chem. 261(20):9202-9205). Moreover, the presence of this helix breaker (Pro) close to the middle of the peptide reduced its affinity for phospholipid surfaces as well as its ability to activate LCAT. While certain of the LAP peptides were

shown to bind phospholipids (Sparrow et al., supra), controversy exists as to the extent to which LAP peptides are helical in the presence of lipids (Buchko et al., 1996, J. Biol. Chem. 271(6):3039-3045; Zhong et al., 1994, Peptide Research 7(2):99-106).

Segrest et al. have synthesized peptides composed of 18 to 24 amino acid residues that share no sequence homology with the helices of ApoA-I (Kannelis et al., 1980, J. Biol. Chem. 255(3):11464-11472; Segrest et al., 1983, J. Biol. Chem. 258:2290-2295). The sequences were specifically designed to mimic the amphipathic helical domains of class A exchangeable apolipoproteins in terms of hydrophobic moment (Eisenberg et al., 1982, Nature 299:371-374) and charge distribution (Segrest et al., 1990, Proteins 8:103-117; U.S. Patent No. 4,643,988). One 18-residue peptide, the "18A" peptide, was designed to be a model class-A α-helix (Segrest et al., 1990, supra). Studies with these peptides and other peptides having a reversed charged distribution, like the "18R" peptide, have consistently shown that charge distribution is critical for activity; peptides with a reversed charge distribution exhibit decreased lipid affinity relative to the 18A class-A mimics and a lower helical content in the presence of lipids (Kanellis et al., 1980, J. Biol. Chem. 255:11464-11472; Anantharamaiah et al., 1985, J. Biol. Chem. 260:10248-10255; Chung et al., 1985, J. Biol. Chem. 260:10256-10262; Epand et al., 1987, J. Biol. Chem. 262:9389-9396; Anantharamaiah et al., 1991, Adv. Exp. Med. Biol. 285:131-140).

Other synthetic peptides sharing no sequence homology with the apolipoproteins which have been proposed with limited success include dimers and trimers of the 18A peptide (Anantharamaiah et al., 1986, Proteins of Biological Fluids 34:63-66), GALA and EALA peptides (Subbarao et al., 1988, PROTEINS: Structure, Function and Genetics 3:187-198) and ID peptides (Labeur et al., 1997, Arteriosclerosis, Thrombosis and Vascular Biology 17:580-588) and the 18AM4

peptide (Brasseur et al., 1993, Biochim. Biophys. Acta 1170:1-7).

A "consensus" peptide containing 22-amino acid residues based on the sequences of the helices of human ApoA-I has also been designed (Anantharamaiah et al., 1990, Arteriosclerosis 10(1):95-105; Venkatachalapathi et al., 1991, Mol. Conformation and Biol. Interactions, Indian Acad. Sci. B:585-596). The sequence was constructed by identifying the most prevalent residue at each position of the hypothesized helices of human ApoA-I. Like the peptides described above, the helix formed by this peptide has positively charged amino acid residues clustered at the hydrophilic-hydrophobic interface, negatively charged amino acid residues clustered at the center of the hydrophilic face and a hydrophobic angle of less than 180°. While a dimer of this peptide is somewhat effective in activating LCAT, the monomer exhibited poor lipid binding properties (Venkatachalapathi et al., 1991, supra).

Based primarily on in vitro studies with the peptides described above, a set of "rules" has emerged for designing peptides which mimic the function of apoA-I. Significantly, it is thought that an amphipathic α-helix having positively charged residues clustered at the hydrophilic-hydrophobic interface and negatively charged amino acid residues clustered at the center of the hydrophilic face is required for lipid affinity and LCAT activation (Venkatachalapathi et al., 1991, supra). Anantharamaiah et al. have also indicated that the negatively charged Glu residue at position 13 of the consensus 22-mer peptide, which is positioned within the hydrophobic face of the α-helix, plays an important role in LCAT activation (Anantharamaiah et al., 1991, supra). Furthermore, Brasseur has indicated that a hydrophobic angle (pho angle) of less than 180° is required for optimal lipid-apolipoprotein complex stability, and also accounts for the formation of discoidal particles having the peptides around the edge of the lipid bilayer (Brasseur, 1991, J. Biol. Chem. 66(24):16120-16127). Rosseneu et al. have also

insisted that a hydrophobic angle of less than 180° is required for LCAT activation (WO93/25581).

However, despite these "rules" to date, no one has designed or produced a peptide as active as ApoA-I -- the best having less than 40% of the activity of ApoA-I as measured by the LCAT activation assay described herein. None of the peptide "mimetics" described in the literature have been demonstrated to be useful as a drug.

In view of the foregoing, there is a need for the development of a stable ApoA-I agonist that mimics the activity of ApoA-I and which is relatively simple and cost-effective to produce. However, the "rules" for designing efficacious ApoA-I mimetics have not been unraveled and the principles for designing organic molecules with the function of ApoA-I are unknown.

3. SUMMARY OF THE INVENTION

The invention relates to ApoA-I agonists capable of forming amphipathic α-helices that mimic the activity of ApoA-I, with specific activities, i.e., units of activity (activation of LCAT)/unit of mass), approaching or exceeding that of the native molecule. In particular, the ApoA-I agonists of the invention are peptides or peptide analogues that: form amphipathic helices (in the presence of lipids), bind lipids, form pre-β-like or HDL-like complexes, activate LCAT, increase serum levels of HDL fractions, and promote cholesterol efflux.

The invention is based, in part, on the applicants' design and discovery of peptides that mimic the function of ApoA-I. The peptides of the invention were designed based on the supposed helical structure and amphipathic properties of the 22 amino acid consensus sequence which was derived from the helical repeats of ApoA-I. Surprisingly, the peptides of the invention have a specific activity well above that reported for ApoA-I-derived peptides described in the literature. Indeed, some embodiments of the invention

approach 100% of the activity of native ApoA-I, whereas superagonists described herein exceed the specific activity of ApoA-I.

The invention is illustrated by way of working examples that describe the structure, preparation and use of particular amphipathic peptides that form helices (in the presence of lipids), bind lipids, form complexes and increase LCAT activity. Based upon the structure and activity of the exemplified embodiments, the applicants have devised a set of "rules" which can be used to design altered or mutated forms that are also within the scope of the invention.

The invention also relates to pharmaceutical formulations containing such ApoA-I agonists (either as peptides or peptide-lipid complexes) as the active ingredient, as well as methods for preparing such formulations and their use to treat diseases associated with dyslipoproteinemia (e.g., cardiovascular diseases, atherosclerosis, metabolic syndrome), restenosis, or endotoxemia (e.g., septic shock).

3.1. ABBREVIATIONS

As used herein, the abbreviations for the genetically encoded L-enantiomeric amino acids are conventional and are as follows:

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu

Amino Acid	One-Letter Symbol	Common Abbreviation
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

The abbreviations used for the D-enantiomers of the genetically encoded amino acids are lower-case equivalents of the one-letter symbols. For example, "R" designates L-arginine and "r" designates D-arginine.

3.2. DEFINITIONS

As used herein, the following terms shall have the following meanings:

"Alkyl:" refers to a saturated branched, straight chain or cyclic hydrocarbon radical. Typical alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, hexyl, and the like. In preferred embodiments, the alkyl groups are (C_1-C_6)alkyl.

"Alkenyl:" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon double bond. The radical may be in either the *cis* or *trans* conformation about the double bond(s). Typical alkenyl groups include, but are not limited to, ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, *tert*-butenyl, pentenyl, hexenyl and the like. In preferred embodiments, the alkenyl group is (C_1-C_6) alkenyl.

"Alkynyl:" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon triple bond. Typical alkynyl groups include, but are not limited to, ethynyl, propynyl, butynyl, isobutynyl, pentynyl, hexynyl and the like. In preferred embodiments, the alkynyl group is (C_1-C_6) alkynyl.

"Aryl:" refers to an unsaturated cyclic hydrocarbon radical having a conjugated π electron system. Typical aryl groups include, but are not limited to, penta-2,4-diene, phenyl, naphthyl, anthracyl, azulenyl, chrysenyl, coronenyl, fluoranthenyl, indacenyl, idenyl, ovalenyl, perylenyl, phenalenyl, phenanthrenyl, picenyl, pleiadenyl, pyrenyl, pyranthrenyl, rubicenyl, and the like. In preferred embodiments, the aryl group is (C_5-C_{20}) aryl, with (C_5-C_{10}) being particularly preferred.

"Alkaryl:" refers to a straight-chain alkyl, alkenyl or alkynyl group wherein one of the hydrogen atoms bonded to a terminal carbon is replaced with an aryl moiety. Typical alkaryl groups include, but are not limited to, benzyl, benzylidene, benzylidyne, benzenobenzyl, naphthenobenzyl and the like. In preferred embodiments, the alkaryl group is (C_6-C_{26}) alkaryl, *i.e.*, the alkyl, alkenyl or alkynyl moiety of the alkaryl group is (C_1-C_6) and the aryl moiety is (C_5-C_{20}). In particularly preferred embodiments, the alkaryl group is (C_6-C_{13}) alkaryl, *i.e.*, the alkyl, alkenyl or alkynyl moiety of the alkaryl group is (C_1-C_3) and the aryl moiety is (C_5-C_{10}).

"Heteroaryl:" refers to an aryl moiety wherein one or more carbon atoms is replaced with another atom, such as N, P, O, S, As, Se, Si, Te, etc. Typical heteroaryl groups include, but are not limited to, acridarsine, acridine, arsanthridine, arsindole, arsindoline, carbazole, β-carboline, chromene, cinnoline, furan, imidazole, indazole, indole, indolizine, isoarsindole, isoarsinoline, isobenzofuran,

isochromene, isoindole, isophosphoindole, isophosphinoline, isoquinoline, isothiazole, isoxazole, naphthyridine, perimidine, phenanthridine, phenanthroline, phenazine, phosphoindole, phosphinoline, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, quinazoline, quinoline, quinolizine, quinoxaline, selenophene, tellurophene, thiophene and xanthene. In preferred embodiments, the heteroaryl group is a 5-20 membered heteroaryl, with 5-10 membered aryl being particularly preferred.

"Alkheteroaryl:" refers to a straight-chain alkyl, alkenyl or alkynyl group where one of the hydrogen atoms bonded to a terminal carbon atom is replaced with a heteroaryl moiety. In preferred embodiments, the alkheteroaryl group is 6-26 membered alkheteroaryl, i.e., the alkyl, alkenyl or alkynyl moiety of the alkheteroaryl is (C_1-C_6) and the heteroaryl is a 5-20-membered heteroaryl. In particularly preferred embodiments the alkheteroaryl is 6-13 membered alkheteroaryl, i.e., the alkyl, alkenyl or alkynyl moiety is a 5-10 membered heteroaryl.

"Substituted Alkyl, Alkenyl, Alkynyl, Aryl, Alkaryl, Heteroaryl or Alkheteroaryl:" refers to an alkyl, alkenyl, alkynyl, aryl, alkaryl, heteroaryl or alkheteroaryl group in which one or more hydrogen atoms is replaced with another substituent. Preferred substituents include -OR, -SR, -NRR, $-NO_2$, -CN, halogen, -C(O)R, -C(O)OR and -C(O)NR, where each R is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl, heteroaryl or alkheteroaryl.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A is a Schiffer-Edmundson helical wheel diagram of an idealized amphipathic α-helix in which open circles represent hydrophilic amino acid residues and shaded circles represent hydrophobic amino acid residues.

FIG. 1B is a helical net diagram of the idealized amphipathic helix of FIG. 1A.

FIG. 1C is a helical cylinder diagram of the idealized amphipathic helix of FIG. 1A.

FIG. 2A is a Schiffer-Edmundson helical wheel diagram of the core peptide of structure (I) illustrating the amphipathicity of the helix (open circles represent hydrophilic amino acid residues, shaded circles represent hydrophobic amino acid residues and partially shaded circles represent either hydrophilic or hydrophobic amino acid residues).

FIG. 2B is a helical net diagram of the core peptide of structure (I) illustrating the hydrophobic face of the helix.

FIG. 2C is a helical net diagram of the core peptide of structure (I) illustrating the hydrophilic face of the helix.

FIG. 3A is a helical net diagram illustrating the hydrophilic face of Segrest's consensus 22-mer peptide (PVLDEFREKLNEELEALKQKLK; SEQ ID NO:75).

FIG. 3B is a helical net diagram illustrating the hydrophilic face of exemplary core peptide 4 (PVLDLFRELLNELLEALKQKLK; SEQ ID NO:4).

FIG. 4A is a helical net diagram illustrating the hydrophobic face of Segrest's consensus 22-mer peptide (SEQ ID NO:75).

FIG. 4B is a helical net diagram illustrating the hydrophobic face of exemplary core peptide 4 (SEQ ID NO:4).

FIG. 5A is a Schiffer-Edmundson helical wheel diagram of Segrest's consensus 22-mer peptide (SEQ ID NO:75).

FIG. 5B is a Schiffer-Edmundson helical wheel diagram of exemplary core peptide 4 (SEQ ID NO:4).

FIG. 6A is a computer model of two peptides 4 (SEQ ID NO:4) arranged in an antiparallel fashion in which residues Glu-8 and Gln-19 are highlighted to illustrate the ability of

these two peptides to form intermolecular hydrogen-bonds when bound to lipids.

FIG. 6B is a computer model of two peptides **102** (PVLDLFRELLNLXLEALKEKLK; SEQ ID NO:102) arranged in an antiparallel fashion in which residues Glu-8 and Glu-19 are highlighted to illustrate the inability of these two peptides to form intermolecular hydrogen-bonds when bound to lipids.

FIG. 7A illustrates a tertiary-order branched network of the invention.

FIG. 7B illustrates a quaternary-order branched network of the invention.

FIG. 7C illustrates a mixed-order branched network of the invention.

FIG. 7D illustrates exemplary "Lys-tree" branched networks of the invention.

FIG. 8A is a graph illustrating the differences between the observed Hα chemical shifts and the tabulated random coil Hα chemical shifts for peptide **4** (SEQ ID NO:4) and Segrest's consensus 22-mer peptide (SEQ ID NO:75).

FIG. 8B is a graph illustrating the differences between the observed amide proton chemical shifts and the tabulated random coil amide proton chemical shifts for peptide **4** (SEQ ID NO:4) and Segrest's consensus 22-mer peptide (SEQ ID NO:75).

FIG. 8C is a cartoon illustrating the periodic relationship between the Hα proton chemical shifts of peptide **4** (SEQ ID NO:4) and its α-helical conformation.

FIGS. 9A-9D provide gel chromatograms of isolated human HDL incubated for 2 hr. at 37°C in buffer as measured by absorbance (—) and in the presence of ^{14}C-labeled peptide **4** as measured by absorbance (---) or ^{14}C-radiometric counting (♦). Chromatograms were obtained at peptide:HDL mass ratios of 1:15 (FIG. 9A), 1:10 (FIG. 9B), 1:5 (FIG. 9C) and 1:3 (FIG. 9D).

FIG. 9E is a control gel filtration chromatogram of free, unbound ^{14}C-labeled peptide **4** as measured by absorbance (---) and ^{14}C radiometric counting (♦).

FIG. 9F provides gel filtration difference chromatograms illustrating the difference between each of the peptide-treated HDL absorbance chromatograms presented in FIGS. 9A (---), 9B (—), 9C (---) and 9D (—) and the control chromatogram of FIG. 9E (positive values indicate a higher absorbance in the treated sample; negative values indicate a greater absorbance in the control sample).

FIG. 10 is a graph illustrating the lipoprotein profile of rabbits injected with 10 mg/kg peptide 4 (SEQ ID NO: 4) (in the form of peptide/DPPC complexes).

FIG. 11A is a cartoon depicting the various aggregation states and peptide-lipid complexes that can be obtained with the ApoA-I agonists of the invention. **Left:** Multimerization process of the peptides resulting from the interaction of several peptide helices and leading to the formation of oligomers in conditions of defined peptide concentration, pH and ionic strength. **Center:** The interaction of the peptides (in any of these states of aggregation) with lipidic entities (such as SUVs) leads to lipid reorganization. **Right:** By changing the lipid:peptide molar ratio, different types of peptide-lipid complexes can be obtained, from lipid-peptide comicelles at low lipid-peptide ratios, to discoidal particles and finally to large multilamellar complexes at increasingly higher lipid:peptide ratios.

FIG. 11B illustrates the generally-accepted model for discoidal peptide-lipid complexes formed in a defined range of lipid:peptide ratios. Each peptide surrounding the disc edge is in close contact with its two nearest neighbors.

5. DETAILED DESCRIPTION OF THE INVENTION

The ApoA-I agonists of the invention mimic ApoA-I function and activity. They form amphipathic helices (in the presence of lipids), bind lipids, form pre-β-like or HDL-like complexes, activate LCAT, increase serum HDL concentration and promote cholesterol efflux. The biological function of the

peptides correlates with their helical structure, or conversion to helical structures in the presence of lipids.

The ApoA-I agonists of the invention can be prepared in stable bulk or unit dosage forms, e.g., lyophilized products, that can be reconstituted before use in vivo or reformulated. The invention includes the pharmaceutical formulations and the use of such preparations in the treatment of hyperlipidemia, hypercholesterolemia, coronary heart disease, atherosclerosis, and other conditions such as endotoxemia causing septic shock.

The invention is illustrated by working examples which demonstrate that the ApoA-I agonists of the invention associate with the HDL component of plasma, and can increase the concentration of HDL and pre-β particles. The ApoA-I agonists of the invention increase cellular cholesterol efflux. The agonists are also extremely efficient at activating LCAT, and thus promote RCT. Use of the ApoA-I agonists of the invention in vivo in animal models results in an increase in serum HDL concentration.

The invention is set forth in more detail in the subsections below, which describe: the composition and structure of the ApoA-I peptide agonists; structural and functional characterization; methods of preparation of bulk and unit dosage formulations; and methods of use.

5.1. PEPTIDE STRUCTURE AND FUNCTION

The ApoA-I agonists of the invention are generally peptides, or analogues thereof, which are capable of forming amphipathic α-helices in the presence of lipids and which mimic the activity of ApoA-I. The agonists have as their main feature a "core" peptide composed of 15 to 29 amino acid residues, preferably 22 amino acid residues, or an analogue thereof wherein at least one amide linkage in the peptide is replaced with a substituted amide, an isostere of an amide or an amide mimetic.

The ApoA-I agonists of the invention are based, in part, on the applicants' surprising discovery that altering certain amino acid residues in the primary sequence of the 22-mer consensus sequence of Venkatachalapathi et al., 1991, Mol. Conformation and Biol. Interactions, Indian Acad. Sci. B:585-596 (PVLDEFREKLNEELEALKQKLK; SEQ ID NO:75; hereinafter "Segrest's consensus 22-mer" or "consensus 22-mer") that were thought to be critical for activity yields synthetic peptides which exhibit activities that approach, or in some embodiments even exceed, the activity of native ApoA-I. In particular, the applicants have discovered that replacing three charged amino acid residues in Segrest's consensus 22-mer peptide (Glu-5, Lys-9 and Glu-13) with a hydrophobic Leu residue provides peptides that mimic the structural and functional properties of ApoA-I to a degree that is unprecedented in the art.

While not intending to be bound by any particular theory, it is believed that the helix formed by the ApoA-I agonists of the invention more closely mimics the structural and functional properties of the amphipathic helical regions of native ApoA-I that are important for effecting lipid-binding, cholesterol efflux and LCAT activation than does the α-helix formed by the ApoA-I mimetic peptides described in the literature, thereby resulting in peptides that exhibit significantly higher ApoA-I-like activity than these other peptides. Indeed, whereas many of the ApoA-I agonists of the invention approach, and in some embodiments even exceed, the activity of ApoA-I, to date, the best peptide ApoA-I mimics described in the literature-- peptide 18AM4 (EWLEAFYKKVLEKLKELF; SEQ ID NO: 246) (Corinjn et al., 1993, Biochim. Biophys. Acta 1170:8-16; Labeur et al., Oct. 1994, Arteriosclerosis: Abstract Nos. 186 and 187) and N-acetylated, C-amidated peptide 18AM4 (SEQ ID NO: 239) (Brasseur, 1993, Biochim. Biophys. Acta 1170:1-7)-- exhibit less than 4% and 11%, respectively, of the activity of ApoA-I as measured by the LCAT activation assay described herein.

Generally, the core peptides (or analogues thereof) that compose the ApoA-I agonists of the invention have the following structural formula (I):

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-X_{18}-X_{19}-X_{20}-X_{21}-X_{22}

wherein:

X_1 is Pro (P), Ala (A), Gly (G), Gln (Q), Asn (N), Asp (D) or D-Pro (p);

X_2 is an aliphatic amino acid;

X_3 is Leu (L) or Phe (F);

X_4 is an acidic amino acid;

X_5 is Leu (L) or Phe (F);

X_6 is Leu (L) or Phe (F);

X_7 is a hydrophilic amino acid;

X_8 is an acidic or a basic amino acid;

X_9 is Leu (L) or Gly (G);

X_{10} is Leu (L), Trp (W) or Gly (G);

X_{11} is a hydrophilic amino acid;

X_{12} is a hydrophilic acid;

X_{13} is Gly (G) or an aliphatic amino acid;

X_{14} is Leu (L), Trp (W), Gly (G) or Nal;

X_{15} is a hydrophilic amino acid;

X_{16} is a hydrophobic amino acid;

X_{17} is a hydrophobic amino acid;

X_{18} is a basic amino acid, Gln (Q) or Asn (N);

X_{19} is a basic amino acid, Gln (Q) or Asn (N);

X_{20} is a basic amino acid;

X_{21} is an aliphatic amino acid; and

X_{22} is a basic amino acid.

The core peptides of structure (I) are defined, in part, in terms of amino acids of designated classes. The definitions of the various designated classes are provided infra in connection with the description of mutated or altered embodiments of structure (I).

In the core peptides of structure (I), the symbol "—" between amino acid residues X_n generally designates a backbone constitutive linking function. Thus, the symbol "-" usually represents a peptide bond or amide linkage (-C(O)NH-). It is to be understood, however, that the present invention contemplates peptide analogues wherein one or more amide linkages is optionally replaced with a linkage other than amide, preferably a substituted amide or an isostere of amide. Thus, while the various X_n residues within structure (I) are generally described in terms of amino acids, and preferred embodiments of the invention are exemplified by way of peptides, one having skill in the art will recognize that in embodiments having non-amide linkages, the term "amino acid" or "residue" as used herein refers to other bifunctional moieties bearing groups similar in structure to the side chains of the amino acids.

Substituted amides generally include, but are not limited to, groups of the formula -C(O)NR-, where R is (C_1-C_6) alkyl, substituted (C_1-C_6) alkyl, (C_1-C_6) alkenyl, substituted (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, substituted (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl, substituted (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl, substituted 5-20 membered heteroaryl, 6-26 membered alkheteroaryl and substituted 6-26 membered alkheteroaryl.

Isosteres of amide generally include, but are not limited to, $-CH_2NH-$, $-CH_2S-$, $-CH_2CH_2-$, -CH=CH- (cis and trans), $-C(O)CH_2-$, $-CH(OH)CH_2-$ and $-CH_2SO-$. Compounds having such non-amide linkages and methods for preparing such compounds are well-known in the art (see, e.g., Spatola, March 1983, Vega Data Vol. 1, Issue 3; Spatola, 1983, "Peptide Backbone Modifications" *In*: Chemistry and Biochemistry of Amino Acids Peptides and Proteins, Weinstein, ed., Marcel Dekker, New York, p. 267 (general review); Morley, 1980, Trends Pharm. Sci. 1:463-468; Hudson et al., 1979, Int. J. Prot. Res. 14:177-185 ($-CH_2NH-$, $-CH_2CH_2-$); Spatola et al., 1986, Life Sci. 38:1243-1249 ($-CH_2-S$); Hann, 1982, J. Chem. Soc. Perkin

Trans. I. 1:307-314 (-CH=CH-, cis and trans); Almquist *et al.*, 1980, J. Med. Chem. 23:1392-1398 (-$COCH_2$-); Jennings-White *et al.*, Tetrahedron. Lett. 23:2533 (-$COCH_2$-); European Patent Application EP 45665 (1982) CA 97:39405 (-$CH(OH)CH_2$-); Holladay *et al.*, 1983, Tetrahedron Lett. 24:4401-4404 (-$C(OH)CH_2$-); and Hruby, 1982, Life Sci. 31:189-199 (-CH_2-S-).

Additionally, one or more amide linkages can be replaced with peptidomimetic or amide mimetic moieties which do not significantly interfere with the structure or activity of the peptides. Suitable amide mimetic moieties are described, for example, in Olson et al., 1993, J. Med. Chem. 36:3039-3049.

A critical feature of the core peptides of structure (I), is their ability to form an amphipathic α-helix in the presence of lipids. By amphipathic is meant that the α-helix has opposing hydrophilic and hydrophobic faces oriented along its long axis, i.e., one face of the helix projects mainly hydrophilic side chains while the opposite face projects mainly hydrophobic side chains. FIGS. 1A and 1B present two illustrative views of the opposing hydrophilic and hydrophobic faces of an exemplary idealized amphipathic α-helix. FIG. 1A is a Schiffer-Edmundson helical wheel diagram (Schiffer and Edmundson, 1967, Biophys. J. 7:121-135). In the wheel, the long axis of the helix is perpendicular to the page. Starting with the N-terminus, successive amino acid residues (represented by circles) are radially distributed about the perimeter of a circle at 100° intervals. Thus, amino acid residue n+1 is positioned 100° from residue n, residue n+2 is positioned 100° from residue n+1, and so forth. The 100° placement accounts for the 3.6 amino acid residues per turn that are typically observed in an idealized α-helix. In FIG. 1A, the opposing hydrophilic and hydrophobic faces of the helix are clearly visible; hydrophilic amino acids are represented as open circles and hydrophobic amino acid residues are represented as shaded circles.

FIG. 1B presents a helical net diagram of the idealized amphipathic helix of FIG. 1A. (Lim, 1978, FEBS Lett. 89:10-14). In a typical helical net diagram, the α-helix is presented as a cylinder that has been cut along the center of its hydrophilic face and flattened. Thus, the center of the hydrophobic face, determined by the hydrophobic moment of the helix (Eisenberg et al., 1982, Nature 299:371-374), lies in the center of the figure and is oriented so as to rise out of the plane of the page. An illustration of the helical cylinder prior to being cut and flattened is depicted in FIG. 1C. By cutting the cylinder along different planes, different views of the same amphipathic helix can be observed, and different information about the properties of the helix obtained.

The amphipathic nature of the α-helix formed by the core peptides of structure (I) in the presence of lipids is illustrated in FIG. 2. FIG. 2A presents a Schiffer-Edmundson helical wheel diagram, FIG. 2B presents a helical net diagram illustrating the hydrophobic face and FIG. 2C presents a helical net diagram illustrating the hydrophilic face. In each of FIGS. 2A, 2B and 2C, hydrophilic residues are represented as open circles and hydrophobic residues as shaded circles. As will be discussed more thoroughly below in conjunction with altered or mutated forms of the peptides of structure (I), certain amino acid residues can be replaced with other amino acid residues such that the hydrophilic and hydrophobic faces of the helix formed by the peptides may not be composed entirely of hydrophilic and hydrophobic amino acids, respectively. Thus, it is to be understood that when referring to the amphipathic α-helix formed by the core peptides of the invention, the phrase "hydrophilic face" refers to a face of the helix having overall net hydrophilic character. The phrase "hydrophobic face" refers to a face of the peptide having overall net hydrophobic character.

While not intending to be bound by any particular theory, it is believed that certain structural and/or

physical properties of the amphipathic helix formed by the core peptides of structure (I), are important for activity. These properties include the degree of amphipathicity, overall hydrophobicity, mean hydrophobicity, hydrophobic and hydrophilic angles, hydrophobic moment, mean hydrophobic moment, and net charge of the α-helix.

While the helical wheel diagrams of FIG. 2A provide a convenient means of visualizing the amphipathic nature of the core peptides of structure (I), the degree of amphipathicity (degree of asymmetry of hydrophobicity) can be conveniently quantified by calculating the hydrophobic moment (μ_H) of the helix. Methods for calculating μ_H for a particular peptide sequence are well-known in the art, and are described, for example in Eisenberg, 1984, Ann. Rev. Biochem. 53:595-623. The actual μ_H obtained for a particular peptide will depend on the total number of amino acid residues composing the peptide. Thus, it is generally not informative to directly compare μ_H for peptides of different lengths.

The amphipathicities of peptides of different lengths can be directly compared by way of the mean hydrophobic moment ($<\mu_H>$). The mean hydrophobic moment can be obtained by dividing μ_H by the number of residues in the helix (i.e., $<\mu_H> = \mu_H/N$). Generally, core peptides which exhibit a $<\mu_H>$ in the range of 0.45 to 0.65, as determined using the normalized consensus hydrophobicity scale of Eisenberg (Eisenberg, 1984, J. Mol. Biol. 179:125-142) are considered to be within the scope of the present invention, with a $<\mu_H>$ in the range of 0.50 to 0.60 being preferred.

The overall or total hydrophobicity (H_o) of a peptide can be conveniently calculated by taking the algebraic sum of the hydrophobicities of each amino acid residue in the peptide (i.e., $H_o = \sum_{i=1}^{N} H_i$), where N is the

number of amino acid residues in the peptide and H_i is the hydrophobicity of the *i*th amino acid residue). The mean hydrophobicity ($<H_o>$) is the hydrophobicity divided by the number of amino acid residues (i.e., $<H_o> = H_o/N$). Generally, core peptides that exhibit a mean hydrophobicity in the range of -0.050 to -0.070, as determined using the normalized consensus hydrophobicity scale of Eisenberg (Eisenberg, 1984, J. Mol. Biol. 179:125-142) are considered to be within the scope of the present invention, with a mean hydrophobicity in the range of -0.030 to -0.055 being preferred.

The total hydrophobicity of the hydrophobic face (H_o^{pho}) of an amphipathic helix can be obtained by taking the sum of the hydrophobicities of the hydrophobic amino acid residues which fall into the hydrophobic angle as defined below (*i.e.*, $H_o^{pho} = \sum_{i=1}^{N} H_i$, where H_i is as previously defined and N_H is the total number of hydrophobic amino acids in the hydrophobic face). The mean hydrophobicity of the hydrophobic face ($<H_o^{pho}>$) is H_o^{pho}/N_H where N_H is as defined above. Generally, core peptides which exhibit a $<H_o^{pho}>$ in the range of 0.90 to 1.20, as determined using the consensus hydrophobicity scale of Eisenberg (Eisenberg, 1984, supra; Eisenberg et al., 1982, supra) are considered to be within the scope of the present invention, with a $<H_o^{pho}>$ in the range of 0.94 to 1.10 being preferred.

The hydrophobic angle (pho angle) is generally defined as the angle or arc covered by the longest continuous stretch of hydrophobic amino acid residues when the peptide is arranged in the Schiffer-Edmundson helical wheel representation (i.e., the number of contiguous hydrophobic residues on the wheel multiplied by 20°). The hydrophilic angle (phi angle) is the difference between 360° and the pho angle (i.e., 360°-pho angle). Those of skill in the art will recognize that the pho and phi angles will depend, in part,

on the number of amino acid residues in the peptide. For example, referring to FIGS. 5A and 5B, it can be seen that only 18 amino acids fit around one rotation of the Schiffer-Edmundson helical wheel. Fewer amino acids leave a gap in the wheel; more amino acids cause certain positions of the wheel to be occupied by more than one amino acid residue.

In the case of peptides containing more than 18 amino acid residues, such as the core peptides of structure (I), a "continuous" stretch of hydrophobic amino acid residues is meant that at least one amino acid at positions along the wheel containing two or more amino acids is a hydrophobic amino acid. Thus, referring to FIG. 5B, the pho angle is the arc covered by residues 5, 16, 9, 2, 13, 6, 17, 10, 3 and 14 despite the occurrence of a hydrophilic residue at position 20, as the residue at position 2, which shares the same position on the wheel, is a hydrophobic residue. Typically, core peptides having a pho angle in the range of 160° to 220° are considered to be within the scope of the invention, with a pho angle in the range of 180° to 200° being preferred.

Certain structural and/or physical characteristics of the core peptides of structure (I) are illustrated in FIGS. 3 and 4. FIG. 3B presents a helical net diagram of an exemplary core peptide of the invention, peptide 4 (PVLDLFRELLNELLEALKQKLK; SEQ ID NO:4), illustrating the charge distribution along the hydrophilic face of the helix. In FIG. 3B, the helical cylinder has been cut along the center of the hydrophobic face and flattened. The three hydrophobic Leu (L) residues that replace hydrophilic residues in Segrest's consensus 22-mer (FIG. 3A) are shaded. As can be seen in FIG. 3B, positively-charged amino acid residues are clustered at the last C-terminal turn of the helix (the C-terminus is at the top of the page). While not intending to be bound by any particular theory, it is believed that the cluster of basic residues at the C-terminus stabilizes the helix through charge (NH_3^+)-helix dipole

electrostatic interactions. It is also thought that stabilization occurs through hydrophobic interactions between lysine side chains and the helix core (see, Groebke et al., 1996, Proc. Natl. Acad. Sci. U.S.A. 93:4025-4029; Esposito et al., 1997, Biopolymers 41:27-35).

With the exception of the positively-charged C-terminal cluster, negative charges are distributed on the rest of the hydrophilic face, with at least one negatively charged (acidic) amino acid residue per turn, resulting in a continuous stretch of negative charges along the hydrophilic face of the helix. One positive charge is located at residue 7, which potentially contributes to helix stability by forming a salt bridge with an acidic residue one turn away on the helix.

FIG. 4B presents a helical net diagram illustrating the hydrophobic face of the amphipathic helix formed by exemplary core peptide 4 (SEQ ID NO:4). In FIG. 4B, the helical cylinder is cut along the center of the hydrophilic face and flattened. The hydrophobic face of the core peptide consists of two hydrophobic residues per turn, except for the last C-terminal turn, where basic residues dominate. NMR studies indicate that amino acid residues 3, 6, 9 and 10 of this core peptide form a hydrophobic cluster near the N-terminus of the helix. Phe-6 is centered in this cluster and is believed to play an important role in stabilizing the hydrophobic cluster.

While not intending to be bound by any particular theory, it is believed that the hydrophobic cluster formed by residues 3, 6, 9 and 10 is significant in effecting lipid binding and LCAT activation. For example, whereas exemplary peptide 4 (SEQ ID NO:4) exhibits 93% LCAT activation in the assay described herein, a derivative of peptide 4 containing a Lys (K) residue at position 9 (peptide 33; SEQ ID NO:33), which destroys the hydrophobic cluster, exhibits only 33% LCAT activation in the same assay. Amphipathic peptides are expected to bind phospholipids by pointing their hydrophobic

faces towards the alkyl chains of the lipid moieties. Thus, it is believed that this highly hydrophobic cluster contributes to the strong lipid affinities observed for the core peptides of the invention. Since lipid binding is a prerequisite for LCAT activation, it is believed that this hydrophobic cluster is also essential for LCAT activation.

Aromatic residues are often found to be important in anchoring peptides and proteins to lipids (De Kruijff, 1990, Biosci. Rep. 10:127-130; O'Neil and De Grado, 1990, Science 250:645-651; Blondelle et al., 1993, Biochim. Biophys. Acta 1202:331-336). Thus, it is further believed that Phe-6, which is positioned at the center of the hydrophobic cluster, may also play a key role in anchoring the core peptides of structure (I) to lipids.

Interactions between the core peptides of the invention and lipids lead to the formation of peptide-lipid complexes. As illustrated in FIG. 11A, the type of complex obtained (comicelles, discs, vesicles or multilayers) depends on the lipid:peptide molar ratio, with comicelles generally being formed at low lipid:peptide molar ratios and discoidal and vesicular or multilayer complexes being formed with increasing lipid:peptide molar ratios. This characteristic has been described for amphipathic peptides (Epand, The Amphipathic Helix, 1993) and for ApoA-I (Jones, 1992, Structure and Function of Apolipoproteins, Chapter 8, pp. 217-250). The lipid:peptide molar ratio also determines the size and composition of the complexes (*see*, Section 5.3.1, *infra*).

The long axis of the α-helix formed by the core peptides of structure (I) has an overall curved shape. In typical amphipathic helices, it has been found that the lengths of the hydrogen bonds of the hydrophilic and hydrophobic faces vary such that the hydrophobic side of the helix is concave (Barlow and Thornton, 1988, J. Mol. Biol. 201:601-619; Zhou et al., 1992, J. Am. Chem. Soc. 33:11174-11183; Gesell et al., 1997, J. Biomol. NMR 9:127-135). While

not intending to be bound by theory, it is believed that the overall curvature of the hydrophobic face of the helix may be important in binding discoidal complexes -- a curved helix permits the peptide to "fit" better around the edges of discoidal particles, thereby increasing the stability of the peptide-disc complex.

In the generally accepted structural model of ApoA-I, the amphipathic α-helices are packed around the edge of the discoidal HDL (*see*, FIG. 11B). In this model, the helices are assumed to be aligned with their hydrophobic faces pointing towards the lipid acyl chains (Brasseur et al., 1990, Biochim. Biophys. Acta 1043:245-252). The helices are arranged in an antiparallel fashion, and a cooperative effect between the helices is thought to contribute to the stability of the discoidal HDL complex (Brasseur et al., supra). It has been proposed that one factor that contributes to the stability of the HDL discoidal complex is the existence of ionic interactions between acidic and basic residues resulting in the formation of intermolecular salt bridges or hydrogen bonds between residues on adjacent anti-parallel helices. In this model, the peptides are considered not as a single entity, but as in interaction with at least two other neighboring peptide molecules (FIG. 11B).

It is also generally accepted that intramolecular hydrogen bond or salt bridge formation between acidic and basic residues, respectively, at positions i and i+3 of the helix stabilize the helical structure (Marqusee et al., 1985, Proc. Natl. Acad. Sci. USA 84(24):8898-8902).

Thus, additional key features of the core peptides of structure (I) are their ability to form intermolecular hydrogen-bonds with one another when aligned in an antiparallel fashion with their hydrophobic faces pointing in the same direction, such as would be the case when the peptides are bound to lipids (i.e., between the acidic residues at positions 4 and 8 and the basic residues at positions 18, 20 and 22), and also their ability to form

intramolecular hydrogen bonds or salt bridges near the N- and C-termini of the helix (i.e., between the acidic and basic residues at positions 4 and 7 and 15 and 18).

The ability of the core peptides of structure (I) to form intermolecular hydrogen bonds is illustrated in FIG. 6A. In FIG. 6A, two ideal α-helices of exemplary core peptide 4 (SEQ ID NO:4) are aligned in an antiparallel fashion with their respective hydrophobic faces pointing in the same direction (out of the plane of the page). H-bonding interactions could occur between residues E-8 and Q-19 (Huyghues-Despointes et al., 1995, Biochemistry 34(41):13267-13271).

Furthermore, when arranged in this anti-parallel fashion, the helices are closely packed; there is no steric hindrance preventing close contact between the helices. Alterations in the sequence of the core peptides which affect the packing of the helices negatively influences the activity of the core peptides. For example, referring to FIG. 6B, a dimer of peptides having Gln-19 replaced by Glu-19 (peptide 102; PVLDLFRELLNLXLEALKEKLK where X is Aib; SEQ ID NO:102), and which therefore cannot form intermolecular hydrogen bonds, did not activate LCAT. Significantly, whereas peptide 4 (SEQ ID NO:4) exhibited 93% LCAT activation in the assay described herein, peptide 102 (SEQ ID NO:102) exhibited only 2% activity in the same assay.

Thus, while not being bound by any particular theory, it is believed that the ability of the core peptides of structure (I) to closely pack and ionically interact to form intra- and/or inter-molecular salt bridges and/or hydrogen bonds when bound to lipids in an antiparallel fashion is an important feature of the core peptides of the invention.

The ability of the core peptides to form favorable intermolecular peptide-peptide interactions is also thought to be of relevance in the absence of lipids. The core peptides of the invention self-associate, due in part to

their high $<\mu_H>$, $<H_0>$ and hydrophobic angle (*see*, TABLE I, *infra*). The self-association phenomenon depends on the conditions of pH, peptide concentration and ionic strength, and can result in several states of association, from monomeric to several multimeric forms (FIG. 11A). The hydrophobic core of peptide aggregates favors hydrophobic interactions with lipids. The ability of the peptides to aggregate even at very low concentrations may favor their binding to lipids. It is thought that in the core of the peptide aggregates peptide-peptide interactions also occur and may compete with lipid-peptide interactions.

In addition to the above-described properties, other parameters are thought to be important for activity as well, including the total number of hydrophobic residues, the total number of charged residues, and the net charge of the peptides.

A summary of the preferred physical and structural properties of the core peptides of structure (I) is provided in TABLE I, below:

TABLE I
PHYSICAL PROPERTIES OF PREFERRED
ApoA-I AGONISTS OF STRUCTURE (I)

PROPERTY	RANGE	PREFERRED RANGE
% hydrophobic amino acids	40 - 70	50 - 60
$<H_o>$	-0.050 to -0.070	-0.030 to -0.055
$<H_o^{pho}>$	0.90 - 1.2	0.94 - 1.1
$<\mu_H>$	0.45 - 0.65	0.50 - 0.60
pho angle	160° - 220°	180° - 200°
# positively charged amino acids	3 - 5	4
# negatively charged amino acids	3 - 5	4
net charge	-1 to +1	0
hydrophobic cluster	positions 3,6,9,10 are hydrophobic amino acids	
acidic cluster	at least 1 acidic amino acid per turn except for last 5 C-terminal amino acids	
basic cluster	at least 3 basic amino acids in last 5 C-terminal amino acids	

The properties of the amphipathic α-helices formed by the core peptides of the invention differ significantly from the properties of class A amphipathic α-helices, particularly the class A α-helix of Segrest's consensus 22-mer. These differences are illustrated with exemplary core peptide 4 (SEQ ID NO:4) in FIGS. 3-5.

Referring to FIGS. 4A and 4B, it can be seen that the hydrophobic face of peptide 4 has much greater hydrophobic character than the hydrophobic face of Segrest's consensus 22-mer. In particular, residue 5, 9 and 13 (shaded region of FIG. 4B) are hydrophobic Leu (L) residues in peptide 4 (SEQ ID NO:4) as compared to charged residues in the consensus 22-mer (SEQ ID NO:75). The replacement of these three charged residues in Segrest's consensus 22-mer with hydrophobic Leu (L) residues leads to significant differences in the amphipathicity, hydrophobicity, pho angle and other properties of the helix.

A comparison of the physical and structural properties of two exemplary core peptides of structure (I), peptide 4 (SEQ ID NO:4) and peptide 8 (SEQ ID NO:8), and Segrest's consensus 22-mer (SEQ ID NO:75) is provided in TABLE II, below:

TABLE II
COMPARISON OF PROPERTIES OF EXEMPLARY CORE PEPTIDES WITH SEGREST'S CONSENSUS 22-MER

PROPERTY	CONSENSUS	peptide 4	peptide 8
# amino acids	22	22	22
# hydrophilic amino acids	13	10	10
# hydrophobic amino acids	9	12	12
% hydrophobic amino acids	41	55	55
$\langle H_o \rangle$	-0.293	-0.013	-0.040
$\langle H_o^{pho} \rangle$	0.960	0.990	0.940
$\langle \mu_H \rangle$	0.425	0.547	0.521
pho angle	100°	200°	200°
# positively charged amino acids	5	4	4
# negatively charged amino acids	6	4	4
net charge	-1	0	0

Most notably, the core peptides of structure (I) are composed of a larger percentage of hydrophobic residues, have a significantly larger $\langle H_o \rangle$ and $\langle \mu_H \rangle$, and have a two-fold larger pho angle (see FIGS. 5A and 5B). These differences in properties lead to significant differences in activity. Whereas Segrest's consensus 22-mer (SEQ ID NO:75) exhibits only 10% LCAT activation as compared with native ApoA-I in the assays described herein, peptides 4 (SEQ ID NO:4) and 8 (SEQ ID NO:8) exhibit 93% and 83% LCAT activation, respectively, as compared with native ApoA-I in the same assays. Peptide 1 (PVLDLFRELLNELLEZLKQKLK; SEQ ID NO:1) and

peptide 2 (GVLDLFRELLNELLEALKQKLKK; SEQ ID NO:2), exhibited 120% and 105% LCAT activation, respectively, as compared with native ApoA-I in the same assays.

Certain amino acid residues in the core peptides of structure (I) can be replaced with other amino acid residues without significantly deleteriously affecting, and in many cases even enhancing, the activity of the peptides. Thus, also contemplated by the present invention are altered or mutated forms of the core peptides of structure (I) wherein at least one defined amino acid residue in the structure is substituted with another amino acid residue. As one of the critical features affecting the activity of the core peptides of the invention is believed to be their ability to form α-helices in the presence of lipids that exhibit the amphipathic and other properties described above, it will be recognized that in preferred embodiments of the invention, the amino acid substitutions are conservative, i.e., the replacing amino acid residue has physical and chemical properties that are similar to the amino acid residue being replaced.

For purposes of determining conservative amino acid substitutions, the amino acids can be conveniently classified into two main categories -- hydrophilic and hydrophobic--depending primarily on the physical-chemical characteristics of the amino acid side chain. These two main categories can be further classified into subcategories that more distinctly define the characteristics of the amino acid side chains. For example, the class of hydrophilic amino acids can be further subdivided into acidic, basic and polar amino acids. The class of hydrophobic amino acids can be further subdivided into apolar and aromatic amino acids. The definitions of the various categories of amino acids that define structure (I) are as follows:

"Hydrophilic Amino Acid" refers to an amino acid exhibiting a hydrophobicity of less than zero according to the normalized consensus hydrophobicity scale of Eisenberg et

al., 1984, J. Mol. Biol. 179:125-142. Genetically encoded hydrophilic amino acids include Thr (T), Ser (S), His (H), Glu (E), Asn (N), Gln (Q), Asp (D), Lys (K) and Arg (R).

"Acidic Amino Acid" refers to a hydrophilic amino acid having a side chain pK value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Genetically encoded acidic amino acids include Glu (E) and Asp (D).

"Basic Amino Acid" refers to a hydrophilic amino acid having a side chain pK value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Genetically encoded basic amino acids include His (H), Arg (R) and Lys (K).

"Polar Amino Acid" refers to a hydrophilic amino acid having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Genetically encoded polar amino acids include Asn (N), Gln (Q) Ser (S) and Thr (T).

"Hydrophobic Amino Acid" refers to an amino acid exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg, 1984, J. Mol. Biol. 179:125-142. Genetically encoded hydrophobic amino acids include Pro (P), Ile (I), Phe (F), Val (V), Leu (L), Trp (W), Met (M), Ala (A), Gly (G) and Tyr (Y).

"Aromatic Amino Acid" refers to a hydrophobic amino acid with a side chain having at least one aromatic or heteroaromatic ring. The aromatic or heteroaromatic ring may contain one or more substituents such as -OH, -SH, -CN, -F, -Cl, -Br, -I, $-NO_2$, -NO, $-NH_2$, -NHR, -NRR, -C(O)R, -C(O)OH, -C(O)OR, $-C(O)NH_2$, -C(O)NHR, -C(O)NRR and the like where each R is independently (C_1-C_6) alkyl, substituted (C_1-C_6) alkyl, (C_1-C_6) alkenyl, substituted (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, substituted (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20})

aryl, (C_6-C_{26}) alkaryl, substituted (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl, substituted 5-20 membered heteroaryl, 6-26 membered alkheteroaryl or substituted 6-26 membered alkheteroaryl. Genetically encoded aromatic amino acids include Phe (F), Tyr (Y) and Trp (W).

"Nonpolar Amino Acid" refers to a hydrophobic amino acid having a side chain that is uncharged at physiological pH and which has bonds in which the pair of electrons shared in common by two atoms is generally held equally by each of the two atoms (i.e., the side chain is not polar). Genetically encoded apolar amino acids include Leu (L), Val (V), Ile (I), Met (M), Gly (G) and Ala (A).

"Aliphatic Amino Acid" refers to a hydrophobic amino acid having an aliphatic hydrocarbon side chain. Genetically encoded aliphatic amino acids include Ala (A), Val (V), Leu (L) and Ile (I).

The amino acid residue Cys (C) is unusual in that it can form disulfide bridges with other Cys (C) residues or other sulfanyl-containing amino acids. The ability of Cys (C) residues (and other amino acids with -SH containing side chains) to exist in a peptide in either the reduced free -SH or oxidized disulfide-bridged form affects whether Cys (C) residues contribute net hydrophobic or hydrophilic character to a peptide. While Cys (C) exhibits a hydrophobicity of 0.29 according to the normalized consensus scale of Eisenberg (Eisenberg, 1984, supra), it is to be understood that for purposes of the present invention Cys (C) is categorized as a polar hydrophilic amino acid, notwithstanding the general classifications defined above.

As will be appreciated by those of skill in the art, the above-defined categories are not mutually exclusive. Thus, amino acids having side chains exhibiting two or more physical-chemical properties can be included in multiple categories. For example, amino acid side chains having aromatic moieties that are further substituted with polar substituents, such as Tyr (Y), may exhibit both aromatic

hydrophobic properties and polar or hydrophilic properties, and can therefore be included in both the aromatic and polar categories. The appropriate categorization of any amino acid will be apparent to those of skill in the art, especially in light of the detailed disclosure provided herein.

Certain amino acid residues, called "helix breaking" amino acids, have a propensity to disrupt the structure of α-helices when contained at internal positions within the helix. Amino acid residues exhibiting such helix-breaking properties are well-known in the art (see, e.g., Chou and Fasman, Ann. Rev. Biochem. 47:251-276) and include Pro (P), Gly (G) and potentially all D-amino acids (when contained in an L-peptide; conversely, L-amino acids disrupt helical structure when contained in a D-peptide). While these helix-breaking amino acid residues fall into the categories defined above, with the exception of Gly (G) (discussed infra), these residues should not be used to substitute amino acid residues at internal positions within the helix -- they should only be used to substitute 1-3 amino acid residues at the N-terminus and/or C-terminus of the peptide.

While the above-defined categories have been exemplified in terms of the genetically encoded amino acids, the amino acid substitutions need not be, and in certain embodiments preferably are not, restricted to the genetically encoded amino acids. Indeed, many of the preferred peptides of structure (I) contain genetically non-encoded amino acids. Thus, in addition to the naturally occurring genetically encoded amino acids, amino acid residues in the core peptides of structure (I) may be substituted with naturally occurring non-encoded amino acids and synthetic amino acids.

Certain commonly encountered amino acids which provide useful substitutions for the core peptides of structure (I) include, but are not limited to, β-alanine (β-Ala) and other omega-amino acids such as 3-aminopropionic acid, 2,3-diaminopropionic acid (Dpr), 4-aminobutyric acid

and so forth; α-aminoisobutyric acid (Aib); ε-aminohexanoic acid (Aha); δ-aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly); ornithine (Orn); citrulline (Cit); t-butylalanine (t-BuA); t-butylglycine (t-BuG); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); naphthylalanine (Nal); 4-chlorophenylalanine (Phe(4-Cl)); 2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β-2-thienylalanine (Thi); methionine sulfoxide (MSO); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); *p*-aminophenylalanine (Phe(*p*NH_2)); N-methyl valine (MeVal); homocysteine (hCys), homophenylalanine (hPhe) and homoserine (hSer); hydroxyproline (Hyp), homoproline (hPro), N-methylated amino acids and peptoids (N-substituted glycines).

The classifications of the genetically encoded and common non-encoded amino acids according to the categories defined above are summarized in TABLE III, below. It is to be understood that TABLE III is for illustrative purposes only and does not purport to be an exhaustive list of amino acid residues that can be used to substitute the core peptides described herein. Other amino acid residues not specifically mentioned herein can be readily categorized based on their observed physical and chemical properties in light of the definitions provided herein.

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TABLE III
CLASSIFICATIONS OF COMMONLY ENCOUNTERED AMINO ACIDS

Classification	Genetically Encoded	Non-Genetically Encoded
Hydrophobic		
Aromatic	F, Y, W	Phg, Nal, Thi, Tic, Phe(4-Cl), Phe(2-F), Phe(3-F), Phe(4-F), hPhe
Apolar	L, V, I, M, G, A, P	t-BuA, t-BuG, MeIle, Nle, MeVal, Cha, McGly, Aib
Aliphatic	A, V, L, I	b-Ala, Dpr, Aib, Aha, MeGly, t-BuA, t-BuG, MeIle, Cha, Nle, MeVal
Hydrophilic		
Acidic	D, E	
Basic	H, K, R	Dpr, Orn, hArg, Phe(p-NH_2), Dbu, Dab
Polar	C, Q, N, S, T	Cit, AcLys, MSO, bAla, hSer
Helix-Breaking	P, G	D-Pro and other D-amino acids (in L-peptides)

While in most instances, the amino acids of the core peptides of structure (I) will be substituted with L-enantiomeric amino acids, the substitutions are not limited to L-enantiomeric amino acids. Thus, also included in the definition of "mutated" or "altered" forms are those situations where an L-amino acid is replaced with an identical D-amino acid (e.g., L-Arg → D-Arg) or with a D-amino acid of the same category or subcategory (e.g., L-Arg → D-Lys), and vice versa. Indeed, in certain preferred embodiments that are suitable for oral administration to animal subjects, the peptides may advantageously be composed of at least one D-enantiomeric amino acid. Peptides containing such D-amino acids are thought to be more stable to degradation in the oral cavity, gut or serum than are peptides composed exclusively of L-amino acids.

As noted above, D-amino acids tend to disrupt the structure of α-helices when contained at internal positions with an α-helical L-peptide. Furthermore, it has been observed that certain mutated forms of the core peptides of

structure (I) that are composed entirely of D-amino acids exhibit significantly lower LCAT activation in the assay described herein than identical peptides composed entirely of L-amino acids. As a consequence, D-amino acids should not be used to substitute internal L-amino acids; D-amino acid substitutions should be limited to 1-3 amino acid residues at the N-terminus and/or C-terminus of the peptide.

As previously discussed, the amino acid Gly (G) generally acts as a helix-breaking residue when contained at internal positions of a peptide. Quite surprisingly, the applicants have discovered that while the helical structure of the core peptides of the invention is disrupted in the absence of lipids when internal amino acid residues are substituted with Gly (G), in the presence of lipids such Gly (G) containing peptides exhibit significant helical structure, as well as activity. For example, whereas peptide 8 (SEQ ID NO:8) exhibits only 20% helical structure in buffer, 61-93% helical structure was observed in the presence of lipids and 93% helicity was observed in the presence of tri-fluoroethanol (TFE). The helical structure of this peptide in the presence of TFE was confirmed via NMR (*see*, Section 7.3.5, infra). Notably, this peptide also exhibited 83% LCAT activation. Other core peptides containing internal glycine residues also exhibited ≥38% LCAT activation (*see*, e.g., TABLE X, Section 8.3, infra). Thus, although Gly (G) is generally considered to be a helix-breaking residue, Gly (G) can be used to substitute amino acids at internal positions of the core peptides of structure (I). Preferably, only internal residues positioned within about ± 1 helical turn of the center of the peptide (particularly for peptides composed of an even number of amino acids) are substituted with Gly (G). Additionally, it is preferred that only one internal amino acid residue in the peptide be substituted with Gly (G). Preferred embodiments of the ApoA-I agonists of the invention containing internal glycines are described in Section 5.1.2, infra.

Using the amino acid residue classifications described above in conjunction with the Schiffer-Edmundson helical wheel and helical net diagram presentations of the core peptides of structure (I), as well as the detailed description of the desired properties provided herein, altered or mutated forms of the core peptides of structure (I) that substantially retain the amphipathic and other properties of the helix, and which are therefore considered to be within the scope of the present invention, can be readily obtained.

In a preferred embodiment of the invention, altered or mutated forms of the core peptides of structure (I) are obtained by fixing the the hydrophilic or hydrophobic residues according to structure (I) and substituting at least one non-fixed residue with another amino acid, preferably with another amino acid of the same category or sub-category. The residues composing the basic and/or hydrophobic clusters can also be fixed, and at least one non-fixed residue substituted.

In another preferred embodiment, altered or mutated forms of the core peptides of structure (I) are obtained by fixing the hydrophilic amino acid residues positioned within the hydrophilic face of the helix according to structure (I) and substituting at least one non-fixed amino acid residue with another amino acid, preferably with another amino acid residue of the same category or sub-category. Referring to FIG. 2A, it can be seen that residues 1, 4, 7, 8, 11, 12, 15, 18, 19 and 22 are positioned within the hydrophilic face of the amphipathic helix formed by the core peptides of structure (I). Of these residues, all are hydrophilic except for residue 1, which may be either hydrophilic or hydrophobic. Thus, in one preferred embodiment, residues 4, 7, 8, 11, 12, 15, 18, 19 and 22 are fixed according to structure (I) and at least one of residues 2, 3, 5, 6, 9, 10, 13, 14, 16, 17, 20 and 21 is substituted with another amino acid of the same category, preferably with another amino acid

of the same sub-category. Alternatively, residue 1 is also fixed according to structure (I) and at least one of residues 2, 3, 5, 6, 9, 10, 13, 14, 16, 17, 20 and 21 is substituted as described.

In a particularly preferred embodiment, the C-terminal basic cluster (residues 18, 19, 20 and 22) is also fixed according to structure (I), and only residues 2, 3, 5, 6, 9, 10, 13, 14, 16, 17 and/or 21 are substituted.

In another particularly preferred embodiment, the hydrophobic cluster is also fixed, and only residues 2, 5, 13, 14, 16, 17, 20 and/or 21 are substituted.

In still another particularly preferred embodiment, both the basic and hydrophobic clusters are fixed and only residues 2, 5, 13, 14, 16, 17 and/or 21 are substituted.

In another preferred embodiment of the invention, altered or mutated forms of the core peptides of the invention are obtained by fixing the hydrophobic amino acid residues positioned within the hydrophobic face of the helix and substituting at least one non-fixed amino acid residue with another amino acid residue, preferably with another residue of the same category or sub-category.

Referring to FIG. 2A, it can be seen that residues 2, 3, 5, 6, 9, 10, 13, 14, 16, 17, 20 and 21 are positioned within the hydrophobic face. Of these, all are hydrophobic except for residue 20, which is hydrophilic. Thus, in one preferred embodiment residues 2, 3, 5, 6, 9, 10, 13, 14, 16, 17 and 21 are fixed according to structure (I) and at least one of residues 1, 4, 7, 8, 11, 12, 15, 18, 19, 20 and 22 is substituted with another amino acid residue, preferably with another amino acid of the same category or subcategory.

In a particularly preferred embodiment, the C-terminal basic cluster is also fixed, and only residues 1, 4, 7, 8, 11, 12 and/or 15 are substituted.

In another embodiment, altered or mutated forms of the peptides of structure (I) are obtained by fixing all of the amino acid residues residing within the hydrophobic or

hydrophilic face of the helix and substituting, preferably conservatively, at least one amino acid residue residing in the other face with another amino acid residue. The residues comprising the hydrophobic cluster and/or the basic cluster may also be optionally fixed according to structure (I), as previously defined.

In another embodiment of the invention, the altered or mutated forms of structure (I) are obtained by substituting at least one amino acid with a non-conservative amino acid. Those of skill in the art will recognize that such substitutions should not substantially alter the amphipathic and/or structural properties of the helix discussed, supra. Thus, in certain instances it may be desirable to substitute one or more pairs of amino acids so as to preserve the net properties of the helix. Further guidance for selecting appropriate amino acid substitutions is provided by the peptide sequences listed in TABLE X (see, Section 8.3, infra).

In still another embodiment of the invention, the first one to four amino acid residues at the N-terminus and/or C-terminus of the core peptides of structure (I) are substituted with one or more amino acid residues, or one or more peptide segments, that are known to confer stability to regions of α-helical secondary structure ("end-cap" residues or segments). Such end-cap residues and segments are well-known in the art (see, e.g., Richardson and Richardson, 1988, Science 240:1648-1652; Harper et al., 1993, Biochemistry 32(30):7605-7609; Dasgupta and Bell, 1993, Int. J. Peptide Protein Res. 41:499-511; Seale et al., 1994, Protein Science 3:1741-1745; Doig et al., 1994, Biochemistry 33:3396-3403; Zhou et al., 1994, Proteins 18:1-7; Doig and Baldwin, 1995, Protein Science 4:1325-1336; Odaert et al., 1995, Biochemistry 34:12820-12829; Petrukhov et al., 1996, Biochemistry 35:387-397; Doig et al., 1997, Protein Science 6:147-155). Alternatively, the first one to four N-terminal and/or C-terminal amino acid residues of structure (I) can be

replaced with peptidomimetic moieties that mimic the structure and/or properties of end-cap residues or segments. Suitable end-cap mimetics are well-known in the art, and are described, for example, in Richardson and Richardson, 1988, Science 240:1648-1652; Harper et al., 1993, Biochemistry 32(30):7605-7609; Dasgupta and Bell, 1993, Int. J. Peptide Protein Res. 41:499-511; Seale et al., 1994, Protein Science 3:1741-1745; Doig et al., 1994, Biochemistry 33:3396-3403; Zhou et al., 1994, Proteins 18:1-7; Doig and Baldwin, 1995, Protein Science 4:1325-1336; Odaert et al., 1995, Biochemistry 34:12820-12829; Petrukhov et al., 1996, Biochemistry 35:387-397; Doig et al., 1997, Protein Science 6:147-155).

While structure (I) contains 22 specified amino acid residue positions, it is to be understood that the core peptides of the invention can contain fewer than 22 amino acid residues. Indeed, truncated or internally deleted forms of structure (I) containing as few as 18 or even 15 amino acid residues that substantially retain the overall characteristics and properties of the amphipathic helix formed by the core peptides of structure (I) are considered to be within the scope of the present invention.

Truncated forms of the peptides of structure (I) are obtained by deleting one or more amino acids from the N- and/or C-terminus of structure (I). Internally deleted forms of structure (I) are obtained by deleting one or more amino acids from internal positions within the peptide of structure (I). The internal amino acid residues deleted may or may not be consecutive residues.

Those of skill in the art will recognize that deleting an internal amino acid residue from a core peptide of structure (I) will cause the plane of the hydrophilic-hydrophobic interface of the helix to rotate by 100° at the point of the deletion. As such rotations can significantly alter the amphipathic properties of the resultant helix, in a preferred embodiment of the invention amino acid residues are

deleted so as to substantially retain the alignment of the plane of the hydrophilic-hydrophobic interface along the entire long axis of the helix.

This can be conveniently achieved by deleting a sufficient number of consecutive or non-consecutive amino acid residues such that one complete helical turn is deleted. An idealized α-helix contains 3.6 residues per turn. Thus, in a preferred embodiment, groups of 3-4 consecutive or non-consecutive amino acid residues are deleted. Whether 3 amino acids or 4 amino acids are deleted will depend upon the position within the helix of the first residue to be deleted. Determining the appropriate number of consecutive or non-consecutive amino acid residues that constitute one complete helical turn from any particular starting point within an amphipathic helix is well within the capabilities of those of skill in the art.

Due to the surmised importance of the basic cluster at the C-terminus of the core peptides of structure (I) in stabilizing the helix and the importance of the hydrophobic cluster in effecting lipid binding and LCAT activation, in preferred embodiments of the invention, residues comprising the basic and hydrophobic clusters are not deleted. Thus, in preferred embodiments, residues 18, 19, 20 and 22 (basic cluster) and residues 3, 6, 9 and 10 (hydrophobic cluster) are not deleted.

The core peptides of structure (I) can also be extended at one or both termini or internally with additional amino acid residues that do not substantially interfere with, and in some embodiments even enhance, the structural and/or functional properties of the peptides. Indeed, extended core peptides containing as many as 23, 25, 26, 29 or even more amino acid residues are considered to be within the scope of the present invention. Preferably, such extended peptides will substantially retain the net amphipathicity and other properties of the peptides of structure (I). Of course, it will be recognized that adding amino acids internally will

rotate the plane of the hydrophobic-hydrophilic interface at the point of the insertion in a manner similar to that described above for internal deletions. Thus, the considerations discussed above in connection with internal deletions apply to internal additions, as well.

In one embodiment, the core peptides are extended at the N- and/or C-terminus by least one helical turn. Preferably, such extensions will stabilize the helical secondary structure in the presence of lipids, such as the end-cap amino acids and segments previously described.

In a particularly preferred embodiment, the core peptide of structure (I) is extended at the C-terminus by a single basic amino acid residue, preferably Lys (K). When so extended, X_1 is preferably D-Pro (p) or Gly (G); X_2 is preferably Val (V); X_3 is preferably Leu (L); X_4 is preferably Asp (D); X_5 is preferably Leu (L); X_6 is preferably Phe (F); X_7 is preferably Arg (R); X_8 is preferably Glu (E); X_9 is preferably Leu (L); X_{10} is preferably Leu (L); X_{11} is preferably Asn (N); X_{12} is preferably Glu (E); X_{13} is preferably Leu (L); X_{14} is preferably Leu (L); X_{15} is preferably Glu (E); X_{16} is preferably Ala (A); X_{17} is preferably Leu (L); X_{18} is preferably Lys (K); X_{19} is preferably Gln (Q); X_{20} is preferably Lys (K); X_{21} is preferably Leu (L); and/or X_{22} is preferably Lys (K).

Also included within the scope of the present invention are "blocked" forms of the ApoA-I agonist, i.e., forms of the ApoA-I agonists in which the N- and/or C-terminus is blocked with a moiety capable of reacting with the N-terminal $-NH_2$ or C-terminal -C(O)OH. It has been discovered that removing the N- and/or C-terminal charges of the ApoA-I agonists of the invention containing 18 or fewer amino acid residues (by synthesizing N-acylated peptide amides/ester/hydrazides/alcohols and substitutions thereof) results in agonists which approach, and in some embodiments even exceed, the activity of the unblocked form of the agonist. In some embodiments containing 22 or more amino

acids, blocking the N- or C-terminus results in ApoA-I agonists which exhibit lower activity than the unblocked forms. However, blocking both the N- and C-termini of ApoA-I agonists composed of 22 or more amino acids is expected to restore activity. Thus, in a preferred embodiment of the invention, either the N- and/or C-terminus (preferably both termini) of core peptides containing 18 or fewer amino acids are blocked, whereas the N- and C-termini of peptides containing 22 or more amino acids are either both blocked or both unblocked. Typical N-terminal blocking groups include RC(O)-, where R is -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl or 6-26 membered alkheteroaryl. Preferred N-terminal blocking groups include acetyl, formyl and dansyl. Typical C-terminal blocking groups include -C(O)NRR and -C(O)OR, where each R is independently defined as above. Preferred C-terminal blocking groups include those where each R is independently methyl. While not intending to be bound by any particular theory, it is believed that such terminal blocking groups stabilize the α-helix in the presence of lipids (see, e.g., Venkatachelapathi et al., 1993, PROTEINS: Structure, Function and Genetics 15:349-359).

The native structure of ApoA-I contains eight helical units that are thought to act in concert to bind lipids (Nakagawa et al., 1985, J. Am. Chem. Soc. 107:7087-7092; Anantharamaiah et al., 1985, J. Biol. Chem. 260:10248-10262; Vanloo et al., 1991, J. Lipid Res. 32:1253-1264; Mendez et al., 1994, J. Clin. Invest. 94:1698-1705; Palgunari et al., 1996, Arterioscler. Thromb. Vasc. Biol. 16:328-338; Demoor et al., 1996, Eur. J. Biochem. 239:74-84). Thus, also included in the present invention are ApoA-I agonists comprised of dimers, trimers, tetramers and even higher order polymers ("multimers") of the core peptides described herein. Such multimers may be in the form of tandem repeats, branched networks or combinations thereof. The core peptides may be

directly attached to one another or separated by one or more linkers.

The core peptides that comprise the multimers may be the peptides of structure (I), analogues of structure (I), mutated forms of structure (I), truncated or internally deleted forms of structure (I), extended forms of structure (I) and/or combinations thereof. The core peptides can be connected in a head-to-tail fashion (*i.e.*, N-terminus to C-terminus), a head-to-head fashion, (*i.e.*, N-terminus to N-terminus), a tail-to-tail fashion (*i.e.*, C-terminus to C-terminus), or combinations thereof.

In one embodiment of the invention, the multimers are tandem repeats of two, three, four and up to about ten core peptides. Preferably, the multimers are tandem repeats of from 2 to 8 core peptides. Thus, in one embodiment, the ApoA-I agonists of the invention comprise multimers having the following structural formula:

(II) $HH\{LL_m\text{-}HH\}_nLL_m\text{-}HH$

wherein:

each m is independently an integer from 0 to 1, preferably 1;

n is an integer from 0 to 10, preferably 0 to 8;

each "HH" independently represents a core peptide or peptide analogue of structure (I) or a mutated, truncated, internally deleted or extended form thereof as described herein;

each "LL" independently represents a linker; and

each " - " independently designates a covalent linkage.

In structure (II), the linker LL can be any bifunctional molecule capable of covalently linking two peptides to one another. Thus, suitable linkers are bifunctional molecules in which the functional groups are capable of being covalently attached to the N- and/or C-

terminus of a peptide. Functional groups suitable for attachment to the N- or C-terminus of peptides are well known in the art, as are suitable chemistries for effecting such covalent bond formation.

The linker may be flexible, rigid or semi-rigid, depending on the desired properties of the multimer. Suitable linkers include, for example, amino acid residues such as Pro or Gly or peptide segments containing from about 2 to about 5, 10, 15 or 20 or even more amino acids, bifunctional organic compounds such as $H_2N(CH_2)_nCOOH$ where n is an integer from 1 to 12, and the like. Examples of such linkers, as well as methods of making such linkers and peptides incorporating such linkers are well-known in the art (see, e.g., Hünig et al., 1974, Chem. Ber. 100:3039-3044; Basak et al., 1994, Bioconjug. Chem. 5(4):301-305).

In a preferred embodiment of the invention, the tandem repeats are internally punctuated by a single proline residue. To this end, in those instances where the core peptides are terminated at their N- or C-terminus with proline, such as, e.g., where X_1 in structure (I) is Pro (P) or D-Pro (p), m in structure (II) is preferably 0. In those instances where the core peptides do not contain an N- or C-terminal proline, LL is preferably Pro (P) or D-Pro (p) and m is preferably 1.

In certain embodiments of the invention, it may be desirable to employ cleavable linkers that permit the release of one or more helical segments (HH) under certain conditions. Suitable cleavable linkers include peptides having amino acid sequences that are recognized by proteases, oligonucleotides that are cleaved by endonucleases and organic compounds that can be cleaved via chemical means, such as under acidic, basic or other conditions. Preferably, the cleavage conditions will be relatively mild so as not to denature or otherwise degrade the helical segments and/or non-cleaved linkers composing the multimeric ApoA-I agonists.

Peptide and oligonucleotide linkers that can be selectively cleaved, as well as means for cleaving the linkers are well known and will be readily apparent to those of skill in the art. Suitable organic compound linkers that can be selectively cleaved will be apparent to those of skill in the art, and include those described, for example, in WO 94/08051, as well as the references cited therein.

In a preferred embodiment, the linkers employed are peptides that are substrates for endogenous circulatory enzymes, thereby permitting the multimeric ApoA-I agonists to be selectively cleaved in vivo. Endogenous enzymes suitable for cleaving the linkers include, for example, proapolipoprotein A-I propeptidase. Appropriate enzymes, as well as peptide segments that act as substrates for such enzymes, are well-known in the art (*see, e.g.*, Edelstein et al., 1983, J. Biol. Chem. 258:11430-11433; Zanis, 1983, Proc. Natl. Acad. Sci. USA 80:2574-2578).

As discussed above, a key feature of the core peptides of the invention is their ability to form intermolecular hydrogen-bonds or salt bridges when arranged in an antiparallel fashion. Thus, in a preferred embodiment of the invention, linkers of sufficient length and flexibility are used so as to permit the helical segments (HH) of structure (II) to align in an antiparallel fashion and form intermolecular hydrogen-bonds or salt bridges in the presence of lipids.

Linkers of sufficient length and flexibility include, but are not limited to, Pro (P), Gly (G), Cys-Cys, $H_2N-(CH_2)_n-COOH$ where n is 1 to 12, preferably 4 to 6; H_2N-aryl-COOH and carbohydrates.

Alternatively, as the native apolipoproteins permit cooperative binding between antiparallel helical segments, peptide linkers which correspond in primary sequence to the peptide segments connecting adjacent helices of the native apolipoproteins, including, for example, ApoA-I, ApoA-II, ApoA-IV, ApoC-I, ApoC-II, ApoC-III, ApoD, ApoE and ApoJ can

be conveniently used to link the core peptides. These sequences are well known in the art (see, e.g., Rosseneu et al., "Analysis of the Primary and of the Secondary Structure of the Apolipoproteins," In: Structure and Function of Lipoproteins, Ch. 6, 159-183, CRC Press, Inc., 1992).

Other linkers which permit the formation of intermolecular hydrogen bonds or salt bridges between tandem repeats of antiparallel helical segments include peptide reverse turns such as β-turns and γ-turns, as well as organic molecules that mimic the structures of peptide β-turns and/or γ-turns. Generally, reverse turns are segments of peptide that reverse the direction of the polypeptide chain so as to allow a single polypeptide chain to adopt regions of antiparallel β-sheet or antiparallel α-helical structure. β-turns generally are composed of four amino acid residues and γ-turns are generally composed of three amino acid residues.

The conformations and sequences of many peptide β-turns have been well-described in the art and include, by way of example and not limitation, type-I, type-I', type-II, type-II', type-III, type-III', type-IV, type-V, type-V', type-VIa, type-VIb, type-VII and type-VIII (see, Richardson, 1981, Adv. Protein Chem. 34:167-339; Rose et al., 1985, Adv. Protein Chem. 37:1-109; Wilmot et al., 1988, J. Mol. Biol. 203:221-232; Sibanda et al., 1989, J. Mol. Biol. 206:759-777; Tramontano et al., 1989, Proteins: Struct. Funct. Genet. 6:382-394).

The specific conformations of short peptide turns such as β-turns depend primarily on the positions of certain amino acid residues in the turn (usually Gly, Asn or Pro). Generally, the type-I β-turn is compatible with any amino acid residue at positions 1 through 4 of the turn, except that Pro cannot occur at position 3. Gly predominates at position 4 and Pro predominates at position 2 of both type-I and type-II turns. Asp, Asn, Ser and Cys residues frequently occur at position 1, where their side chains often hydrogen-bond to the NH of residue 3.

In type-II turns, Gly and Asn occur most frequently at position 3, as they adopt the required backbone angles most easily. Ideally, type-I' turns have Gly at positions 2 and 3, and type-II' turns have Gly at position 2. Type-III turns generally can have most amino acid residues, but type-III' turns usually require Gly at positions 2 and 3. Type-VIa and VIb turns generally have a *cis* peptide bond and Pro as an internal residue. For a review of the different types and sequences of β-turns in proteins and peptides the reader is referred to Wilmot et al., 1988, J. Mol. Biol. 203:221-232.

The conformation and sequences of many peptide γ-turns have also been well-described in the art (see, e.g., Rose et al., 1985, Adv. Protein Chem. 37:1-109; Wilmer-White et al., 1987, Trends Biochem. Sci. 12:189-192; Wilmot et al., 1988, J. Mol. Biol. 203:221-232; Sibanda et al., 1989, J. Mol. Biol. 206:759-777; Tramontano et al., 1989, Proteins: Struct. Funct. Genet. 6:382-394). All of these types of β-turns and γ-turn structures and their corresponding sequences, as well as later discovered peptide β-turns and γ-turn structures and sequences, are specifically contemplated by the invention.

Alternatively, the linker (LL) may comprise an organic molecule or moiety that mimics the structure of a peptide β-turn or γ-turn. Such β-turn and/or γ-turn mimetic moieties, as well as methods for synthesizing peptides containing such moieties, are well known in the art, and include, among others, those described in Giannis and Kolter, 1993 Angew. Chem. Intl. Ed. Eng. 32:1244-1267; Kahn et al., 1988, J. Molecular Recognition 1:75-79; and Kahn et al., 1987, Tetrahedron Lett. 28:1623-1626.

In still another embodiment of the invention, the multimers are in the form of branched networks (see, e.g., FIG. 7). Such networks are conveniently obtained through the use of multifunction linking moieties that permit more than two helical units to be attached to a simple linking moiety.

Thus, branched networks employ molecules having three, four or even more functional groups that are capable of covalently attaching to the N- and/or C-terminus of a peptide. Suitable linking moieties include, for example, amino acid residues having side chains bearing hydroxyl, sulfanyl, amino, carboxyl, amide and/or ester functionalities, such as, for example, Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q), Lys (K), Arg (R), Orn, Asp (D) and Glu (E); or other organic molecules containing such functional groups.

The helical segments attached to a single linking moiety need not be attached via like termini. Indeed, in some embodiments the helical segments are attached to a single linking moiety so as to be arranged in an antiparallel fashion, i.e., some of the helices are attached via their N-termini, others via their C-termini.

The helical segments can be attached directly to the linking moiety, or may be spaced from the linking moiety by way of one or more bifunctional linkers (LL), as previously described.

Referring to FIGS. 7A and 7B, it can be seen that a branched network can be described in terms of the number of "nodes" comprising the network, where each multifunctional linking moiety constitutes a node. In FIGS. 7A and 7B, helical segments (i.e., core peptides of the invention) are illustrated as cylinders, and multifunctional linking moieties (or nodes) as circles (●), where the number of lines emanating from the circle indicates the "order" (or number of functional groups) of the multifunctional linking moiety.

The number of nodes in the network will generally depend on the total desired number of helical segments, and will typically be from about 1 to 2. Of course, it will be appreciated that for a given number of desired helical segments, networks having higher order linking moieties will have fewer nodes. For example, referring to FIGS. 7A and 7B, a tertiary-order network (i.e., a network having trifunctional linking moieties) of seven helical units has

three nodes (FIG. 7A), whereas a quaternary order network (i.e., a network having tetrafunctional linking moieties) of seven helical units has only two nodes (FIG. 7B).

The networks may be of uniform order, i.e., networks in which all nodes are, for example, trifunctional or tetrafunctional linking moieties, or may be of mixed order, e.g., networks in which the nodes are mixtures of, for example, trifunctional and tetrafunctional linking moieties. Of course, it is to be understood that even in uniform order networks the linking moieties need not be identical. A tertiary order network may employ, for example, two, three, four or even more different trifunctional linking moieties.

Like the linear multimers, the helical segments comprising the branched network may be, but need not be, identical.

An example of such a mixed order branched network is illustrated in FIG. 7C. In FIG. 7C, helical segments (i.e., core peptides of the invention) are illustrated as cylinders and multifunctional linking moieties as circles (●), where the number of lines emanating from the circle indicates the "order" (or number of functional groups) of the multifunctional linking moiety. Lines connecting helical segments represent bifunctional linkers LL, as previously described. Helical segments which comprise the branched networks may be tandem repeats of core peptides, as previously described.

In one illustrative embodiment, the branched networks of the invention are described by the formula:

(III) $X\text{-}N_{ya}\text{-}X_{(ya-1)}\text{-}(N_{yb}\text{-}X_{(yb-1)})_p$

wherein:

each X is independently $HH\text{-}(LL_m\text{-}HH)_nLL_m\text{-}HH$;

each HH is independently a core peptide of structure (I) or an analogue or mutated, truncated,

internally deleted or extended form thereof as described herein;

each LL is independently a bifunctional linker;

each m is independently an integer from 0 to 1;

each n is independently an integer from 0 to 8;

N_{y_a} and N_{y_b} are each independently a multifunctional linking moiety where y_a and y_b represent the number of functional groups on N_{y_a} and N_{y_b}, respectively;

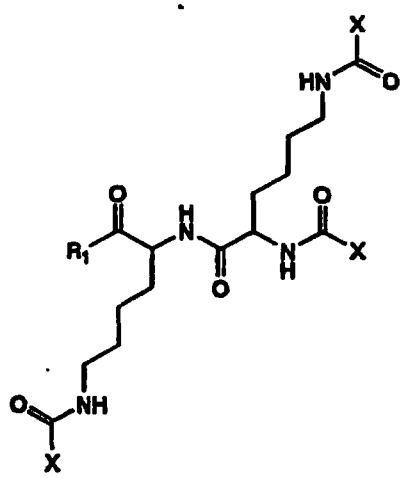
each y_a or y_b is independently an integer from 3 to 8;

p is an integer from 0 to 7; and

each "—" independently designates a covalent bond.

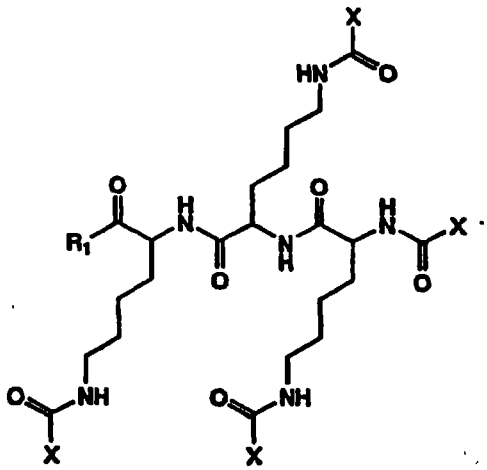
In a preferred embodiment, the branched network comprises a "Lys-tree," i.e., a network wherein the multifunctional linking moiety is one or more Lys (K) residues (see, e.g., FIG. 7D).

In one illustrative embodiment, the "Lys tree" branched networks of the invention are described by the formulae:



(IV)

-OR-



(V)

wherein:

each X is independently HH-(LL$_m$-HH)$_n$LL$_m$-HH;

each HH is independently a core peptide or peptide analogue of structure (I) or a mutated, truncated, internally deleted or extended form thereof as described herein;

each LL is independently a bifunctional linker;

each n is independently an integer from 0 to 8;

each m is independently an integer from 0 to 1;

R_1 is -OR or -NRR; and

each R is independently -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl; (C_5-C_{20}) aryl (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl or 6-26 membered alkheteroaryl.

5.1.1. ANALYSIS OF STRUCTURE AND FUNCTION

The structure and function of the core peptides or peptide analogues of the invention, as well as ApoA-I agonists composed of such core peptides, including the multimeric forms described above, can be assayed in order to select active agonists or mimetics of ApoA-I. For example, the core peptides or peptide analogues can be assayed for their ability to form α-helices in the presence of lipids, to bind lipids, to form complexes with lipids, to activate LCAT, to promote cholesterol efflux, etc.

Methods and assays for analyzing the structure and/or function of the peptides are well-known in the art. Preferred methods are provided in the working examples, infra. For example, the circular dichroism (CD) and nuclear magnetic resonance (NMR) assays described in Section 7, infra, can be used to analyze the structure of the peptides or peptide analogues -- particularly the degree of helicity in the presence of lipids. The ability to bind lipids can be determined using the fluorescence spectroscopy assay described in Section 7, infra. The ability of the peptides and/or peptide analogues to activate LCAT can be readily determined using the LCAT activation described in Section 8, infra. The in vitro and in vivo assays described in Section 9, 10 and 11, infra, can be used to evaluate the half-life, distribution, cholesterol efflux and effects on RCT.

Generally, core peptides and/or peptide analogues according to the invention which exhibit the properties listed in TABLE IV, infra, are considered to be active.

TABLE IV

PROPERTIES OF ACTIVE PEPTIDES

	Range	Preferred Range
% Helicity in the presence of lipids (Ri=30) (unblocked 22-amino acid residue peptides)	≥ 60%	≥ 80%
% Helicity in the presence of lipids (Ri=30) (unblocked 18-amino acid residue peptides)	≥ 40%	≥ 60%
% Helicity in the presence of lipids (Ri=30) (blocked 18-amino acid residue peptides and shorter peptides)	≥ 60%	≥ 80%
Lipid Binding (in the presence of SUVs)	0.5 - 10μM peptide R_i=1-50	
LCAT activation	≥ 38%	≥ 80%

R_i is lipid:peptide molar ratio

As illustrated in the working examples, infra, core peptides which exhibit a high degree of LCAT activation (≥ 38%) generally possess significant α-helical structure in the presence of lipidic small unilamellar vesicles (SUVs) (≥ 60% helical structure in the case of unblocked peptides containing 22 or more amino acid residues and blocked peptides containing 18 or fewer amino acid residues; ≥ 40% helical structure in the case of unblocked peptides containing 18 or fewer amino acids), and those peptides which exhibit little or no LCAT activation possess little α-helical structure. However, in certain instances, peptides which

exhibit significant helical structure in the presence of lipids do not effect significant LCAT.

Similarly, while core peptides that exhibit significant LCAT activation typically bind lipids, in certain instances peptides which exhibit lipid binding do not effect significant LCAT activation.

As a consequence, it will be recognized by those of skill in the art that while the ability of the core peptides described herein to form α-helices (in the presence of lipids) and to bind lipids is critical for activity, in many instances these properties may not be sufficient. Thus, in a preferred embodiment core peptides of the invention are subjected to a series of screens to select for core peptides exhibiting significant pharmacological activity.

In a first step, a core peptide is screened for its ability to form an α-helix in the presence of lipids using the CD assay described in Section 7, infra. Those peptides which are at least 40% helical (unblocked peptides containing 18 or fewer amino acids) or 60% helical (blocked peptides containing 18 or fewer amino acids; unblocked peptides containing 22 or more amino acids) in the presence of lipids (at a conc. of about 5 μM and a lipid:peptide molar ratio of about 30) are then screened for their ability to bind lipids using the fluorescence assay described in Section 7, infra. Of course, only those core peptides which contain a fluorescent Trp (W) or Nal residue are screened for lipid binding via fluorescence. However, for peptides which do not contain fluorescent residues, binding to lipids is obvious when helicity increases in the presence of lipids.

Core peptides which exhibit lipid binding in the presence of SUVs (0.5-10 μM peptide; lipid:peptide molar ratio in the range of 1 to 50) are then screened for pharmacological activity. Of course, the pharmacological activity screened for will depend upon the desired use of the ApoA-I agonists. In a preferred embodiment, the core peptides are screened for their ability to activate LCAT, as

peptides which activate LCAT are particularly useful in the methods described herein. Core peptides which exhibit at least about 38% LCAT activation as compared with native human ApoA-I (as determined using the LCAT activation assay described in Section 8, infra), are preferred, with core peptides exhibiting 50%, 60%, 70%, 80% or even 90% or more being particularly preferred.

5.1.2. PREFERRED EMBODIMENTS

The ApoA-I agonists of the invention can be further defined by way of preferred embodiments.

In one preferred embodiment, the ApoA-I agonists are 22 amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof.

In another preferred embodiment, the ApoA-I agonists are 22 amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_7 is a basic amino acid, Asn (N) or Glu (E); X_8 is an acidic amino acid or Arg (R); X_{12} is an acidic amino acid or Asn (N); and/or X_{15} is an acidic amino acid, Gln (Q) or Lys (K); and X_1, X_2, X_3, X_4, X_5, X_6, X_9, X_{10}, X_{11}, X_{13}, X_{14}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20} and X_{21} are as previously defined for Structure (I).

In another preferred embodiment, the ApoA-I agonists are 22 amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which:

X_1 is Pro (P), Gly (G), Ala (A), Gln (Q), Asn (N), Asp (D) or D-Pro (p);

X_2 is Ala (A), Val (V) or Leu (L);

X_4 is Asp (D) or Glu (E);

X_7 is Lys (K), Arg (R), Orn, Asn (N) or Glu (E);

X_8 is Asp (D), Arg (R) or Glu (E);

X_{11} is Asn (N), Gln (Q), Glu (E) or Arg (R);

X_{12} is Asp (D), Glu (E) or Asn (N);

X_{13} is Leu (L), Gly (G) or Aib;

X_{15} is Asp (D), Glu (E), Gln (Q) or Lys (K);

X_{16} is Ala (A), Trp (W), Gly (G), Leu (L), Phe (F) or Nal;

X_{17} is Leu (L), Gly (G) or Nal;

X_{18} is Lys (K), Orn, Gln (Q) or Asn (N);

X_{19} is Lys (K), Orn, Gln (Q) or Asn (N);

X_{20} is Lys (K) or Orn;

X_{21} is Leu (L); and/or

X_{22} is Lys (K) or Orn, and X_3, X_5, X_6, X_9, X_{10} and X_{14} are as previously defined for structure (I).

An even more preferred embodiment according to this aspect of the invention are those peptides in which:

X_2 is Val (V);

X_3 is Leu (L);

X_5 is Leu (L);

X_6 is Phe (F);

X_7 is Arg (R) or Lys (K);

X_8 is Glu (E);

X_9 is Leu (L);

X_{10} is Leu (L);

X_{11} is Asn (N) or Glu (Q);

X_{12} is Glu (E); and/or

X_{15} is Glu (E);

and X_1, X_4, X_{13}, X_{14}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, X_{21} and X_{22} are as previously defined for structure (I) or are as defined in the preceding paragraph.

In yet another preferred embodiment, the ApoA-I agonists are 22 amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which only one of X_{18} or X_{19} is a basic amino acid and the other one of X_{18} or X_{19} is Gln (Q) or Asn (N).

In yet another preferred embodiment, the ApoA-I agonists are peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified

forms thereof, in which one of X_{18} or X_{19} is Lys (K) or Orn and the other one of X_{18} or X_{19} is Gln (Q) or Asn (N).

In still another preferred embodiment, the ApoA-I agonists are 22 amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which one of X_9, X_{10}, X_{13}, X_{14}, X_{16} or X_{17} is Gly (G) and the others are other than Gly (G).

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_{13} is Gly (G) and each of X_9, X_{10}, X_{14}, X_{16} and X_{17} is other than Gly (G).

In yet another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which:

X_1 is Pro (P), Gly (G) or D-Pro (p);
X_2 is Val (V);
X_3 is Leu (L);
X_4 is Asp (D) or Glu (E);
X_5 is L (Leu) or Phe (F);
X_6 is Phe (F);
X_7 is Arg (R);
X_8 is Glu (E);
X_9 is Leu (L);
X_{10} is Leu (L) or Trp (W);
X_{11} is Asn (N);
X_{12} is Glu (E);
X_{13} is Gly (G);
X_{14} is Leu (L);
X_{15} is Glu (E);
X_{16} is Ala (A) or Trp (W);
X_{17} is Leu (L) or Nal;
X_{18} is Lys (K) or Orn;
X_{19} is Gln (Q);

X_{20} is Lys (K) or Orn;

X_{21} is Leu (L); and

X_{22} is Lys (K) or Orn.

Particularly preferred ApoA-I agonists according to this aspect of the invention are selected from the group consisting of:

peptide 3	PVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:3);
peptide 13	GVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:13);
peptide 19	pVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:19);
peptide 137	PVLDLFRELLNEGLEAZKQKLK	(SEQ ID NO:137);
peptide 138	PVLDLFRELLNEGLEWLKQKLK	(SEQ ID NO:138);
peptide 139	PVLDLFRELWNEGLEALKQKLK	(SEQ ID NO:139);
peptide 140	PVLDLFRELLNEGLEALOQOLO	(SEQ ID NO:140);
peptide 141	PVLDFFRELLNEGLEALKQKLK	(SEQ ID NO:141);
peptide 142	PVLELFRELLNEGLEALKQKLK	(SEQ ID NO:142);

and the C-terminal amidated or esterified and/or N-terminal acylated forms thereof.

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_9 is Gly (G) and each of X_{10}, X_{13}, X_{14}, X_{16} and X_{17} is other than Gly (G). A particularly preferred ApoA-I agonist according to this aspect of the invention is peptide 20: PVLDLFREGLNELLEALKQKLK (SEQ ID NO:20).

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_{10} is Gly (G) and each of X_9, X_{13}, X_{14}, X_{16} and X_{17} is other than Gly (G). A particularly preferred ApoA-I agonist according to this aspect of the invention is peptide 9: PVLDLFRELGNELLEALKQKLK (SEQ ID NO:9).

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_{14} is Gly (G) and each of X_9, X_{10}, X_{13}, X_{16} and X_{17} is other than Gly (G). A particularly preferred ApoA-I agonist according to this aspect of the invention is peptide 126:
PVLDLFRELLNELGEALKQKLK (SEQ ID NO:126).

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_{16} is Gly (G) and each of X_9, X_{10}, X_{13}, X_{14} and X_{17} is other than Gly (G). A particularly preferred ApoA-I agonist according to this aspect of the invention is peptide 22:
PVLDLFRELLNELLEGLKQKLK (SEQ ID NO:22).

In still another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which X_{17} is Gly (G) and each of X_9, X_{10}, X_{13}, X_{14} and X_{16} is other than Gly (G). A particularly preferred ApoA-I agonist according to this aspect of the invention is peptide 12:
PVLDLFRELLNELLEAGKQKLK (SEQ ID NO:12).

Embodiments containing internal glycine residues can be readily synthesized in high yield by way of segment condensation, thereby providing significant advantages for large-scale production. Segment condensation, i.e., the joining together of small constituent peptide chains to form a larger peptide chain, has been used to prepare many biologically active peptides, including 44-amino acid residue mimics of ApoA-I (see, e.g., Nakagawa et al., 1985, J. Am Chem. Soc. 107:7087-7083; Nokihara et al., 1989, Peptides 1988:166-168; Kneib-Cordonnier et al., 1990, Int. J. Pept. Protein Res. 35:527-538), and is considered to be the most

cost-effective method for high-yield bulk synthesis of the core peptides of the invention.

Advantages of synthesis via segment condensation include the ability to condense pre-formed segments in the solution phase and the ease of purification of the final product. Drawbacks of the method include low coupling efficiency and yield at the condensation step and low solubility of certain peptide sequences.

The coupling efficiency of the condensation step can be significantly increased by increasing the coupling time. Typically, increasing the coupling time results in increased racemezation of the product (Sieber et al., 1970, Helv. Chim. Acta 53:2135-2150). However, since glycine lacks a chiral center it does not undergo racemezation (proline residues, due to steric hindrance, also undergo little or no racemezation at long coupling times). Thus, embodiments containing internal glycine residues can be synthesized in bulk in high yield via segment condensation by synthesizing constituent segments which take advantage of the fact that glycine residues do not undergo racemezation. Thus, embodiments containing internal glycine residues provide significant synthetic advantages for large-scale bulk preparation.

In yet another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which each of X_9, X_{10}, X_{13}, X_{14}, X_{16} and X_{17} is other than Gly (G).

In yet another preferred embodiment, the ApoA-I agonists are 22-amino acid residue peptides according to structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which:

X_1 is Pro (P), Gly (G), Ala (A) or D-Pro (p);

X_2 is Val (V) or Leu (L);

X_3 is Leu (L);

X_4 is Asp (D) or Glu (E);

X_5 is Leu (L) or Phe (F);
X_6 is Leu (L) or Phe (F);
X_7 is Arg (R) or Lys (K);
X_8 is Glu (E);
X_9 is Leu (L);
X_{10} is Leu (L) or Trp (W);
X_{11} is Asn (N) or Gln (Q);
X_{12} is Glu (E);
X_{13} is Leu (L) or Aib;
X_{14} is Leu (L), Trp (W) or Nal;
X_{15} is Glu (E);
X_{16} is Ala (A), Leu (L), Trp (W) or Nal;
X_{17} is Leu (L) or Nal;
one of X_{18} or X_{19} is Gln (Q) and the other is Lys (K) or Orn;
X_{20} is Lys (K) or Orn;
X_{21} is Leu (L); and
X_{22} is Lys (K) or Orn.

In a particularly preferred embodiment according to this aspect of the invention, X_2 is Val (V); X_4 is Asp (D); X_5 is Leu (L); X_6 is Phe (F); X_7 is Arg R); X_{10} is Leu (L); X_{11} is Asn (N); X_{13} is Leu (L); X_{14} is Leu (L); X_{16} is Ala (A); X_{17} is Leu (L); X_{18} is Lys (K); X_{19} is Gln (Q); X_{20} is Lys (K) and/or X_{22} is Lys (K).

In still another preferred embodiment, the ApoA-I agonists are altered or mutated forms of the peptides of structure (I), or the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, in which:

X_1 is other than Aib, Val (V) or Leu (L);
X_2 is other than D-Val (v);
X_5 is other than Lys (K), Glu (E), Trp (W) or Nal;
X_6 is other than Trp (W);
X_7 is other than Trp (W) or Leu (L);
X_8 is other than Trp (W);
X_9 is other than Lys (K) or Trp (W);
X_{11} is other than Trp (W);

X_{12} is other than Trp (W) or Leu (L);
X_{13} is other than Glu (E) or Trp (W);
X_{15} is other than Trp (W); and/or
X_{21} is other than Lys (K).

In yet another preferred embodiment, the ApoA-I agonists of the invention are selected from the group of peptides set forth below:

peptide 1	PVLDLFRELLNELLEZLKQKLK	(SEQ ID NO:1);
peptide 2	GVLDLFRELLNELLEALKQKLKK	(SEQ ID NO:2);
peptide 3	PVLDLFRELLNELLEWLKQKLK	(SEQ ID NO:3);
peptide 4	PVLDLFRELLNELLEALKQKLK	(SEQ ID NO:4);
peptide 5	pVLDLFRELLNELLEALKQKLKK	(SEQ ID NO:5);
peptide 6	PVLDLFRELLNEXLEALKQKLK	(SEQ ID NO:6);
peptide 7	PVLDLFKELLNELLEALKQKLK	(SEQ ID NO:7);
peptide 8	PVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:8);
peptide 9	PVLDLFRELGNELLEALKQKLK	(SEQ ID NO:9);
peptide 10	PVLDLFRELLNELLEAZKQKLK	(SEQ ID NO:10);
peptide 11	PVLDLFKELLQELLEALKQKLK	(SEQ ID NO:11);
peptide 12	PVLDLFRELLNELLEAGKQKLK	(SEQ ID NO:12);
peptide 13	GVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:13);
peptide 14	PVLDLFRELLNELLEALOQOLO	(SEQ ID NO:14);
peptide 15	PVLDLFRELWNELLEALKQKLK	(SEQ ID NO:15);
peptide 16	PVLDLLRELLNELLEALKQKLK	(SEQ ID NO:16);
peptide 17	PVLELFKELLQELLEALKQKLK	(SEQ ID NO:17);
peptide 18	GVLDLFRELLNELLEALKQKLK	(SEQ ID NO:18);
peptide 19	pVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:19);
peptide 20	PVLDLFREGLNELLEALKQKLK	(SEQ ID NO:20);
peptide 21	pVLDLFRELLNELLEALKQKLK	(SEQ ID NO:21);
peptide 22	PVLDLFRELLNELLEGLKQKLK	(SEQ ID NO:22);
peptide 23	PLLELFKELLQELLEALKQKLK	(SEQ ID NO:23);
peptide 24	PVLDLFRELLNELLEALQKKLK	(SEQ ID NO:24);
peptide 25	PVLDFFRELLNEXLEALKQKLK	(SEQ ID NO:25);
peptide 26	PVLDLFRELLNELLELLKQKLK	(SEQ ID NO:26);

peptide 27 PVLDLFRELLNELZEALKQKLK (SEQ ID NO:27);
peptide 28 PVLDLFRELLNELWEALKQKLK (SEQ ID NO:28);
peptide 29 AVLDLFRELLNELLEALKQKLK (SEQ ID NO:29);
peptide 123 QVLDLFRELLNELLEALKQKLK (SEQ ID NO:123);
peptide 124 PVLDLFOELLNELLEALOQOLO (SEQ ID NO:124);
peptide 125 NVLDLFRELLNELLEALKQKLK (SEQ ID NO:125);
peptide 126 PVLDLFRELLNELGEALKQKLK (SEQ ID NO:126);
peptide 127 PVLDLFRELLNELLELLKQKLK (SEQ ID NO:127);
peptide 128 PVLDLFRELLNELLEFLKQKLK (SEQ ID NO:128);
peptide 129 PVLELFNDLLRELLEALQKKLK (SEQ ID NO:129);
peptide 130 PVLELFNDLLRELLEALKQKLK (SEQ ID NO:130);
peptide 131 PVLELFKELLNELLDALRQKLK (SEQ ID NO:131);
peptide 132 PVLDLFRELLENLLEALQKKLK (SEQ ID NO:132);
peptide 133 PVLELFERLLEDLLQALNKKLK (SEQ ID NO:133);
peptide 134 PVLELFERLLEDLLKALNQKLK (SEQ ID NO:134);
peptide 135 DVLDLFRELLNELLEALKQKLK (SEQ ID NO:135);
peptide 136 PALELFKDLLQELLEALKQKLK (SEQ ID NO:136);
peptide 137 PVLDLFRELLNEGLEAZKQKLK (SEQ ID NO:137);
peptide 138 PVLDLFRELLNEGLEWLKQKLK (SEQ ID NO:138);
peptide 139 PVLDLFRELWNEGLEALKQKLK (SEQ ID NO:139);
peptide 140 PVLDLFRELLNEGLEALOQOLO (SEQ ID NO:140);
peptide 141 PVLDFFRELLNEGLEALKQKLK (SEQ ID NO:141);
peptide 142 PVLELFRELLNEGLEALKQKLK (SEQ ID NO:142);

and the N-terminal acylated (particularly acetylated or dansylated) and/or C-terminal amidated or esterified forms thereof, wherein X is Aib; Z is Nal; and O is Orn.

In still another preferred embodiment, the ApoA-I agonists of the invention are selected from the group of peptides set forth below:

peptide 1 PVLDLFRELLNELLEZLKQKLK (SEQ ID NO:1);
peptide 2 GVLDLFRELLNELLEALKQKLKK (SEQ ID NO:2);
peptide 3 PVLDLFRELLNELLEWLKQKLK (SEQ ID NO:3);

peptide 4 PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4);
peptide 5 pVLDLFRELLNELLEALKQKLKK (SEQ ID NO:5);
peptide 6 PVLDLFRELLNEXLEALKQKLK (SEQ ID NO:6);
peptide 7 PVLDLFKELLNELLEALKQKLK (SEQ ID NO:7);
peptide 8 PVLDLFRELLNEGLEALKQKLK (SEQ ID NO:8);
peptide 9 PVLDLFRELGNELLEALKQKLK (SEQ ID NO:9);
peptide 10 PVLDLFRELLNELLEAZKQKLK (SEQ ID NO:10);
peptide 11 PVLDLFKELLQELLEALKQKLK (SEQ ID NO:11);
peptide 12 PVLDLFRELLNELLEAGKQKLK (SEQ ID NO:12);
peptide 13 GVLDLFRELLNEGLEALKQKLK (SEQ ID NO:13);
peptide 14 PVLDLFRELLNELLEALOQOLO (SEQ ID NO:14);
peptide 15 PVLDLFRELWNELLEALKQKLK (SEQ ID NO:15);
peptide 16 PVLDLLRELLNELLEALKQKLK (SEQ ID NO:16);
peptide 17 PVLELFKELLQELLEALKQKLK (SEQ ID NO:17);
peptide 18 GVLDLFRELLNELLEALKQKLK (SEQ ID NO:18);
peptide 19 pVLDLFRELLNEGLEALKQKLK (SEQ ID NO:19);
peptide 20 PVLDLFREGLNELLEALKQKLK (SEQ ID NO:20);
peptide 21 pVLDLFRELLNELLEALKQKLK (SEQ ID NO:21);
peptide 22 PVLDLFRELLNELLEGLKQKLK (SEQ ID NO:22);
peptide 23 PLLELFKELLQELLEALKQKLK (SEQ ID NO:23);
peptide 24 PVLDLFRELLNELLEALQKKLK (SEQ ID NO:24);
peptide 25 PVLDFFRELLNEXLEALKQKLK (SEQ ID NO:25);
peptide 26 PVLDLFRELLNELLELLKQKLK (SEQ ID NO:26);
peptide 27 PVLDLFRELLNELZEALKQKLK (SEQ ID NO:27);
peptide 28 PVLDLFRELLNELWEALKQKLK (SEQ ID NO:28);
peptide 29 AVLDLFRELLNELLEALKQKLK (SEQ ID NO:29);

and the N-terminal acylated (particularly acetylated or dansylated) and/or C-terminal amidated or esterified forms thereof, wherein X is Aib; Z is Nal; and O is Orn.

In yet another preferred embodiment, the ApoA-I agonists are multimeric forms according to structures II, III and/or IV in which HH is a peptide according to structure (I), or an N-terminal acylated and/or C-terminal amidated or

esterified form thereof, or any of the preferred peptides according to structure (I) described herein.

In yet another preferred embodiment, the core peptides that compose the ApoA-I agonists are not any of the following peptides:

peptide 75:	PVLDEFREKLNEELEALKQKLK	(SEQ ID NO:75);
peptide 94:	PVLDEFREKLNEALEALKQKLK	(SEQ ID NO:94);
peptide 109:	PVLDEFREKLNERLEALKQKLK	(SEQ ID NO:109);
peptide 237:	LDDLLQKWAEAFNQLLKK	(SEQ ID NO:237);
peptide 238:	EWLKAFYEKVLEKLKELF*	(SEQ ID NO:238);
peptide 241:	DWFKAFYDKVFEKFKEFF	(SEQ ID NO:241);
peptide 242:	GIKKFLGSIWKFIKAFVG	(SEQ ID NO:242);
peptide 243:	DWFKAFYDKVAEKFKEAF	(SEQ ID NO:243);
peptide 244:	DWLKAFYDKVAEKLKEAF	(SEQ ID NO:244);
peptide 245:	DWLKAFYDKVFEKFKEFF	(SEQ ID NO:245);
peptide 246:	EWLEAFYKKVLEKLKELF	(SEQ ID NO:246);
peptide 247:	DWFKAFYDKFFEKFKEFF	(SEQ ID NO:247);
peptide 248:	EWLKAFYEKVLEKLKELF	(SEQ ID NO:248);
peptide 249:	EWLKAEYEKVEEKLKELF*	(SEQ ID NO:249);
peptide 250:	EWLKAEYEKVLEKLKELF*	(SEQ ID NO:250); and
peptide 251:	EWLKAFYKKVLEKLKELF*	(SEQ ID NO:251).

In a final preferred embodiment, the ApoA-I agonists are not any of the peptides listed in TABLE X (Section 8.3, infra) exhibiting an LCAT activation activity of less than 38% as compared with native human ApoA-I.

5.2 SYNTHESIS AND PURIFICATION OF THE ApoA-I PEPTIDE AGONISTS

The core peptides of the invention may be prepared using virtually any art-known technique for the preparation of peptides. For example, the peptides may be prepared using conventional step-wise solution or solid phase peptide syntheses, or recombinant DNA techniques.

5.2.1 CHEMICAL SYNTHESIS

Core peptides may be prepared using conventional step-wise solution or solid phase synthesis (see, *e.g.*, Chemical Approaches to the Synthesis of Peptides and Proteins, Williams *et al.*, Eds., 1997, CRC Press, Boca Raton Florida, and references cited therein; Solid Phase Peptide Synthesis: A Practical Approach, Atherton & Sheppard, Eds., 1989, IRL Press, Oxford, England, and references cited therein).

Alternatively, the peptides of the invention may be prepared by way of segment condensation, as described, for example, in Liu et al., 1996, Tetrahedron Lett. 37(7):933-936; Baca, et al., 1995, J. Am. Chem. Soc. 117:1881-1887; Tam *et al.*, 1995, Int. J. Peptide Protein Res. 45:209-216; Schnölzer and Kent, 1992, Science 256:221-225; Liu and Tam, 1994, J. Am. Chem. Soc. 116(10):4149-4153; Liu and Tam, 1994, Proc. Natl. Acad. Sci. USA 91:6584-6588; Yamashiro and Li, 1988, Int. J. Peptide Protein Res. 31:322-334). This is particularly the case with glycine containing peptides. Other methods useful for synthesizing the peptides of the invention are described in Nakagawa et al., 1985, J. Am. Chem. Soc. 107:7087-7092.

ApoA-I agonists containing N- and/or C-terminal blocking groups can be prepared using standard techniques of organic chemistry. For example, methods for acylating the N-terminus of a peptide or amidating or esterifying the C-terminus of a peptide are well-known in the art. Modes of carrying other modifications at the N- and/or C-terminus will be apparent to those of skill in the art, as will modes of protecting any side-chain functionalities as may be necessary to attach terminal blocking groups.

Pharmaceutically acceptable salts (counter ions) can be conveniently prepared by ion-exchange chromatography or other methods as are well known in the art.

Compounds of the invention which are in the form of tandem multimers can be conveniently synthesized by adding

the linker(s) to the peptide chain at the appropriate step in the synthesis. Alternatively, the helical segments can be synthesized and each segment reacted with the linker. Of course, the actual method of synthesis will depend on the composition of the linker. Suitable protecting schemes and chemistries are well known, and will be apparent to those of skill in the art.

Compounds of the invention which are in the form of branched networks can be conveniently synthesized using the trimeric and tetrameric resins and chemistries described in Tam, 1988, Proc. Natl. Acad. Sci. USA 85:5409-5413 and Demoor et al., 1996, Eur. J. Biochem. 239:74-84. Modifying the synthetic resins and strategies to synthesize branched networks of higher or lower order, or which contain combinations of different core peptide helical segments, is well within the capabilities of those of skill in the art of peptide chemistry and/or organic chemistry.

Formation of disulfide linkages, if desired, is generally conducted in the presence of mild oxidizing agents. Chemical oxidizing agents may be used, or the compounds may simply be exposed to atmospheric oxygen to effect these linkages. Various methods are known in the art, including those described, for example, by Tam et al., 1979, Synthesis 955-957; Stewart et al., 1984, Solid Phase Peptide Synthesis, 2d Ed., Pierce Chemical Company Rockford, IL; Ahmed et al., 1975, J. Biol. Chem. 250:8477-8482; and Pennington et al., 1991 Peptides 1990 164-166, Giralt and Andreu, Eds., ESCOM Leiden, The Netherlands. An additional alternative is described by Kamber et al., 1980, Helv. Chim. Acta 63:899-915. A method conducted on solid supports is described by Albericio, 1985, Int. J. Peptide Protein Res. 26:92-97. Any of these methods may be used to form disulfide linkages in the peptides of the invention.

5.2.2 RECOMBINANT SYNTHESIS

If the peptide is composed entirely of gene-encoded amino acids, or a portion of it is so composed, the peptide or the relevant portion may also be synthesized using conventional recombinant genetic engineering techniques.

For recombinant production, a polynucleotide sequence encoding the peptide is inserted into an appropriate expression vehicle, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation. The expression vehicle is then transfected into a suitable target cell which will express the peptide. Depending on the expression system used, the expressed peptide is then isolated by procedures well-established in the art. Methods for recombinant protein and peptide production are well known in the art (see, e.g., Sambrook et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. each of which is incorporated by reference herein in its entirety.)

To increase efficiency of production, the polynucleotide can be designed to encode multiple units of the peptide separated by enzymatic cleavage sites -- either homopolymers (repeating peptide units) or heteropolymers (different peptides strung together) can be engineered in this way. The resulting polypeptide can be cleaved (e.g., by treatment with the appropriate enzyme) in order to recover the peptide units. This can increase the yield of peptides driven by a single promoter. In a preferred embodiment, a polycistronic polynucleotide can be designed so that a single mRNA is transcribed which encodes multiple peptides (i.e., homopolymers or heteropolymers) each coding region operatively linked to a cap-independent translation control sequence; e.g., an internal ribosome entry site (IRES). When

used in appropriate viral expression systems, the translation of each peptide encoded by the mRNA is directed internally in the transcript; e.g., by the IRES. Thus, the polycistronic construct directs the transcription of a single, large polycistronic mRNA which, in turn, directs the translation of multiple, individual peptides. This approach eliminates the production and enzymatic processing of polyproteins and may significantly increase yield of peptide driven by a single promoter.

A variety of host-expression vector systems may be utilized to express the peptides described herein. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA or plasmid DNA expression vectors containing an appropriate coding sequence; yeast or filamentous fungi transformed with recombinant yeast or fungi expression vectors containing an appropriate coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an appropriate coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus or tobacco mosaic virus) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an appropriate coding sequence; or animal cell systems.

The expression elements of the expression systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedron promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the

promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5 K promoter) may be used; when generating cell lines that contain multiple copies of expression product, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

In cases where plant expression vectors are used, the expression of sequences encoding the peptides of the invention may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, Nature 310:511-514), or the coat protein promoter of TMV (Takamatsu et al., 1987, EMBO J. 6:307-311) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J. 3:1671-1680; Broglie et al., 1984, Science 224:838-843) or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques *see, e.g.*, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

In one insect expression system that may be used to produce the peptides of the invention, *Autographa californica*, nuclear polyhidrosis virus (AcNPV) is used as a vector to express the foreign genes. The virus grows in *Spodoptera frugiperda* cells. A coding sequence may be cloned into non-essential regions (for example the polyhedron gene)

of the virus and placed under control of an AcNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (*e.g.*, see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051). Further examples of this expression system may be found in Current Protocols in Molecular Biology, Vol. 2, Ausubel et al., eds., Greene Publish. Assoc. & Wiley Interscience.

In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing peptide in infected hosts. (*e.g.*, See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. (USA) 81:3655-3659). Alternatively, the vaccinia 7.5 K promoter may be used, (*see, e.g.*, Mackett et al., 1982, Proc. Natl. Acad. Sci. (USA) 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. 79:4927-4931).

Other expression systems for producing the peptides of the invention will be apparent to those having skill in the art.

5.2.3 PURIFICATION OF PEPTIDES

The peptides of the invention can be purified by art-known techniques such as reverse phase chromatography high performance liquid chromatography, ion exchange

chromatography, gel electrophoresis, affinity chromatography and the like. The actual conditions used to purify a particular peptide will depend, in part, on synthesis strategy and on factors such as net charge, hydrophobicity, hydrophilicity, etc., and will be apparent to those having skill in the art. Multimeric branched peptides can be purified, e.g., by ion exchange or size exclusion chromatography.

For affinity chromatography purification, any antibody which specifically binds the peptide may be used. For the production of antibodies, various host animals, including but not limited to rabbits, mice, rats, etc., may be immunized by injection with a peptide. The peptide may be attached to a suitable carrier, such as BSA, by means of a side chain functional group or linkers attached to a side chain functional group. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum*.

Monoclonal antibodies to a peptide may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, 1975, Nature 256:495-497, or Kaprowski, U.S. Patent No. 4,376,110 which is incorporated by reference herein; the human B-cell hybridoma technique) Kosbor et al., 1983, Immunology Today 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030); and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). In addition, techniques developed for the

production of "chimeric antibodies" Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454, Boss, U.S. Patent No. 4,816,397; Cabilly, U.S. Patent No. 4,816,567; which are incorporated by reference herein) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Or "humanized" antibodies can be prepared (see, e.g., Queen, U.S. Patent No. 5,585,089 which is incorporated by reference herein). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce peptide-specific single chain antibodies.

Antibody fragments which contain deletions of specific binding sites may be generated by known techniques. For example, such fragments include but are not limited to $F(ab')_2$ fragments, which can be produced by pepsin digestion of the antibody molecule and Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the peptide of interest.

The antibody or antibody fragment specific for the desired peptide can be attached, for example, to agarose, and the antibody-agarose complex is used in immunochromatography to purify peptides of the invention. *See*, Scopes, 1984, Protein Purification: Principles and Practice, Springer-Verlag New York, Inc., NY, Livingstone, 1974, Methods In Enzymology: Immunoaffinity Chromatography of Proteins 34:723-731.

5.3 PHARMACEUTICAL FORMULATIONS AND METHODS OF TREATMENT

The ApoA-I agonists of the invention can be used to treat any disorder in animals, especially mammals including humans, for which increasing serum HDL concentration, activating LCAT, and promoting cholesterol efflux and RCT is beneficial. Such conditions include, but are not limited to hyperlipidemia, and especially hypercholesterolemia, and cardiovascular disease such as atherosclerosis (including treatment and prevention of atherosclerosis); restenosis (e.g., preventing or treating atherosclerotic plaques which develop as a consequence of medical procedures such as balloon angioplasty); and other disorders, such as endotoxemia, which often results in septic shock.

The ApoA-I agonists can be used alone or in combination therapy with other drugs used to treat the foregoing conditions. Such therapies include, but are not limited to simultaneous or sequential administration of the drugs involved.

For example, in the treatment of hypercholesterolemia or atherosclerosis, the ApoA-I agonist formulations can be administered with any one or more of the cholesterol lowering therapies currently in use; e.g., bile-acid resins, niacin, and/or statins. Such a combined regimen may produce particularly beneficial therapeutic effects since each drug acts on a different target in cholesterol synthesis and transport; i.e., bile-acid resins affect cholesterol recycling, the chylomicron and LDL population; niacin primarily affects the VLDL and LDL population; the statins inhibit cholesterol synthesis, decreasing the LDL population (and perhaps increasing LDL receptor expression); whereas the ApoA-I agonists affect RCT, increase HDL, increase LCAT activity and promote cholesterol efflux.

In another embodiment, the ApoA-I agonists may be used in conjunction with fibrates to treat hyperlipidemia,

hypercholesterolemia and/or cardiovascular disease such as atherosclerosis.

In yet another embodiment, the ApoA-I agonists of the invention can be used in combination with the anti-microbials and anti-inflammatory agents currently used to treat septic shock induced by endotoxin.

The ApoA-I agonists of the invention can be formulated as peptides or as peptide-lipid complexes which can be administered to subjects in a variety of ways to deliver the ApoA-1 agonist to the circulation. Exemplary formulations and treatment regimens are described below.

5.3.1 ApoA-I AGONISTS AND PEPTIDE/LIPID COMPLEX AS THE ACTIVE INGREDIENT

The ApoA-I agonist peptides can be synthesized or manufactured using any technique described in Section 5.2 and its subsections. Stable preparations which have a long shelf life may be made by lyophilizing the peptides -- either to prepare bulk for reformulation, or to prepare individual aliquots or dosage units which can be reconstituted by rehydration with sterile water or an appropriate sterile buffered solution prior to administration to a subject.

In certain embodiments, it may be preferred to formulate and administer the ApoA-I agonist in a peptide-lipid complex. This approach has several advantages since the complex should have an increased half-life in the circulation, particularly when the complex has a similar size and density to HDL, and especially the pre-β-1 or pre-β-2 HDL populations. The peptide-lipid complexes can conveniently be prepared by any of a number of methods described below. Stable preparations having a long shelf life may be made by lyophilization -- the co-lyophilization procedure described below being the preferred approach. The lyophilized peptide-lipid complexes can be used to prepare bulk for pharmaceutical reformulation, or to prepare individual aliquots or dosage units which can be reconstituted by

rehydration with sterile water or an appropriate buffered solution prior to administration to a subject.

A variety of methods well known to those skilled in the art can be used to prepare the peptide-lipid vesicles or complexes. To this end, a number of available techniques for preparing liposomes or proteoliposomes may be used. For example, the peptide can be cosonicated (using a bath or probe sonicator) with appropriate lipids to form complexes. Alternatively the peptide can be combined with preformed lipid vesicles resulting in the spontaneous formation of peptide-lipid complexes. In yet another alternative, the peptide-lipid complexes can be formed by a detergent dialysis method; e.g., a mixture of the peptide, lipid and detergent is dialyzed to remove the detergent and reconstitute or form peptide-lipid complexes (e.g., see Jonas et al., 1986, Methods in Enzymol. 128:553-582).

While the foregoing approaches are feasible, each method presents its own peculiar production problems in terms of cost, yield, reproducibility and safety. The applicants have developed a simple method for preparing peptide or protein-phospholipid complexes which have characteristics similar to HDL. This method can be used to prepare the ApoA-I peptide-lipid complexes, and has the following advantages: (1) Most or all of the included ingredients are used to form the designed complexes, thus avoiding waste of starting material which is common to the other methods. (2) Lyophilized compounds are formed which are very stable during storage. The resulting complexes may be reconstituted immediately before use. (3) The resulting complexes usually need not be further purified after formation and before use. (4) Toxic compounds, including detergents such as cholate, are avoided. Moreover, the production method can be easily scaled up and is suitable for GMP manufacture (i.e., in an endotoxin-free environment).

In accordance with the preferred method, the peptide and lipid are combined in a solvent system which co-

solubilizes each ingredient and can be completely removed by lyophilization. To this end, solvent pairs must be carefully selected to ensure co-solubility of both the amphipathic peptide and the lipid. In one embodiment, the protein(s) or peptide(s) to be incorporated into the particles can be dissolved in an aqueous or organic solvent or mixture of solvents (solvent 1). The (phospho)lipid component is dissolved in an aqueous or organic solvent or mixture of solvents (solvent 2) which is miscible with solvent 1, and the two solutions are mixed. Alternatively, the peptide and lipid can be incorporated into a co-solvent system; i.e., a mixture of the miscible solvents. A suitable proportion of peptide (protein) to lipids is first determined empirically so that the resulting complexes possess the appropriate physical and chemical properties; i.e., usually (but not necessarily) similar in size to HDL. The resulting mixture is frozen and lyophilized to dryness. Sometimes an additional solvent must be added to the mixture to facilitate lyophilization. This lyophilized product can be stored for long periods and will remain stable.

In the working examples describe infra, peptide 4 (SEQ ID NO:4) and phospholipids were dissolved separately in methanol, combined, then mixed with xylene before lyophilization. The peptide and lipid can both be added to a mixture of the two solvents. Alternatively, a solution of the peptide dissolved in methanol can be mixed with a solution of lipid dissolved in xylene. Care should be taken to eliminate salt from the solvent system in order to avoid salting out the peptide. The resulting solution containing the peptide and lipid cosolubilized in methanol/xylene is lyophilized to form a powder.

The lyophilized product can be reconstituted in order to obtain a solution or suspension of the peptide-lipid complex. To this end, the lyophilized powder is rehydrated with an aqueous solution to a suitable volume (often 5 mgs peptide/ml which is convenient for intravenous injection).

In a preferred embodiment the lyophilized powder is rehydrated with phosphate buffered saline or a physiological saline solution. The mixture may have to be agitated or vortexed to facilitate rehydration, and in most cases, the reconstitution step should be conducted at a temperature equal to or greater than the phase transition temperature of the lipid component of the complexes. Within minutes, a clear preparation of reconstituted lipid-protein complexes results.

An aliquot of the resulting reconstituted preparation can be characterized to confirm that the complexes in the preparation have the desired size distribution; e.g., the size distribution of HDL. Gel filtration chromatography can be used to this end. In the working examples described infra, a Pharmacia Superose 6 FPLC gel filtration chromatography system was used. The buffer used contains 150 mM NaCl in 50 mM phosphate buffer, pH 7.4. A typical sample volume is 20 to 200 microliters of complexes containing 5 mgs peptide/ml. The column flow rate is 0.5 mls/min. A series of proteins of known molecular weight and Stokes' diameter as well as human HDL are used as standards to calibrate the column. The proteins and lipoprotein complexes are monitored by absorbance or scattering of light of wavelength 254 or 280 nm.

The ApoA-I agonists of the invention can be complexed with a variety of lipids, including saturated, unsaturated, natural and synthetic lipids and/or phospholipids. Suitable lipids include, but are not limited to, small alkyl chain phospholipids, egg phosphatidylcholine, soybean phosphatidylcholine, dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine, distearoylphosphatidylcholine 1-myristoyl-2-palmitoylphosphatidylcholine, 1-palmitoyl-2-myristoylphosphatidylcholine, 1-palmitoyl-2-stearoylphosphatidylcholine, 1-stearoyl-2-palmitoylphosphatidylcholine, dioleoylphosphatidylcholine dioleophosphatidylethanolamine, dilauroylphosphatidylglycerol

phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, sphingolipids, phosphatidylglycerol, diphosphatidylglycerol, dimyristoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, distearoylphosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoylphosphatidic acid, dipalmitoylphosphatidic acid, dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, dimyristoylphosphatidylserine, dipalmitoylphosphatidylserine, brain phosphatidylserine, brain sphingomyelin, dipalmitoylsphingomyelin, distearoylsphingomyelin, phosphatidic acid, galactocerebroside, gangliosides, cerebrosides, dilaurylphosphatidylcholine, (1,3)-D-mannosyl-(1,3)diglyceride, aminophenylglycoside, 3-cholesteryl-6'-(glycosylthio)hexyl ether glycolipids, and cholesterol and its derivatives.

The Applicants have discovered that when the ApoA-I agonists of the invention are complexed with sphingomyelin, all of the HDL of the pre-β-like particles is removed. Accordingly, in a preferred embodiment of the invention, the ApoA-I agonists are administered as a complex with sphingomyelin.

5.3.2 METHODS OF THE TREATMENT

The ApoA-I peptide agonists or peptide-lipid complexes of the invention may be administered by any suitable route that ensures bioavailability in the circulation. This can best be achieved by parenteral routes of administration, including intravenous (IV), intramuscular (IM), intradermal, subcutaneous (SC) and intraperitoneal (IP) injections. However, other routes of administration may be used. For example, absorption through the gastrointestinal tract can be accomplished by oral routes of administration (including but not limited to ingestion, buccal and sublingual routes) provided appropriate formulations (e.g.,

enteric coatings) are used to avoid or minimize degradation of the active ingredient, e.g., in the harsh environments of the oral mucosa, stomach and/or small intestine. Alternatively, administration via mucosal tissue such as vaginal and rectal modes of administration may be utilized to avoid or minimize degradation in the gastrointestinal tract. In yet another alternative, the formulations of the invention can be administered transcutaneously (e.g., transdermally), or by inhalation. It will be appreciated that the preferred route may vary with the condition, age and compliance of the recipient.

The actual dose of ApoA-I agonists or peptide-lipid complex used will vary with the route of administration, and should be adjusted to achieve circulating plasma concentrations of 100 mg/l to 2 g/l. Data obtained in animal model systems described herein show that the ApoA-I agonists of the invention associate with the HDL component, and have a projected half-life in humans of about five days. Thus, in one embodiment, the ApoA-I agonists can be administered by injection at a dose between 0.5 mg/kg to 100 mg/kg once a week. In another embodiment, desirable serum levels may be maintained by continuous infusion or by intermittent infusion providing about 0.5 mg/kg/hr to 100 mg/kg/hr.

Toxicity and therapeutic efficacy of the various ApoA-I agonists can be determined using standard pharmaceutical procedures in cell culture or experimental animals for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. ApoA-I peptide agonists which exhibit large therapeutic indices are preferred.

5.3.3 PHARMACEUTICAL FORMULATIONS

The pharmaceutical formulation of the invention contain the ApoA-I peptide agonist or the peptide-lipid complex as the active ingredient in a pharmaceutically acceptable carrier suitable for administration and delivery in vivo. As the peptides may contain acidic and/or basic termini and/or side chains, the peptides can be included in the formulations in either the form of free acids or bases, or in the form of pharmaceutically acceptable salts.

Injectable preparations include sterile suspensions, solutions or emulsions of the active ingredient in aqueous or oily vehicles. The compositions may also contain formulating agents, such as suspending, stabilizing and/or dispersing agent. The formulations for injection may be presented in unit dosage form, e.g., in ampules or in multidose containers, and may contain added preservatives.

Alternatively, the injectable formulation may be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, dextrose solution, etc., before use. To this end, the ApoA-I agonist may be lyophilized, or the co-lyophilized peptide-lipid complex may be prepared. The stored preparations can be supplied in unit dosage forms and reconstituted prior to use in vivo.

For prolonged delivery, the active ingredient can be formulated as a depot preparation, for administration by implantation; e.g., subcutaneous, intradermal, or intramuscular injection. Thus, for example, the active ingredient may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives; e.g., as a sparingly soluble salt form of the ApoA-I agonist.

Alternatively, transdermal delivery systems manufactured as an adhesive disc or patch which slowly releases the active ingredient for percutaneous absorption

may be used. To this end, permeation enhancers may be used to facilitate transdermal penetration of the active ingredient. A particular benefit may be achieved by incorporating the ApoA-I agonists of the invention or the peptide-lipid complex into a nitroglycerin patch for use in patients with ischemic heart disease and hypercholesterolemia.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in

conventional manner. For rectal and vaginal routes of administration, the active ingredient may be formulated as solutions (for retention enemas) suppositories or ointments.

For administration by inhalation, the active ingredient can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

5.4 OTHER USES

The ApoA-I agonists of the invention can be used in assays in vitro to measure serum HDL, e.g., for diagnostic purposes. Because the ApoA-I agonists associate with the HDL component of serum, the agonists can be used as "markers" for the HDL population. Moreover, the agonists can be used as markers for the subpopulation of HDL that are effective in RCT. To this end, the agonist can be added to or mixed with a patient serum sample; after an appropriate incubation time, the HDL component can be assayed by detecting the incorporated ApoA-I agonist. This can be accomplished using labeled agonist (e.g., radiolabels, fluorescent labels, enzyme labels, dyes, etc.), or by immunoassays using antibodies (or antibody fragments) specific for the agonist.

Alternatively, labeled agonist can be used in imaging procedures (e.g., CAT scans, MRI scans) to visualize the circulatory system, or to monitor RCT, or to visualize accumulation of HDL at fatty streaks, atherosclerotic lesions, etc. (where the HDL should be active in cholesterol efflux).

6. EXAMPLE: SYNTHESIS OF PEPTIDE AGONISTS OF ApoA-I

The peptides described in TABLE X (Section 8.3, infra) were synthesized and characterized as described in the subsections below. The peptides were also analyzed structurally and functionally as described in Sections 7 and 8, infra.

6.1 SYNTHESIS OF CORE PEPTIDES

Peptides were synthesized on solid phase according to the Merrifield technique (Merrifield, 1969, J. Am. Chem. Soc. 85:2149-2154) using 0.25 mmol ρ-alkoxybenzylalcohol resin (HMP resin) (Wang, 1973, J. Am. Chem. Soc. 95:1328-1333) and Fmoc chemistry. All syntheses were carried out on an Applied Biosystems ABI model 430A automated peptide synthesizer (Perkin-Elmer, Foster City, CA). The solvation and activation times used for each coupling cycle are shown in TABLE V below:

TABLE V

SINGLE COUPLE ACTIVATOR CYCLES

CYCLE NAME	DESIGNATED AMINO ACIDS	DISSOLVING SOLVENT	TIME	ACTIVATION TIME	TRANSFER TIMES*
afmc 31	Asn(trt), His(trt), Lys(Boc),Trp	~0.4ml DCM ~1.2ml NMP ~1.0ml HOBt/NMP	~7 min.	~51 min.	1=50 sec. 2=36 sec.
afmc 32	Arg(Pmc), Gln(trt),Aib	~0.8ml DCM ~1.2ml NMP ~1.0ml HOBt/NMP	~32 min.	~51 min.	1=60 sec. 2=40 sec.
afmc 33	Ala,Asp(OtBu), Glu(OtBu),Gly, Ile,Leu, Met,Phe,Pro	~0.4ml DCM ~0.8ml NMP ~0.1ml HOBt/NMP	~4 min.	~36.5 min.	1=38 sec. 2=27 sec.
afmc 34	Val	~0.4ml DCM ~0.8ml NMP ~0.1ml HOBt/NMP	~4 min.	~61.5 min.	1=38 sec. 2=27 sec.

* 1=Transfer from Cartridge to Activator.
2=Transfer from Activator to Cartridge.

DCC is dicyclohexylcarbodiimide
HOBt is 1-hydroxybenzotriazole
NMP is N-methylpyrrolidone
BOC is t-butyloxycarbonyl
Pmc is pentamethylchroman-6-sulfonyl
OtBu is t-butyl ester
trt is trityl

The resins were washed with NMP between each coupling step. The protocol for one synthesis cycle is shown below in TABLE VI:

TABLE VI

COUPLING PROTOCOL FOR ONE SYNTHESIS CYCLE

	OPERATION	TIME (min.)
1.	Deprotection (10% piperdine in NMP)	20
2.	Wash (NMP)	5
3.	Couple (4 equiv. Fmoc-amino acid-HOBT ester in NMP, preactivated 50 min.)	61
4.	Wash	3
5.	Resin Sample (optional)	3
	TOTAL	92

All amino acids except Fmoc-β-(1-naphthyl)alanine were coupled in this manner. Fmoc-β-(1-naphthyl)alanine was coupled manually. For manual coupling, 1 mmol Fmoc-β-(1-naphthyl)alanine and 1 mmol 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) were dissolved in 5 ml NMP and mixed with the peptide-resin.

Thereafter, 2 mmol of N-ethyldiisopropylamine were added, the mixture shaken for 2 hours and the peptide-resin washed 6 times with 10 ml NMP. The coupling efficiency was monitored using the Kaiser Test (Kaiser, 1970, Anal. Biochem. 34:59577), and the coupling repeated if necessary. After coupling of naphthylalanine, the remainder of the synthesis was performed automatically as described above.

6.2 SYNTHESIS OF PEPTIDE AMIDES

Where indicated in TABLE X (Section 8.3, infra), peptide amides were synthesized using a Rink amide resin containing the Fmoc-Rink amide handle 4-(2',4'-dimethylphenyl)-Fmoc-phenoxymethyl (Rink, 1987, Tetrahedron Lett. 28:3787-3790) and the synthesis protocols described in Section 6.1, supra.

6.3 SYNTHESIS OF N-TERMINAL ACYLATED PEPTIDES

Where indicated in TABLE X (Section 8.3, infra), N-terminal acylated forms of the peptides were prepared by exposing the resin-bound peptide prepared as described in Section 6.1 or 6.2, supra, to an appropriate acylating agent.

For N-terminal acetylated peptides, 15 ml of acetic anhydride solution (10% v/v in NMP) was added to each 1 g of resin-bound peptide, the mixture shaken for 5 min. and the resin recovered by filtration. The recovered resin was washed three times with NMP (15 ml) and three times with ethanol (15 ml).

6.4 CLEAVAGE AND DEPROTECTION

Following synthesis, the peptides described in Sections 6.1, 6.2 and 6.3, supra, were cleaved from the resin

and deprotected with a cleavage solution containing 92.5% trifluroacetic acid (TFA)/3.75% anisole/3.75% dodecanthiol (v/v/v). To effect cleavage, 10 ml of cleavage solution was added to 0.25 mmol peptide resin and stirred for 1.5 hours at room temperature. The resin was removed via filtration and the cleaved/deprotected peptide precipitated with diethyl ether, washed with ether and dried in vacuo.

The cleavage cocktail for peptides containing Trp (W), as well as for peptide amides, was composed of 86.5% TFA, 4.5% H_2O, 4.5% 1,2-ethanedithiol, 4.5% anisole and 3% phenol.

6.5 PURIFICATION

The crude, cleaved peptides of Section 6.4 were purified by reverse phase HPLC. The purity of each peptide was confirmed by different analytical techniques (analytical HPLC, capillary electrophoresis). Capillary electrophoreses were carried out on fused silica capillaries of 70 cm length and an internal diameter of 75 μm (Thermo Separation Products). The separations were performed at 25°C, 15 kV, run time 35 min., in two different buffer systems: Buffer 1 (20 mM $Na_2B_4O_7$, pH 9.2) and Buffer 2 (10 mM Na_2HPO_4, pH 2.5). HPLC separations were carried out on Nucleosil 7C18 or Nucleosil 7C4 columns (Macherey and Nagel, Germany), 250 x 21 mm, at a flow rate of 8 ml/min. The gradient elution was performed using a mixture of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). The gradients used were adjusted to meet the needs of each peptide.

6.6 CHARACTERIZATION

The mass and amino acid analysis of the purified peptides described in Section 6.5 were confirmed via mass spectrometry and amino acid analysis, respectively, as described below. Edman degradation was used for sequencing.

6.6.1 LC-MS

A standard commercially available triple stage quadruple mass spectrometer (model TSQ 700; Finnigan MAT, San Jose CA, USA) was used for mass determination. A pneumatically assisted electrospray (ESI) interface was used for sample introduction to the atmospheric pressure ionization source of the mass spectrometer. The interface sprayer was operated at a positive potential of 4.5 kV. The temperature of the steel capillary was held at 200°C whereas the manifold was at 70°C. Positive ions generated by this ion evaporation process entered the analyzer of the mass spectrometer. The multiplier was adjusted to 1000 V. The analyzer compartment of the mass spectrometer was at 4E-6. All acquisitions were performed at resolution < 1u.

Peptides were analyzed by direct infusion of the purified peptides using an ABI (Applied Biosystems) microbore system consisting of a syringe pump (model 140B), an UV detector (model 785A) and an oven/injector (model 112A). The solvent system consisted of water (solvent A) and acetonitrile (solvent B), each containing 0.1% TFA. Peptides were infused using either a gradient or isocratic conditions and eluted from an Aquapore C18 column. The flow rate was typically 300 µl/min. Concentration of each peptide was about 0.03 mg/ml, 20 µl of which was injected (e.g., 30 pmol).

Full scan MS experiments were obtained by scanning quadruple 1 from m/z 500-1500 in 4s. Data were acquired using an Alpha DEC station and were processed using the software package provided by Finnigan MAT (BIOWORKS).

6.6.2 AMINO ACID ANALYSIS

Amino acid analysis was performed on an ABI (Applied Biosystems) 420 Amino Acid Analyzer. This system consists of three modules: a hydrolysis and derivatisation instrument, a reverse-phase HPLC and a data system. Peptide sample were applied (3 times in triplicate) on porous glass

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slides and subsequently hydrolyzed under gas phase conditions (155° C, 90 min.). After removal of the HCL, the resulting amino acids were converted to PTC-AA (Phenylthiocarbamoyl-amino acids) using PITC (Phenylisothiocyanate). After transfer to the HPC sample loop the resulting mixtures were fractionated on an Aquapore C18 column using the gradient mode (Solvent A: 50 mmol ammonium acetate (NH_4Ac), pH 5.4, in water; Solvent B: 32 mmol of sodium acetate (NaOAc) in aqueous acetonitrile) under conditions of temperature control. The HPLC data were processed by the software package provided by Applied Biosystems. Quantification was performed relative to a peptide standard delivered by Applied Biosystems.

6.7 SYNTHESIS OF BRANCHED NETWORKS

Tetrameric-core peptidyl resin and trimeric-core peptidyl resin are synthesized as described in Demoor et al., 1996, Eur. J. Biochem. 239:74-84. The tetrameric and trimeric core matrix still linked to the 4-methyl benzhydrylamine resin is then used as initial peptidyl-resin for automated synthesis of core peptides as previously described.

Branched networks containing helical segments of different amino acid compositions can be synthesized using orthogonal synthesis and protecting strategies well known in the art.

7. EXAMPLE: STRUCTURAL AND LIPID BINDING ANALYSIS OF ApoA-I PEPTIDES

The structural and lipid binding characteristics of the purified peptides synthesized as described in Section 6, supra, were determined by circular dichroism (CD), fluorescence spectroscopy and nuclear magnetic resonance (NMR).

7.1 CIRCULAR DICHROISM

This Example describes a preferred method for determining the degree of helicity of the core peptides of the invention both free in buffer and in the presence of lipids.

7.1.1 EXPERIMENTAL METHOD

Far UV circular dichroism spectra were recorded between 190 and 260 nm (in 0.5 nm or 0.2 nm increments) with a AVIV62DS spectrometer (AVIV Associates, Lakewood, NJ, USA) equipped with a thermoelectric cell holder and sample changer. The instrument was calibrated with (+)-10-camphoric acid. Between one and three scans were collected for each sample, using 10 cm, 5 cm, 1 cm and 0.1 cm path length quartz Suprasil cells, respectively, for peptide concentrations of 10^{-7} M to 10^{-4} M. The bandwidth was fixed at 1.5 nm and the scan speed to 1s per wavelength step. The reported data are the mean of at least 2 or 3 independent measurements.

After background substraction, spectra were converted to molar ellipticity (θ) per residue in deg. cm^{-2} $dmol^{-1}$. The peptide concentration was determined by amino acid analysis and also by absorption spectrometry on a Perkin Elmer Lambda 17 UV/Visible spectrophotometer when the peptide contained a chromophore (tryptophane, dansyl, naphtylalanine).

CD spectra were obtained with free, unbound peptide (5 μM in 5 mM phosphate buffer, pH 7.4); with peptide-SUV complexes (20:1 EPC:Chol., Ri =30 and Ri =50); with peptide-micelle complexes (1-myristoyl-2-hydroxy-sn-glycero-3-phosphatidyl choline, Ri=100); and with free, unbound peptide in the presence of 2,2,2-trifluoroethanol (TFE) (5 μM peptide, 90% vol TFE).

The SUVs were obtained by dispersing the lipids (10 mM, 20:1 EPC:Chol., Avanti Polar Lipids, AL) in phosphate buffer (5 mM, pH 7.4) with bubbling N_2 for 5 min., followed by

sonication (1.5 hr.) in a bath sonicator. The homogeneity of the preparation was checked by FPLC.

The micelles were obtained by dispersing the lipid (6 mM 1-myristoyl-2-hydroxy-sn-glycero-3-phosphatidyl choline, Avanti Polar Lipids, AL) in phosphate buffer (5 mM, pH 7.4) with bubbling N_2 for 5 min., followed by vortexing.

To obtain the peptide-SUV complexes, SUVs were added to the peptide (5 μM in 5 mM phosphate buffer, pH 7.4) at a phospholipid-peptide molar ratio (Ri) of 30 or 50.

To obtain the peptide-micelle complexes, micelles were added to the peptide (5 μM in 5 mM phosphate buffer, pH 7.4) at a Ri of 100.

All spectra were recorded at 37°C. The stability of peptide 4 (SEQ ID NO:4) as a function of temperature (both free in buffer and in micelles) was determined by recording spectra at a series of different temperatures.

The degree of helicity of peptide 4 (SEQ ID NO:4) as a function of concentration was also determined.

7.1.2 HELICITY DETERMINATION

The degree of helicity of the peptides in the various conditions was determined from the mean residue ellipticity at 222 nm (Chen et al., 1974, Biochemistry 13:3350-3359) or by comparing the CD spectra obtained to reference spectra available on databases (16 helical reference spectra from Provencher & Glockner, 1981, Biochemistry 20:33-37; denatured protein reference spectra from Venyaminov et al., 1993, Anal. Biochem. 214:17-24) using the CONTIN curve-fitting algorithm version 2DP, CD-1 pack (Aug. 1982) (Provencher, 1982, Comput. Phys. Commun. 27:213-227, 229-242). Acceptable fit was determined using the statistical analysis methodology provided by the CONTIN algorithm. The error of all methods was ±5% helicity.

7.1.3 RESULTS

The degree of helicity (%) of the free, unbound peptides (free), the peptide-SUV complexes (SUVs), the peptide-micelle complexes (mics) and the peptide-TFE solution (TFE) are reported in TABLE X, Section 8.3, infra. The degree of helicity of peptide 4 (SEQ ID NO:4) as a function of concentration (free and bound to lipids) is provided in TABLE VII.

TABLE VII

% HELICITY OF PEPTIDE 4 (SEQ ID NO:4) AS A FUNCTION OF CONCENTRATION

Concentration (μM)	Free	% Helicity Bound (micelles)	TFE
0.15	42	65	-
0.40	58	68	-
2.0	63	-	-
5.0	80	95	-
50.0	-	-	94

Peptide 4 (SEQ ID NO:4) contains significant α-helical structure (80% helicity) in buffer at a concentration of 5 μM. While the degree of helicity decreases with decreasing peptide concentration, significant helicity (42%) is maintained even at concentrations as low as 0.15 μM. Moreover, the α-helical structure is completely stable over a temperature range of 5-45°C (data not shown).

The helicity of peptide 4 (SEQ ID NO:4) increases in the presence of both SUVs (97% helicity) and micelles (95% helicity), and also in the presence TFE (94% helicity), which is a solvent that, due to having a significantly lower dielectric constant (ϵ= 26.7) that water (ϵ= 78.4), stabilizes α-helices and intrapeptide hydrogen bonds at concentrations between 5-90% (v/v).

Referring to TABLE X, Section 8.3, infra, it can be seen that those peptides which exhibit a high degree of LCAT activation (≥38%) generally possess significant α-helical structure in the presence of lipids (≥60% helical structure in the case of unblocked peptides containing 22 or more amino acids or blocked peptides containing 18 or fewer amino acids; ≥40% helical structure in the case of unblocked peptides containing 18 or fewer amino acids), whereas peptides which exhibit little or no LCAT activation possess little α-helical structure. However, in some instances, peptides which contain significant α-helical structure in the presence of lipids do not exhibit significant LCAT activation. As a consequence, the ability of the core peptides of the invention to adopt an α-helical structure in the presence of lipids is considered a critical feature of the core peptides of the invention, as the ability to form an α-helix in the presence of lipids appears to be a prerequisite for LCAT activation.

7.2 FLUORESCENCE SPECTROSCOPY

The lipid binding properties of the peptides synthesized in Section 6, *supra*, were tested by fluorescence measurements with labeled peptides, in the present case Tryptophane (Trp or W) or Naphtylalanine (Nal). The fluorescence spectra were recorded on a Fluoromax from Spex (Jobin-Yvon) equipped with a Xenon lamp of 150W, two monochromators (excitation and emission), a photomultiplier R-928 for detection sensitive in the red up to 850 nm and a thermoelectric magnetic stirred cell holder. Quartz Suprasil cuvettes were used for measurements in the micromolar concentration range. A device of variable slits (from 0.4 to 5 nm) allows modulation of the incident and emitted intensities according to the concentration of peptide used. The reported values are in general the average of between 2 to 4 spectra. The peptide concentration is determined by absorption spectrometry on a Philips PU 8800 using the

absorption band of the Trp ($\epsilon_{280\ nm}$=5,550 $M^{-1}cm^{-1}$ in Tris buffer) or the Nal ($\epsilon_{224\ nm}$=92,770 $M^{-1}cm^{-1}$ in methanol).

Fluorescence spectra of the peptides were recorded between 290 nm and 450 nm in Tris-HCl buffer (20 mM, pH = 7.5), in the presence and absence of lipidic vesicles. The small unilamellar vesicles were formed after rehydration in buffer of the lyophilized phospholipids, dispersion and tip sonification under a N_2 stream. The lipids used were either Egg PC/Chol. (20:1) or POPC/Chol. (20:1). The spectra were recorded at a peptide concentration of 2μM and at a temperature of 37°C. The fluorescence reference standard in the case of Trp was N-acetyltryptophanylamide (NATA).

Lipid binding studies were done through progressive lipidic vesicle addition to the peptide in solution at 2 μM (slits:5nm in excitation and 1.5 nm in emission). Dilution effects were taken into account for the fluorescence intensity determination. The lipid concentrations were varied from 10 to 600 μM and the molar ratio of lipid to peptide (Ri) was varied from 5 to 300. The wavelength of excitation was set at 280 nm for both Trp and Nal.

7.2.1 FLUORESCENCE SPECTRAL ANALYSIS

The data were directly recorded and treated by an IBM-PC linked to the spectrofluorimeter through the DM3000F software from Spex. The spectra were corrected by substraction of the solvent contribution and by application of a coefficient given by the constructor taking into account the variation of the photomultiplier response versus the wavelength.

The fluorescence spectra of the peptides were characterized by the wavelength at their maximum of fluorescence emission and by their quantum yield compared to NATA in the case of peptides labeled with a tryptophane. The process of binding to lipids was analyzed by calculating the shift of the wavelength at the maximum of fluorescence emission, (λ_{max}), and the variation of the relative

fluorescence intensity of emission versus the lipid concentration. The relative fluorescence intensity is defined as the following ratio: $(I-I_0)_{\lambda max}/I_{0\lambda max}$. I and I_0 are both measured at the (λ_{max}) corresponding to the initial free state of the peptide, i.e., without lipids. I is the intensity at a defined lipid to peptide ratio and I_0 is the same parameter measured in absence of lipids. The absence of these variations is relevant of the absence of interactions of the peptides with the lipids.

7.2.2 RESULTS AND DISCUSSION

The lipid binding properties of peptide 15 (SEQ ID NO:15) (which is peptide 4 containing a Trp residue at position 10) are presented in TABLE VIII.

TABLE VIII

BINDING PROPERTIES OF PEPTIDE 15 (SEQ ID NO:15) TO LIPIDIC VESICLES AS MEASURED BY FLUORESCENCE

Lipid:Peptide Molar Ratio (Ri)	I/I_o	λ_{max} (nm)
0	0	332
5	10.8	323
10	13.2	323.5
30	17.5	323
100	26.4	323
200	43.5	323

The maximum of the tryptophane emission (λ_{max}) at 332 nm indicates that in the buffer at a concentration of 2μM the peptide is slightly self-associated. The peptide binds to the lipidic vesicles (EPC/Chol 5%) with a very high affinity as demonstrated by the burying of the Trp (the maximum of the Trp emission wavelength shifts from 332 nm down to 323 nm) and the high fluorescence intensity exaltation (see TABLE VIII). The maximum burying of the

Tryptophane residue is obtained for a very low lipid to peptide molar ratio lower than 5.

Other peptides which exhibited a high degree of helicity in the presence of lipids (≥60% for unblocked peptides of ≥ 22 amino acids, or blocked peptides of ≤ 18 amino acids; ≥ 40% for unblocked peptides of ≤ 18 amino acids) as measured by circular dichroism as disclosed in Section 7.1, supra, also demonstrated good lipid binding. Of course, among all the peptides selected by the circular dichroism screening, only the ones that could be followed by fluorescence were tested for their lipid binding properties.

7.3 NUCLEAR MAGNETIC RESONANCE (NMR)

This Example describes an NMR method for analyzing the structure of the core peptides of the invention.

7.3.1 NMR SAMPLE PREPARATION

Samples were prepared by dissolving 5 mg of peptide in 90% H_2O/10% D_2O containing trace amounts of 2,2-Dimethyl-2-sila-5-pentane sulfonate (DSS) as an internal chemical shift reference. Some of the samples contained trifluoroethanol (TFE) (expressed as % vol). The total sample volume was 500 µl and the concentration of peptide was approximately 5 mM.

7.3.2 NMR SPECTROSCOPY

^1H NMR spectra were acquired at 500 MHz using a Bruker DRX500 spectrometer equipped with a B-VT2000 temperature control unit. One and two-dimensional experiments were recorded using standard pulse sequences. (Two Dimensional NMR Spectroscopy, Eds. W.R. Croasmun and RMK Carlson, 1994, VCH Publishers, New York, USA). Water suppression was achieved with low power presaturation for 2 sec. Two-dimensional experiments were carried out in the phase sensitive mode using time proportional phase incrementation (TPPI) and a spectral width of 6000 Hz in both dimensions. Typically, 40 scans were co-added for 400 t_1

increments with 2048 data points. Data were processed using FELIX95 software (Molecular Simulations) on an INDIGO2 workstation (Silicon Graphics). Data were zero-filled to give a 2K x 2K data matrix and apodized by a 45° shifted squared sine-bell function.

7.3.3 NMR ASSIGNMENT

Complete proton resonance assignments were obtained by applying the sequential assignment technique using DQFCOSY, TOCSY and NOESY spectra as described in the literature (Wüthrich, NMR of Proteins and Nucleic Acids, 1986, John Wiley & Sons, New York, USA). Secondary chemical shifts were calculated for HN and Hα protons by subtracting the tabulated random coil chemical shifts (Wishart and Sykes, 1994, Method. Enz. 239:363-392) from the corresponding experimental values.

7.3.4 RESULTS AND DISCUSSION

General Consideration. Amphipathic helical peptides tend to aggregate in aqueous solutions at the high concentrations necessary for NMR spectroscopy, making it difficult to obtain high resolution spectra. For example, NMR spectra of exemplary core peptide 4 (SEQ ID NO:4) in water exhibit very broad lines. Thus, the resonances of each amino acid residue cannot be resolved. Addition of TFE to the sample improves the resolution of the spectra. TFE is known to solubilize peptides, and in addition stabilizes helical conformations of peptides having helical propensity. The findings from NMR spectroscopy are demonstrated for peptide 4 (SEQ ID NO:4) as a representative example. The consensus 22-mer of Segrest (SEQ ID NO:75) was studied in comparison.

Secondary chemical shifts. Proton chemical shifts of amino acids depend both on the type of residue and on the local secondary structure within a peptide or protein (Szlagyi, 1995, Progress in Nuclear Magnetic Resonance Spectroscopy 27:325-443). Therefore, identification of regular secondary structure is possible by comparing experimental shifts with tabulated values for random coil conformation.

Formation of an α-helix typically results in an upfield (negative) shift for the Hα resonance. Observation of an upfield Hα shift for several sequential residues is generally taken as evidence of a helical structure. The Hα secondary shifts for peptide 4 (SEQ ID NO:4) in 25% TFE at 295 K show a significant negative shift for residues 4 through 19 (FIG. 9A), demonstrating a highly helical conformation. Small differences are observed in the Hα chemical shifts of the consensus 22-mer (SEQ ID NO:75) compared to peptide 4 (SEQ ID NO:4).

The chemical shifts of amide hydrogens of amino acid residues residing in regions of α-helix are also shifted upfield with respect to the chemical shifts observed for random coil. In addition, a periodicity of the HN shifts can be observed, and it reflects the period of the helical turns. The amplitude of the shift variation along the sequence is related to the amphipathicity of a helical peptide. A higher hydrophobic moment leads to a more pronounced oscillation (Zhou et al., 1992, J. Am. Chem. Soc. 114:4320-4326). The HN secondary shifts for peptide 4 (SEQ ID NO:4) in 25% TFE at 295 K show an oscillatory behavior in agreement with the amphipathic nature of the helix (FIG. 9B). The amide chemical shift pattern of the consensus 22-mer peptide (SEQ ID NO:75) differs significantly from that of peptide 4 (SEQ ID NO:4). In particular, replacement of residues 5, 9 and 13 with Leu (L) results in chemical shifts with a more pronounced periodicity in the case of peptide 4 (SEQ ID NO:4). The effect even extends to residue 17, and to a

lesser extent to residue 21. From the NMR data, the existence of 5-6 turns in the sequence can be discerned. Thus, the replacement of three amino acids affects the folding of the peptide along the entire sequence. The NMR pattern clearly reflects the stronger amphipathic nature of peptide 4 (SEQ ID NO:4) as compared to the consensus 22-mer peptide of Segrest (SEQ ID NO:75).

The secondary shift of an amide proton is influenced by the length of the hydrogen bond to the carbonyl oxygen one turn away from the helix. Therefore, the periodicity of observed chemical shift values reflects different hydrogen bond lengths. This difference is associated with an overall curved helical shape of the helix backbone. The hydrophobic residues are situated on the concave side. The secondary shifts of peptide 4 (SEQ ID NO:4) indicate a curved α-helical conformation.

7.3.5 PEPTIDES CONTAINING INTERNAL GLYCINES ARE HELICAL IN THE PRESENCE OF TFE

The three-dimensional structure of peptide 8 (SEQ ID NO:8) was determined in the presence of TFE using Nuclear Overhauser Effects (NOEs) derived interproton distance constraints. In particular, the presence of medium range NOE's ($d\alpha N_3$, $d\alpha\beta_3$, daN_4) along the entire sequence are consistent with an overall α-helical structure. A family of conformers was generated from the NMR data set and the structures superimpose well from residue 4 to 19 with a backbone RMSD < 0.8 Å. A stereo ribbon representation of the average structure together with the backbone trace of the 15 lowest energy conformers confirm a curved helical shape. In an approximate estimation, the bend results in a 20° angle between the N-terminal and the C-terminal half of the peptide. The hydrophobic residues are mainly found on the concave side with the exception of Leu-5. A well defined hydrophobic cluster is centered around Phe-6 including Leu-3, Leu-9 and Leu-10. While the helical turns start around

residue 3 at the N-terminus, the existence of many NOE's up to residue 22 indicate that the helix extends towards the end of the C-terminus.

8. EXAMPLE: LCAT ACTIVATION ASSAY

The peptides synthesized as described in Section 6, supra, were analyzed in vitro for their ability to activate LCAT. In the LCAT assay, substrate vesicles (small unilamellar vesicles or "SUVs") composed of egg phophatidylcholine (EPC) or 1-Palmitoyl-2-oleyl-phosphatidyl-choline (POPC) and radiolabelled cholesterol are preincubated with equivalent masses either of peptide or ApoA-I (isolated from human plasma). The reaction is initiated by addition of LCAT (purified from human plasma). Native ApoA-I, which was used as positive control, represents 100% activation activity. "Specific activity" (i.e., units of activity (LCAT activation)/unit of mass) of the peptides can be calculated as the concentration of peptide that achieves maximum LCAT activation. For example, a series of concentrations of the peptide (e.g., a limiting dilution) can be assayed to determine the "specific activity" for the peptide -- the concentration which achieves maximal LCAT activation (i.e., percentage conversion of cholesterol to cholesterol ester) at a specific timepoint in the assay (e.g., 1 hr.). When plotting percentage conversion of cholesterol at, e.g., 1 hr., against the concentration of peptide used, the "specific activity" can be identified as the concentration of peptide that achieves a plateau on the plotted curve.

8.1 PREPARATION OF SUBSTRATE VESICLES

The vesicles used in the LCAT assay are SUVs composed of egg phosphatidylcholine (EPC) or 1-palmitoyl-2-oleyl-phosphatidylcholine (POPC) and cholesterol with a molar ratio of 20:1. To prepare a vesicle stock solution sufficient for 40 assays, 7.7 mg EPC (or 7.6 mg POPC; 10 μmol), 78 μg (0.2 μmol) 4-^{14}C-cholesterol, 116 μg

cholesterol (0.3 μmol) are dissolved in 5 ml xylene and lyophilized. Thereafter 4 ml of assay buffer is added to the dry powder and sonicated under nitrogen atmosphere at 4°C. Sonication conditions: Branson 250 sonicator, 10 mm tip, 6 x 5 minutes; Assay buffer: 10 mM Tris, 0.14 M NaCl, 1 mM EDTA, pH 7.4). The sonicated mixture is centrifuged 6 times for 5 minutes each time at 14,000 rpm (16,000x g) to remove titanium particles. The resulting clear solution is used for the enzyme assay.

8.2 PURIFICATION OF LCAT

For the LCAT purification, dextran sulfate/Mg^{2+} treatment of human plasma is used to obtain lipoprotein deficient serum (LPDS), which is sequentially chromatographed on Phenylsepharose, Affigelblue, ConcanavalinA sepharose and anti-ApoA-I affinity chromatography, as summarized for a representative purification in TABLE IX, below:

TABLE IX

LCAT PURIFICATION

Fraction	Total Volume (ml)	Total Protein (mg)	Total Activity (nmol CE/mg*hr)	Yield (%)	Purification (fold)
Plasma	550	44550	63706		
LPDS	500	31000	62620	98	1.4
Phenyl sepharose	210	363	51909	82	100
Affigel blue	95	153	25092	39	115
ConA sepharose	43	36	11245	18	220
Anti-A-I Affinity	120	3.5	5500	9	1109

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8.2.1 PREPARATION OF LPDS

To prepare LPDS, 500 ml plasma is added to 50 ml dextran sulfate (MW=500000) solution. Stir 20 minutes. Centrifuge for 30 minutes at 3000 rpm (16,000 x g) at 4°C. Use supernatant (LPDS) for further purification (ca. 500 ml).

8.2.2 PHENYLSEPHAROSE CHROMATOGRAPHY

The following materials and conditions were used for the phenylsepharose chromatography.

solid phase:	Phenylsepharose fast flow, high subst. grade, Pharmacia
column:	XK26/40, gel bed height: 33 cm, V=ca. 175 ml
flow rates:	200 ml/hr (sample)
wash:	200 ml/hr (buffer)
elution:	80 ml/hr (distilled water)
buffer:	10 mM Tris, 140 mM NaCl, 1 mM EDTA pH7.4, 0.01% sodium azide.

Equilibrate the column in Tris-buffer, add 29 g NaCl to 500 ml LPDS and apply to the column. Wash with several volumes of Tris buffer until the absorption at 280 nm wavelength is approximately at the baseline, then start the elution with distilled water. The fractions containing protein are pooled (pool size: 180 ml) and used for Affigelblue chromatography.

8.2.3 AFFIGELBLUE CHROMATOGRAPHY

The Phenylsepharose pool is dialyzed overnight at 4°C against 20 mM Tris-HCl, pH7.4, 0.01% sodium azide. The pool volume is reduced by ultrafiltration (Amicon YM30) to 50 - 60 ml and loaded on an Affigelblue column.

solid phase:	Affigelblue, Biorad, 153-7301 column, XK26/20, gel bed height: ca. 13 cm; column volume: approx. 70 ml.
flow rates:	loading: 15 ml/h
	wash: 50 ml/h

Equilibrate column in Tris-buffer. Apply Phenylsepharose pool to column. Start in parallel to collect fractions.

Wash with Tris-buffer. The pooled fractions (170 ml) were used for ConA chromatography.

8.2.4 ConA CHROMATOGRAPHY

The Affigelblue pool was reduced via Amicon (YM30) to 30-40 ml and dialyzed against ConA starting buffer (1mM Tris HCl pH7.4; 1mM $MgCl_2$, 1mM $MnCl_2$, 1mM $CaCl_2$, 0.01% sodium azide) overnight at 4°C.

solid phase: ConA sepharose (Pharmacia)
column: XK26/20, gel bed height: 14 cm (75 ml)
flow rates: loading 40 ml/h
washing (with starting buffer): 90 ml/h
elution: 50 ml/h, 0.2M Methyl-α-D-mannoside in 1mM Tris, pH 7.4.

The protein fractions of the mannoside elutions were collected (110 ml), and the volume was reduced by ultrafiltration (YM30) to 44 ml. The ConA pool was divided in 2 ml aliquots, which are stored at -20°C.

8.2.5 ANTI-ApoA-I AFFINITY CHROMATOGRAPHY

Anti-ApoA-I affinity chromatography was performed on Affigel-Hz material (Biorad), to which the anti-ApoA-I abs have been coupled covalently.

column: XK16/20, V=16 ml. The column was equilibrated with PBS pH 7.4. Two ml of the ConA pool was dialyzed for 2 hours against PBS before loading onto the column.
flow rates: loading: 15 ml/hour washing (PBS) 40 ml/hour.

The pooled protein fractions (V=14 ml) are used for LCAT assays.

The column is regenerated with 0.1 M. Citrate buffer (pH 4.5) to elute bound A-I (100 ml), and immediately after this procedure reequilibrated with PBS.

8.3 RESULTS

The results of the LCAT activation assay are presented in TABLE X, infra.

TABLE X

LCAT ACTIVATION EXHIBITED BY EXEMPLARY CORE PEPTIDES

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
1	(SEQ ID NO:1)	PVLDLFRELLNELLEZLKQKLK	120%	77	85	81	69
2	(SEQ ID NO:2)	GVLDLFRELLNELLEALKQKLKK	105%				
3	(SEQ ID NO:3)	PVLDLFRELLNELLEWLKQKLK	98%	70	95	80	95
4	(SEQ ID NO:4)	PVLDLFRELLNELLEALKQKLK	93%	80	95	97	94
5	(SEQ ID NO:5)	pVLDLFRELLNELLEALKQKLKK	90%				
6	(SEQ ID NO:6)	PVLDLFRELLNEXLEALKQKLK	80%	57	93	70	99
7	(SEQ ID NO:7)	PVLDLFKELLNELLEALKQKLK	83%	77	89	85	73
8	(SEQ ID NO:8)	PVLDLFRELLNEGLEALKQKLK	83%	20	90	61	93
9	(SEQ ID NO:9)	PVLDLFRELGNELLEALKQKLK	83%				
10	(SEQ ID NO:10)	PVLDLFRELLNELLEAZKQKLK	79%	60	87	70	71
11	(SEQ ID NO:11)	PVLDLFKELLQELLEALKQKLK	72%				
12	(SEQ ID NO:12)	PVLDLFRELLNELLEAGKQKLK	70%				
13	(SEQ ID NO:13)	GVLDLFRELLNEGLEALKQKLK	67%				
14	(SEQ ID NO:14)	PVLDLFRELLNELLEALOQOLO	61%	70	96	80	
15	(SEQ ID NO:15)	PVLDLFRELWNELLEALKQKLK	60%	55	60	64	68
16	(SEQ ID NO:16)	PVLDLLRELLNELLEALKQKLK	59%				
17	(SEQ ID NO:17)	PVLELFKELLQELLEALKQKLK	59%				

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
18	(SEQ ID NO:18)	GVLDLFRELLNELLEALKQKLK	58%				
19	(SEQ ID NO:19)	pVLDLFRELLNEGLEALKQKLK	58%				
20	(SEQ ID NO:20)	PVLDLFREGLNELLEALKQKLK	57%				
21	(SEQ ID NO:21)	pVLDLFRELLNELLEALKQKLK	57%				
22	(SEQ ID NO:22)	PVLDLFRELLNELLEGLKQKLK	54%				
23	(SEQ ID NO:23)	PLLELFKELLQELLEALKQKLK	54%				
24	(SEQ ID NO:24)	PVLDLFRELLNELLEALQKKLK	53%				
25	(SEQ ID NO:25)	PVLDFFRELLNEXLEALKQKLK	51%	46	82		93
26	(SEQ ID NO:26)	PVLDLFRELLNELLELLKQKLK	47%				
27	(SEQ ID NO:27)	PVLDLFRELLNELZEALKQKLK	44%	72	92	82	81
28	(SEQ ID NO:28)	PVLDLFRELLNELWEALKQKLK	40%	82	98	90	81
29	(SEQ ID NO:29)	AVLDLFRELLNELLEALKQKLK	39%				
30	(SEQ ID NO:30)	PVLDLFRELLNELLEALKQKLK'	38%	85	90	98	90
31	(SEQ ID NO:31)	PVLDLFLELLNEXLEALKQKLK	34%	49	98		90
32	(SEQ ID NO:32)	XVLDLFRELLNELLEALKQKLK	33%				
33	(SEQ ID NO:33)	PVLDLFREKLNELLEALKQKLK	33%				
34	(SEQ ID NO:34)	PVLDZFRELLNELLEALKQKLK	32%	58	67	68	62
35	(SEQ ID NO:35)	PVLDWFRELLNELLEALKQKLK	31%	49 (sp)	59	61	
36	(SEQ ID NO:36)	PLLELLKELLQELLEALKQKLK	31%	95	100		95

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
37	(SEQ ID NO:37)	PVLDLFREWLNELLEALKQKLK	29%	65	75	76	73
38	(SEQ ID NO:38)	PVLDLFRELLNEXLEAWKQKLK	29%	25	49	21	49
39	(SEQ ID NO:39)	PVLDLFRELLEELLKALKKKLK	25%	66	69	68	72
40	(SEQ ID NO:40)	PVLDLFNELLRELLEALQKKLK	25%	66	84	79	77
41	(SEQ ID NO:41)	PVLDLWRELLNEXLEALKQKLK	25%	53	73	85	69
42	(SEQ ID NO:42)	PVLDEFREKLNEXWEALKQKLK	25%	15	74	27	76
43	(SEQ ID NO:43)	PVLDEFREKLWEXLEALKQKLK	25%				
44	(SEQ ID NO:44)	pvldefreklneXlealkqklk	25%	20	86		
45	(SEQ ID NO:45)	PVLDEFREKLNEXLEALKQKLK	24%	24	84	25	86
46	(SEQ ID NO:46)	PVLDLFREKLNEXLEALKQKLK	23%	30	86	58	85
47	(SEQ ID NO:47)	-VLDLFRELLNEGLEALKQKLK	23%				
48	(SEQ ID NO:48)	pvLDLFRELLNELLEALKQKLK	22%				
49	(SEQ ID NO:49)	PVLDLFRNLLEKLLEALEQKLK	22%	57	65	52	57
50	(SEQ ID NO:50)	PVLDLFRELLWEXLEALKQKLK	21%	68	84	89	76
51	(SEQ ID NO:51)	PVLDLFWELLNEXLEALKQKLK	20%	63	82	81	73
52	(SEQ ID NO:52)	PVWDEFREKLNEXLEALKQKLK	20%	sp	sp	sp	
53	(SEQ ID NO:53)	VVLDLFRELLNELLEALKQKLK	19%				
54	(SEQ ID NO:54)	PVLDLFRELLNEWLEALKQKLK	19%	76	71	84	78
55	(SEQ ID NO:55)	P---LFRELLNELLEALKQKLK	19%	38	72	78	75

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
56	(SEQ ID NO:56)	PVLDLFRELLNELLEALKQKKK	18%				
57	(SEQ ID NO:57)	PVLDLFRNLLEELLKALEQKLK	18%				
58	(SEQ ID NO:58)	PVLDEFREKLNEXLEALKQKL~	18%				
59	(SEQ ID NO:59)	LVLDLFRELLNELLEALKQKLK	17%				
60	(SEQ ID NO:60)	PVLDLFRELLNELLEALKQ~~~	16%	39	83	66	84
61	(SEQ ID NO:61)	PVLDEFRWKLNEXLEALKQKLK	16%				
62	(SEQ ID NO:62)	PVLDEWREKLNEXLEALKQKLK	16%	15	85	43	
63	(SEQ ID NO:63)	PVLDFFREKLNEXLEALKQKLK	16%				
64	(SEQ ID NO:64)	PWLDEFREKLNEXLEALKQKLK	15%				
65	(SEQ ID NO:65)	~VLDEFREKLNEXLEALKQKLK	15%				
66	(SEQ ID NO:66)	PVLDLFRNLLEELLEALQKKLK	15%	64	82	66	70
67	(SEQ ID NO:67)	~VLDLFRELLNELLEALKQKLK	14%	81	90	84	94
68	(SEQ ID NO:68)	PVLDEFRELLKEXLEALKQKLK	14%				
69	(SEQ ID NO:69)	PVLDEFRKKLNEXLEALKQKLK	13%				
70	(SEQ ID NO:70)	PVLDEFRELLYEXLEALKQKLK	12%	27	78	33	66
71	(SEQ ID NO:71)	PVLDEFREKLNELXEALKQKLK	11%				
72	(SEQ ID NO:72)	PVLDLFRELLNEXLWALKQKLK	11%	sp	sp	sp	
73	(SEQ ID NO:73)	PVLDEFWEKLNEXLEALKQKLK	10%				
74	(SEQ ID NO:74)	PVLDKFREKLNEXLEALKQKLK	10%				

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
75[1/]	(SEQ ID NO:75)	PVLDEFREKLNEELEALKQKLK	10%	18	28	23	55
76	(SEQ ID NO:76)	PVLDEFRELLFEXLEALKQKLK	9%	41	88		66
77	(SEQ ID NO:77)	PVLDEFREKLNKXLEALKQKLK	9%				
78	(SEQ ID NO:78)	PVLDEFRDKLNEXLEALKQKLK					
79	(SEQ ID NO:79)	PVLDEFRELLNELLEALKQKLK	9%				
80	(SEQ ID NO:80)	PVLDLFERLLNELLEALQKKLK	9%				
81	(SEQ ID NO:81)	PVLDEFREKLNWXLEALKQKLK					
82	(SEQ ID NO:82)	--LDEFREKLNEXLEALKQKLK	8%				
83	(SEQ ID NO:83)	PVLDEFREKLNEXLEALWQKLK					
84	(SEQ ID NO:84)	PVLDEFREKLNELLEALKQKLK	7%				
85	(SEQ ID NO:85)	P-LDLFRELLNELLEALKQKLK	7%	58	61	64	69
86	(SEQ ID NO:86)	PVLELFERLLDELLNALQKKLK	7%				
87	(SEQ ID NO:87)	pllellkellqellealkqklk	7%	100	100		100
88	(SEQ ID NO:88)	PVLDKFRELLNEXLEALKQKLK	7%				
89	(SEQ ID NO:89)	PVLDEFREKLNEXLWALKQKLK	6%				
90	(SEQ ID NO:90)	---DEFREKLNEXLEALKQKLK	6%				
91	(SEQ ID NO:91)	PVLDEFRELLNEXLEALKQKLK	6%	43	100		100

[1/] Segrest's Consensus 22-mer peptide (Anantharamaiah et al., 1990, Arteriosclerosis 10(1):95-105).

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
92	(SEQ ID NO:92)	PVLDEFRELYNEXLEALKQKLK	5%				
93	(SEQ ID NO:93)	PVLDEFREKLNEXLKALKQKLK	5%				
94[2/]	(SEQ ID NO:94)	PVLDEFREKLNEALEALKQKLK	5%	18	70	27	63
95	(SEQ ID NO:95)	PVLDLFRELLNLXLEALKQKLK	5%		sp	sp	
96	(SEQ ID NO:96)	pvldlfrellneXlealkqklk	5%	52	85	63	81
97	(SEQ ID NO:97)	PVLDLFRELLNELLE~~~~~~~	4%				
98	(SEQ ID NO:98)	PVLDLFRELLNEELEALKQKLK	2%				
99	(SEQ ID NO:99)	KLKQKLAELLENLLERFLDLVP	2%	72	88	80	80
100	(SEQ ID NO:100)	pvldlfrellnellealkqklk	2%	83	92		98
101	(SEQ ID NO:101)	PVLDLFRELLNWXLEALKQKLK	2%		sp	sp	
102	(SEQ ID NO:102)	PVLDLFRELLNLXLEALKEKLK	2%	sp			
103	(SEQ ID NO:103)	PVLDEFRELLNEELEALKQKLK	1%				
104	(SEQ ID NO:104)	P~~~~~~~LLNELLEALKQKLK	1%	21	49	29	55
105	(SEQ ID NO:105)	PAADAFREAANEAAEAAKQKAK	1%	29	28	32	65
106	(SEQ ID NO:106)	PVLDLFREKLNEELEALKQKLK	0%				
107	(SEQ ID NO:107)	klkqklaellenllerfldlvp	0%	sp	sp		77
108	(SEQ ID NO:108)	PVLDLFRWLLNEXLEALKQKLK	0%	28	55		54

[2/] [A^{11}]-Consensus 22-mer peptide (Anantharamaiah et al., 1990, Arteriosclerosis 10(1):95-105).

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
109[3/]	(SEQ ID NO:109)	PVLDEFREKLNERLEALKQKLK	0%	19	45	23	58
110	(SEQ ID NO:110)	PVLDEFREKLNEXXEALKQKLK	0%				
111	(SEQ ID NO:111)	PVLDEFREKLWEXWEALKQKLK	0%				
112	(SEQ ID NO:112)	PVLDEFREKLNEXSEALKQKLK	0%				
113	(SEQ ID NO:113)	PVLDEFREKLNEPLEALKQKLK	0%	6	22		
114	(SEQ ID NO:114)	PVLDEFREKLNEXMEALKQKLK	0%				
115	(SEQ ID NO:115	PKLDEFREKLNEXLEALKQKLK	0%				
116	(SEQ ID NO:116)	PHLDEFREKLNEXLEALKQKLK	0%				
117	(SEQ ID NO:117)	PELDEFREKLNEXLEALKQKLK	0%				
118	(SEQ ID NO:118)	PVLDEFREKLNEXLEALEQKLK	0%				
119	(SEQ ID NO:119)	PVLDEFREKLNEELEAXKQKLK	0%				
120	(SEQ ID NO:120)	PVLDEFREKLNEELEXLKQKLK	0%				
121	(SEQ ID NO:121)	PVLDEFREKLNEELEALWQKLK	0%				
122	(SEQ ID NO:122)	PVLDEFREKLNEELEWLKQKLK	0%				
123	(SEQ ID NO:123)	QVLDLFRELLNELLEALKQKLK					
124	(SEQ ID NO:124)	PVLDLFOELLNELLEALOQOLO					
125	(SEQ ID NO:125)	NVLDLFRELLNELLEALKQKLK					

[3/] [R[13]]-Consensus 22-mer peptide (Anantharamaiah et al., 1990, Arteriosclerosis 10(1):95-105).

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PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
126	(SEQ ID NO:126)	PVLDLFRELLNELGEALKQKLK					
127	(SEQ ID NO:127)	PVLDLFRELLNELLELLKQKLK	47%				
128	(SEQ ID NO:128)	PVLDLFRELLNELLEFLKQKLK					
129	(SEQ ID NO:129)	PVLELFNDLLRELLEALQKKLK					
130	(SEQ ID NO:130)	PVLELFNDLLRELLEALKQKLK					
131	(SEQ ID NO:131)	PVLELFKELLNELLDALRQKLK					
132	(SEQ ID NO:132)	PVLDLFRELLENLLEALQKKLK					
133	(SEQ ID NO:133)	PVLELFERLLEDLLQALNKKLK					
134	(SEQ ID NO:134)	PVLELFERLLEDLLKALNQKLK					
135	(SEQ ID NO:135)	DVLDLFRELLNELLEALKQKLK					
136	(SEQ ID NO:136)	PALELFKDLLQELLEALKQKLK					
137	(SEQ ID NO:137)	PVLDLFRELLNEGLEAZKQKLK					
138	(SEQ ID NO:138)	PVLDLFRELLNEGLEWLKQKLK					
139	(SEQ ID NO:139)	PVLDLFRELWNEGLEALKQKLK					
140	(SEQ ID NO:140)	PVLDLFRELLNEGLEALOQOLQ					
141	(SEQ ID NO:141)	PVLDFFRELLNEGLEALKQKLK					
142	(SEQ ID NO:142)	PVLELFRELLNEGLEALKQKLK					
143	(SEQ ID NO:143)	PVLDLFRELLNEGLEALKQKLK*					
144	(SEQ ID NO:144)	pVLELFENLLERLLDALQKKLK	111%	89	88		95

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
145	(SEQ ID NO:145)	GVLELFENLLERLLDALQKKLK	100%	55	51		58
146	(SEQ ID NO:146)	PVLELFENLLERLLDALQKKLK	86%	97	100	100	95
147	(SEQ ID NO:147)	PVLELFENLLERLFDALQKKLK	76%				
148	(SEQ ID NO:148)	PVLELFENLLERLGDALQKKLK	75%	10	76	23	80
149	(SEQ ID NO:149)	PVLELFENLWERLLDALQKKLK	63%	28	54		47
150	(SEQ ID NO:150)	PLLELFENLLERLLDALQKKLK	57%				
151	(SEQ ID NO:151)	PVLELFENLGERLLDALQKKLK	55%				
152	(SEQ ID NO:152)	PVFELFENLLERLLDALQKKLK	50%				
153	(SEQ ID NO:153)	AVLELFENLLERLLDALQKKLK	49%				
154	(SEQ ID NO:154)	PVLELFENLLERGLDALQKKLK	39%	13	76	25	80
155	(SEQ ID NO:155)	PVLELFLNLWERLLDALQKKLK	38%				
156	(SEQ ID NO:156)	PVLELFLNLLERLLDALQKKLK	35%				
157	(SEQ ID NO:157)	PVLEFFENLLERLLDALQKKLK	30%				
158	(SEQ ID NO:158)	PVLELFLNLLERLLDWLQKKLK	30%				
159	(SEQ ID NO:159)	PVLDLFENLLERLLDALQKKLK	28%				
160	(SEQ ID NO:160)	PVLELFENLLERLLDWLQKKLK	28%	65	73	75	61
161	(SEQ ID NO:161)	PVLELFENLLERLLEALQKKLK	27%				
162	(SEQ ID NO:162)	PVLELFENWLERLLDALQKKLK	27%	68	83	81	
163	(SEQ ID NO:163)	PVLELFENLLERLWDALQKKLK	26%	27	53		55

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
164	(SEQ ID NO:164)	PVLELFENLLERLLDAWQKKLK	24%	37	66	51	61
165	(SEQ ID NO:165)	PVLELFENLLERLLDLLQKKLK	23%				
166	(SEQ ID NO:166)	PVLELFLNLLEKLLDALQKKLK	22%				
167	(SEQ ID NO:167)	PVLELFENGLERLLDALQKKLK	18%				
168	(SEQ ID NO:168)	PVLELFEQLLEKLLDALQKKLK	17%				
169	(SEQ ID NO:169)	PVLELFENLLEKLLDALQKKLK	17%				
170	(SEQ ID NO:170)	PVLELFENLLEOLLDALQOOLO	17%				
171	(SEQ ID NO:171)	PVLELFENLLEKLLDLLQKKLK	16%				
172	(SEQ ID NO:172)	PVLELFLNLLERLGDALQKKLK	16%				
173	(SEQ ID NO:173)	PVLDLFDNLLDRLLDLLNKKLK	15%				
174	(SEQ ID NO:174)	pvlelfenllerlldalqkklk	13%				
175	(SEQ ID NO:175)	PVLELFENLLERLLELLNKKLK	13%				
176	(SEQ ID NO:176)	PVLELWENLLERLLDALQKKLK	11%				
177	(SEQ ID NO:177)	GVLELFLNLLERLLDALQKKLK	10%				
178	(SEQ ID NO:178)	PVLELFDNLLEKLLEALQKKLR	9%				
179	(SEQ ID NO:179)	PVLELFDNLLERLLDALQKKLK	8%				
180	(SEQ ID NO:180)	PVLELFDNLLDKLLDALQKKLR	8%				
181	(SEQ ID NO:181)	PVLELFENLLERWLDALQKKLK	8%				
182	(SEQ ID NO:182)	PVLELFENLLEKLLEALQKKLK	7%				

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
183	(SEQ ID NO:183)	PLLELFENLLEKLLDALQKKLK	6%				
184	(SEQ ID NO:184)	PVLELFLNLLERLLDAWQKKLK	4%				
185	(SEQ ID NO:185)	PVLELFENLLERLLDALQOOLO	3%				
186	(SEQ ID NO:186)	PVLELFEQLLERLLDALQKKLK					
187	(SEQ ID NO:187)	PVLELFENLLERLLDALNKKLK					
188	(SEQ ID NO:188)	PVLELFENLLDRLLDALQKKLK					
189	(SEQ ID NO:189)	DVLELFENLLERLLDALQKKLK					
190	(SEQ ID NO:190)	PVLEFWDNLLDKLLDALQKKLR					
191	(SEQ ID NO:191)	PVLDLLRELLEELKQKLK*	100%				
192	(SEQ ID NO:192)	PVLDLFKELLEELKQKLK*	100%	36			56
193	(SEQ ID NO:193)	PVLDLFRELLEELKQKLK*	96%	34	88	87	87
194	(SEQ ID NO:194)	PVLELFRELLEELKQKLK*	88%	38	93		93
195	(SEQ ID NO:195)	PVLELFKELLEELKQKLK*	87%				
196	(SEQ ID NO:196)	PVLDLFRELLEELKNKLK*	81%				
197	(SEQ ID NO:197)	PLLDLFRELLEELKQKLK*	81%	43	70		69
198	(SEQ ID NO:198)	GVLDLFRELLEELKQKLK*	80%				
199	(SEQ ID NO:199)	PVLDLFRELWEELKQKLK*	76%	35	77	80	79
200	(SEQ ID NO:200)	NVLDLFRELLEELKQKLK*	75%				
201	(SEQ ID NO:201)	PLLDLFKELLEELKQKLK*	74%				

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
202	(SEQ ID NO:202)	PALELFKDLLEELRQKLR*	70%				
203	(SEQ ID NO:203)	AVLDLFRELLEELKQKLK*	66%				
204	(SEQ ID NO:204)	PVLDFFRELLEELKQKLK*	63%				
205	(SEQ ID NO:205)	PVLDLFREWLEELKQKLK*	60%				
206	(SEQ ID NO:206)	PLLELLKELLEELKQKLK*	57%				
207	(SEQ ID NO:207)	PVLELLKELLEELKQKLK*	50%				
208	(SEQ ID NO:208)	PALELFKDLLEELRQRLK*	48%				
209	(SEQ ID NO:209)	PVLDLFRELLNELLQKLK	47%	54	71	67	62
210	(SEQ ID NO:210)	PVLDLFRELLEELKQKLK	46%	20	63	37	53
211	(SEQ ID NO:211)	PVLDLFRELLEELOQOLO*	45%				
212	(SEQ ID NO:212)	PVLDLFOELLEELOQOLK*	43%				
213	(SEQ ID NO:213)	PALELFKDLLEEFRQRLK*	42%				
214	(SEQ ID NO:214)	pVLDLFRELLEELKQKLK*	39%				
215	(SEQ ID NO:215)	PVLDLFRELLEEWKQKLK*	38%	28	63	53	68
216	(SEQ ID NO:216)	PVLELFKELLEELKQKLK	35%				
217	(SEQ ID NO:217)	PVLDLFRELLELLKQKLK	30%	52	78	76	70
218	(SEQ ID NO:218)	PVLDLFRELLNELLQKLK*	29%				
219	(SEQ ID NO:219)	PVLDLFRELLNELWQKLK	24%				
220	(SEQ ID NO:220)	PVLDLFRELLEELQKKLK	22%	27	64	54	64

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PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
221	(SEQ ID NO:221)	DVLDLFRELLEELKQKLK*	12%				
222	(SEQ ID NO:222)	PVLDAFRELLEALLQLKK	8%				
223	(SEQ ID NO:223)	PVLDAFRELLEALAQLKK	8%	21	56	23	51
224	(SEQ ID NO:224)	PVLDLFREGWEELKQKLK	8%				
225	(SEQ ID NO:225)	PVLDAFRELAEALAQLKK	1%				
226	(SEQ ID NO:226)	PVLDAFRELGEALLQLKK	1%				
227	(SEQ ID NO:227)	PVLDLFRELGEELKQKLK*	0%				
228	(SEQ ID NO:228)	PVLDLFREGLEELKQKLK*	0%				
229	(SEQ ID NO:229)	PVLDLFRELLEEGKQKLK*	0%				
230	(SEQ ID NO:230)	PVLELFERLLEDLQKKLK					
231	(SEQ ID NO:231)	PVLDLFRELLEKLEQKLK					
232	(SEQ ID NO:232)	PLLELFKELLEELKQKLK*					
237[4/]	(SEQ ID NO:237)	LDDLLQKWAEAFNQLLKK	11%	30	66	45	-
238[5/]	(SEQ ID NO:238)	EWLKAFYEKVLEKLKELF*	19%	49	72	60	58
239[6/]	(SEQ ID NO:239)	EWLEAFYKKVLEKLKELF*	11%	44	49		sp

[4/] ID-3 peptide (Labeur et al., 1997, Arteriosclerosis, Thrombosis and Vascular Biology 17(3):580-588).

[5/] Ac-18AMOD-C(O)NH_2 peptide (Epand et al., 1987, J. Biol. Chem. 262(19):9389-9396).

[6/] Ac-18AM4-C(O)NH_2 peptide (Brasseur, 1993, Biochim. Biophys. Acta 1170:1-7).

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PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
240	(SEQ ID NO:240)	DWLKAFYDKVAEKLKEAF*	10%	16	68	59	57
241	(SEQ ID NO:241)	DWFKAFYDKVFEKFKEFF	8%				
242[7/]	(SEQ ID NO:242)	GIKKFLGSIWKFIKAFVG	7%				
243	(SEQ ID NO:243)	DWFKAFYDKVAEKFKEAF	5%	10	64	50	
244[8/]	(SEQ ID NO:244)	DWLKAFYDKVAEKLKEAF	5%	9	40	13	48
245	(SEQ ID NO:245)	DWLKAFYDKVFEKFKEFF	4%	38	77	70	sp
246[9/]	(SEQ ID NO:246)	EWLEAFYKKVLEKLKELF	4%	18	44	47	
247	(SEQ ID NO:247)	DWFKAFYDKFFEKFKEFF	3%				
248[10/]	(SEQ ID NO:248)	EWLKAFYEKVLEKLKELF	3%	18	45	13	
249[11/]	(SEQ ID NO:249)	EWLKAEYEKVEEKLKELF*					
250[12/]	(SEQ ID NO:250)	EWLKAEYEKVLEKLKELF*					

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[7/] 18L peptide (Segrest et al., 1990, Proteins: Structure, Function and Genetics 8:103-117).

[8/] 18A peptide (Anantharamaiah et al., 1985, J. Biol. Chem. 260(18):10248-10255).

[9/] 18AM4 peptide (Rosseneu et al., WO93/25581; Corijn et al., 1993, Biochim. Biophys. Acta 1170:8-16).

[10/] [$Glu^{1,8}$; $Leu^{5,11,17}$]18A peptide (Epand et al., 1987, J. Biol. Chem. 262(19):9389-9396).

[11/] Ac-18AM3-C(O)NH_2 (Rosseneu et al., WO93/25581).

[12/] Ac-18AM2-C(O)NH_2 (Rosseneu et al., WO93/25581).

PEPTIDE		AMINO ACID SEQUENCE	ACTIVITY (%) LCAT	He (%) free	He (%) mics	He (%) SUVs	He (%) TFE
251[13/]	(SEQ ID NO:251)	EWLKAFYKKVLEKLKELF*					
252	(SEQ ID NO:252)	PVLDLFRELLEQKLK*					
253	(SEQ ID NO:253)	PVLDLFRELLEELKQK*					
254	(SEQ ID NO:254)	PVLDLFRELLEKLKQK*					
255	(SEQ ID NO:255)	PVLDLFRELLEKLQK*					
256	(SEQ ID NO:256)	PVLDLFRELLEALKQK*					
257	(SEQ ID NO:257)	PVLDLFENLLERLKQK*					
258	(SEQ ID NO:258)	PVLDLFRELLNELKQK*					

[13/] Ac-18AM1-C(O)NH_2 (Rosseneu et al., WO93/25581).

In TABLE X, * indicates peptides that are N-terminal acetylated and C-terminal amidated; ¹ indicates peptides that are N-terminal dansylated; sp indicates peptides that exhibited solubility problems under the experimental conditions; X is Aib; Z is Nal; O is Orn; He (%) designates percent helicity; mics designates micelles; and ~ indicates deleted amino acids.

9. EXAMPLE: PHARMACOKINETICS OF THE ApoA-I AGONISTS

The following experiments demonstrate that the ApoA-I agonists are stable in the circulation and associate with the HDL component of plasma.

In particular, radioactively labeled peptide 4 injected intraperitoneally into mice associated with the HDL component and remained stable for at least 6 hours. When added to human plasma (ex vivo) peptide 4 also associated with the HDL component.

9.1. SYNTHESIS OF RADIOLABELED PEPTIDES

Radiolabeled peptides 4 (SEQ ID NO:4) and 8 (SEQ ID NO:8) were synthesized by coupling ^{14}C-labeled Fmoc-Pro as the N-terminal amino acid.

L-[U-^{14}C]Proline, specific activity 9.25 GBq/mmol, was used for the synthesis of labeled Fmoc-L-Proline. The synthesis was carried out according to Lapatsanis, Synthesis, 1983, 671-173. Briefly, 250 μM (29.6 mg) of unlabeled L-Proline was dissolved in 225 μl of a 9% Na_2CO_3 solution and added to a solution (9% Na_2CO_3) of 9.25 MBq (250 μM) ^{14}C labeled L-Proline. The liquid was cooled down to 0°C, mixed with 600 μM (202 mg) 9-fluorenylmethyl-N-succinimidylcarbonate (Fmoc-OSu) in 0.75 ml DMF and shaken at room temperature for 4 hr. Thereafter the mixture was extracted with Diethylether (2 x 5ml) and chloroform (1 x 5ml), the remaining aqueous phase was acidified with 30% HCl and extracted with chloroform (5 x 8 ml). The organic phase was dried over Na_2SO_4, filtered off and the volume was reduced

under nitrogen flow to 5 ml. The purity was estimated by TLC ($CHCl_3$:MeOH:Hac, 9:1:0.1 v/v/v, stationary phase HPTLC silicagel 60, Merck, Germany) and showed a single peak (UV detection, radiochemical purity:Linear Analyzer, Berthold, Germany); reaction yield: 90% (determined by LSC).

The chloroform solution containing ^{14}C-Fmoc Proline was used directly for peptide synthesis. A peptide resin containing amino acids 2-22, synthesized automatically as described in Section 6, was used for the synthesis. The sequence of the peptide was determined by Edman degradation. The coupling was performed as described in Section 6.1 (manual coupling of Fmoc-Nal), except that HATU (O-(7-azabenzotriazol-1-yl)1-,1,3,3-tetramethyluroniumhexafluorophosphate) was used instead of TBTU. A second coupling with unlabeled Fmoc-L-Pro was carried out manually as described in Section 6.1. Peptide cleavage and deprotection were as described in Section 6.4. The specific activities of labeled peptides were as follows:

peptide 4 (SEQ ID NO:4) = 3.9×10^5 dpm/mg
peptide 8 (SEQ ID NO:8) = 1.0×10^5 dpm/mg

9.2. PHARMACOKINETICS IN MICE

In each experiment, 2.5 mg/kg radiolabeled peptide was injected intraperitoneally into mice which were fed normal mouse chow or the atherogenic Thomas-Harcroft modified diet (resulting in severely elevated VLDL and IDL cholesterol). Blood samples were taken at multiple time intervals for assessment of radioactivity in plasma. Results are summarized in TABLE XI, below.

TABLE XI

HALF-LIFE OF PEPTIDE 4 (SEQ ID NO:4) IN MICE

SPECIES	PREPARATION	HALF-LIFE (hours)
C57BL/6 (normal diet)	peptide 4/PBS	5.72[1]
C57B1/6 (high fat diet)	peptide 4/PBS	5.98[2]
	peptide 4/POPC complex[3]	6.39[4]
ApoE-[6]	peptide 4/PBS	14.2[5]

1 The maximum serum concentration reached was 25.7% injected cpm at 1.9 hours following injection. r^2=0.960.

2 The maximum serum concentration reached was 20.3% injected cpm at 2.98 hours following injection. r^2=0.973.

3 Complexes consisting of phospholipid (POPC) and peptide 4 were prepared using the cholate dialysis method.

4 The maximum serum concentration reached was 39.4% injected cpm at 4.2 hours following injection. r^2=0.893.

5 The maximum serum concentration reached was 17.4% injected cpm at 1.46 hours following injection. r^2=0.973.

6 ApoE Knockout.

9.3. STABILITY IN HUMAN SERUM

9.3.1. EXPERIMENTAL METHODS

100 μg of ^{14}C-labeled peptide 4 (SEQ ID NO:4) prepared as described in Section 9.1, supra, was mixed with 2 mL of fresh human plasma (at 37°C) and delipidated either immediately (control sample) or after 8 days of incubation at 37°C (test sample). Delipidation was carried out by extracting the lipids with an equal volume of 2:1 (v/v) chloroform:methanol.

The samples were loaded onto a reverse-phase C_{18} HPLC column and eluted with a linear gradient (25-58% over 33 min.) of acetonitrile (containing 0.1% TFA). Elution

profiles were followed by absorbance (220 nm) and radioactivity.

9.3.2. RESULTS

The control sample eluted as a single peak with a retention time of approximately 30 min. All of the radioactivity eluted in this peak.

The test sample eluted as two peaks: one having a retention time of about 3 min., the other having a retention time of about 30 min. The 3 min. peak (degraded peptide) accounted for approximately 15% of the total radioactivity loaded onto the column. The remainder of the radioactivity eluted with the 30 min. peak (intact peptide), indicating that peptide 4 (SEQ ID NO:4) is extremely stable to human serum-- more than 80% of the peptide 4 remained intact even after an 8 day incubation in human serum.

9.4. FORMATION OF PRE-β LIKE PARTICLES

9.4.1. EXPERIMENTAL METHOD

Human HDL was isolated by KBr density ultra centrifugation at density d= 1.21 g/ml to obtain top fraction followed by Superose 6 gel filtration chromatography to separate HDL from other lipoproteins. Isolated HDL was adjusted to a final concentration of 1.0 mg/ml with physiological saline based on protein content determined by Bradford protein assay. An aliquot of 300 µl was removed from the isolated HDL preparation and incubated with 100 µl ^{14}C-labeled peptide 4 (0.2-1.0 µg/µl) for two hours at 37°C. Five separate incubations were analyzed including a blank containing 100 µl physiological saline and four dilutions of ^{14}C-labeled peptide 4: (i) 0.20 µg/µl peptide:HDL ratio=1:15; (ii) 0.30 µg/µl peptide:HDL ratio=1:10; (iii) 0.60 µg/µl peptide:HDL ratio=1:5; and (iv) 1.00 µg/µl peptide:HDL ratio=1:3. Following the two hour incubation, a 200 µl aliquot of the sample (total volume=400 µl) was loaded onto a Superose 6 gel filtration column for lipoprotein separation

and analysis, and 100 μl was used to determine total radioactivity loaded. The conditions for all FPLC chromatographies were as described in Section 9.4, infra.

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9.4.2. RESULTS

The results are presented in TABLE XII, infra, and FIGS. 9A-9F.

TABLE XII

DISPLACEMENT OF HUMAN APOA-I FROM ISOLATED HUMAN HDL BY ^{14}C-LABELED PEPTIDE 4 (SEQ ID NO:4)

		Peak #1		Peak #2	Peak #3	^{14}C loaded (cpm)	^{14}C column (cpm)	Recovery (%)
		1a	1b					
P:LP=1:15 control	EV	30.5						
	AUC	9.95						
peptide	EV	29.4	30.9					
	AUC	4.09	6.26					
	^{14}C	7,920			1,622	10,361	9,542	92.1
P:LP=1:10 control	EV	30.7						
	AUC	9.15						
peptide	EV	30.7		33.0				
	AUC	8.42		0.67				
	^{14}C	N/A			N/A	N/A	N/A	N/A

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		Peak #1		Peak #2	Peak #3	^{14}C loaded (cpm)	^{14}C column (cpm)	Recovery (%)
		1a	1b					
P:LP=1:5 control	EV	30.9						
	AUC	9.87						
peptide	EV	30.8		34.3				
	AUC	8.19		1.53				
	^{14}C	20,768			5,281	28,794	26,049	90.5
P:LP=1:3 control	EV	30.5						
	AUC	10.22						
peptide	EV	30.6		34.3				
	AUC	7.93		1.97				
	^{14}C	43,495			7,832	54,593	51,321	94

P:LP = Peptide to lipoprotein ratio based on mass (μg)
EV = Elution volume (ml)
AUC = Area under the curve (Peakfit program)
^{14}C = Cpms determined from radiometric counting
Peak #1 = Fractions 29 to 33
Peak #2 = Fractions 34 to 35
Peak #3 = Fractions 41 to 47

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From the data presented in TABLE XII, it is evident that greater than 90% of the radioactivity was recovered from the column after separation of the lipoprotein particles. At low concentration of peptide (i.e., peptide:HDL mass ratio of 1:15), the HDL peak was split into two separate peaks (FIG. 9A), suggesting that an interaction between the peptide and HDL occurred which resulted in some remodeling of the lipoprotein particle, but since no displaced peak was noted the interaction was not sufficient to displace native ApoA-I. As the concentration of peptide was increased, a displacement of ApoA-I was observed which increased with increasing concentration of peptide (FIGS. 9B-9D). In addition, all chromatographic runs showed a third peak detected by radiometric counting (except the one with a mass ratio of 1:10, for which results are not shown) which is consistent with the elution volume of free peptide (FIG. 9E).

To further analyze the effect of peptide concentration on the interaction of ^{14}C-labeled peptide 4 and HDL, a difference plot was generated from the four chromatographic runs (FIGS. 9A-9D) and is shown in FIG. 9F. The difference plot demonstrates the shift in the HDL peak as well as the increased displacement of ApoA-I with increasing peptide concentration. The displacement of ApoA-I results in the formation of pre-β like particles.

9.5. ASSOCIATION OF PEPTIDE 4 WITH HUMAN LIPOPROTEINS

9.5.1. EXPERIMENTAL METHODS

The association of peptide 4 (SEQ ID NO:4) with human lipoprotein fractions was determined by incubating ^{14}C-labeled peptide 4 (SEQ ID NO:4) with each lipoprotein class (HDL, LDL and VLDL) and a mixture of the different lipoprotein classes.

HDL, LDL and VLDL were isolated by KBr density gradient ultracentrifugation at d=1.21 g/ml and purified by FPLC on a Superose 6B column size exclusion column (chromatography was carried out with a flow rate of 0.7 ml/min. and a running

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buffer of 10 mM Tris (pH 8), 115 mM NaCl, 2 mM EDTA and 0.01% NaN_3). ^{14}C-labeled peptide 4 was incubated with HDL, LDL and VLDL at a peptide:phospholipid ratio of 1:5 (mass ratio) for 2 h at 37°C. The required amount of lipoprotein (volumes based on amount needed to yield 1000 μg) was mixed with 0.2 ml of peptide stock solution (1 mg/ml) and the solution was brought up to 2.2 ml using 0.9% of NaCl according to TABLE XIII:

TABLE XIII

PREPARATION OF PEPTIDE-LIPOPROTEIN SAMPLES

	HDL (ml)	LDL (ml)	VLDL (ml)	0.9% NaCl (ml)	^{14}C-labeled peptide 4 (ml)	Total (ml)
Control				2.0	0.2	2.2
LP/Peptide	0.4	0.3	0.9	0.4	0.2	2.2
HDL/Peptide	0.4			1.6	0.2	2.2
LDL/Peptide		0.3		1.7	0.2	2.2
VLDL/Peptide			0.9	1.1	0.2	2.2

After incubating for 2 hr. at 37°C, an aliquot (0.1 ml) was removed for liquid scintillation counting to determine the total radioactivity, the density of the remaining incubation mixture was adjusted to 1.21 g/ml with KBr, and the samples centrifuged at 100,000 rpm (300,000 g) for 24 hours at 4°C in a TLA 100.3 rotor using a Beckman tabletop ultracentrifuge. The resulting supernatant was fractionated by removing 0.3 ml aliquots from the top of each sample for a total of 5 fractions, and 0.05 ml of each fraction was used for liquid scintillation counting. The top two fractions contain the floating lipoproteins, the other fractions (3-5) correspond to proteins/peptides in solution.

9.5.2. RESULTS

The results are presented in TABLE XIV, infra. The incubation of ^{14}C-labeled peptide 4 with the isolated lipoprotein fractions revealed a strong association with HDL (88% of total radioactivity in fractions 1 and 2) as well as

with VLDL (85%), and a substantially weaker affinity to LDL (66%). The incubation mixture consisting of all lipoprotein fractions and ^{14}C-labeled peptide 4 (LP/Peptide) showed 88% of the labeled peptide associated with the lipoprotein fraction. FPLC analysis of the LP/peptide incubation mixture demonstrated that nearly all of the ^{14}C-labeled peptide 4 was associated with the HDL fraction, indicating a high selectivity for this lipoprotein class.

TABLE XIV

ASSOCIATION OF PEPTIDE 4 (SEQ ID NO:4) WITH VARIOUS LIPIDS

Fraction	Control	LP/ Peptide	HDL/ Peptide	LDL/ Peptide	VLDL/ Peptide
1	650	60250	61510	43740	42920
2	1000	12990	17620	15140	19280
3	6590	3810	2260	11880	3930
4	36550	1510	3130	10860	2600
5	34380	5090	5450	7710	4550
Total	79170	83650	89970	89330	73280
Total*	88550	96646	98494	100452	97306
Recovery (%)	89	87	91	89	75

* before centrifugation

9.6. PEPTIDE 4 (SEQ ID NO:4) SELECTIVELY BINDS HDL LIPIDS IN HUMAN PLASMA

9.6.1. EXPERIMENTAL METHOD

Human plasma (2 ml) was incubated with 20, 40, 60, 80, and 100 µg of ^{14}C-labeled peptide peptide 4 for 2 hr. at 37°C. The lipoproteins were separated by adjusting the density to 1.21 g/ml and centrifugation in TLA 100.3 rotor at 100,000 rpm (300,000 g) for 36 hr. at 4°C. The top 900 µl (in 300 µl fractions) was taken for the analysis. 50 µl from each 300 µl fraction was counted for radioactivity and 200 µl

from each fraction was analyzed by FPLC (Superose 6/Superose 12 combination column).

9.6.2. RESULTS

The amount of radioactivity recovered for each fraction is presented in TABLE XV, infra. Most of the radioactivity was recovered in the top three fractions. All the lipoprotein separation profiles of the plasma sample incubated with ^{14}C-labeled peptide 4 (SEQ ID NO:4) (not shown) indicate that, in every case, almost all of the radioactivity is present in the HDL fraction. Thus, although other lipoproteins are present in the human serum, peptide 4 (SEQ ID NO:4) exhibits highly selective binding to HDL.

TABLE XV

RADIOACTIVITY RECOVERED FROM INCUBATION OF ^{14}C-LABELED PEPTIDE 4 WITH HUMAN SERUM

Peptide	Fraction	Radioactivity (cpm*)		Recovery**
(μg)	No.	in 50 μl	in 300 μl	(%)
20	1	1407	8442	71.6
	2	405	2430	20.6
	3	152	912	7.7
40	1	2538	15228	70.4
	2	721	4326	20
	3	347	2082	9.6
60	1	4492	26952	75.5
	2	1107	6642	18.6
	3	354	2124	5.9
80	1	4715	28290	60
	2	2251	13506	28.6
	3	851	5346	11.3
100	1	5424	32544	67.5
	2	1708	10248	21.2
	3	907	5442	11.3

* Value for 300 μl was obtained by multiplying the 50 μl value by 6.

** Based on the initial total counts used for mixing with plasma.

10. EXAMPLE: THE ApoA-I AGONISTS PROMOTE CHOLESTEROL EFFLUX

HepG2 hepatoma cells were plated into 6-well culture dishes and grown to confluence. Cells were labeled with ^{3}H-cholesterol by drying the cholesterol, then adding 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS), sonicating the solution, and adding 0.2 ml labeling solution and 1.8 ml growth medium to the cells, so that each well contained 2 μCi of radioactivity. Cells were incubated for 24 h with the labeling medium.

Peptide (or protein):DMPC complexes were prepared at a 1:2 peptide (or protein):DMPC ratio (w:w). To prepare the complexes, peptide 4 (SEQ ID NO:4) or native human ApoA-I protein was added to a DMPC solution in PBS and incubated at room temperature overnight, by which time the solution has clarified. Peptide or protein concentration in the final solution was 1 mg/ml.

Labeling media was removed from the cells and the cells were washed with PBS prior to addition of complexes. 1.6 ml of growth medium was added to each well, followed by peptide (or protein):DMPC complex and sufficient PBS to bring the final volume to 2 ml per well. The final peptide or ApoA-I concentrations were 1, 2.5, 5, 7.5 and 25 μg/ml medium. After 24 hours of incubation at 37°C, the medium was removed, and the cells washed with 2 ml of 1% BSA/PBS, followed by 2 washes with 2 ml each of PBS. The amount of ^{3}H-cholesterol effluxed into the medium was determined by liquid scintillation counting.

The results demonstrate that peptide 4 (SEQ ID NO:4) was more efficient at cholesterol efflux than ApoA-I.

11. EXAMPLE: USE OF THE ApoA-I AGONISTS IN ANIMAL MODEL SYSTEMS

The efficacy of the ApoA-I agonists of the invention was demonstrated in rabbits. The results show that

administration of the ApoA-I agonists increases serum concentration of HDL-like particles.

11.1. PREPARATION OF THE PHOSPHOLIPID/PEPTIDE COMPLEXES

Small discoidal particles consisting of phospholipid (DPPC) and peptide were prepared following the cholate dialysis method. The phospholipid was dissolved in chloroform and dried under a stream of nitrogen. The peptide was dissolved in buffer (saline) at a concentration of 1-2 mg/ml. The lipid film was redissolved in buffer containing cholate (43°C) and the peptide solution was added at a 3:1 phospholipid/peptide weight ratio. The mixture was incubated overnight at 43°C and then dialyzed at 43°C (24 hr.), room temperature (24 hr.) and 4°C (24 hr.), with three changes of buffer (large volumes) at temperature point. The complexes were filter sterilized (0.22μm) for injection and storage at 4°C.

11.2. ISOLATION AND CHARACTERIZATION OF THE PEPTIDE/PHOSPHOLIPID PARTICLES

The particles were separated on a gel filtration column (Superose 6 HR). The position of the peak containing the particles was identified by measuring the phospholipid concentration in each fraction. From the elution volume, the Stokes radius was determined. The concentration of peptide in the complex was determined by measuring the phenylalanine content (by HPLC) following a 16 hr. acid hydrolysis.

11.3. INJECTION IN THE RABBIT

Male New Zealand White rabbits (2.5-3 kg) were injected intravenously with a dose of phospholipid/peptide complex (5 or 10 mg/kg bodyweight, expressed as peptide) in a single bolus injection not exceeding 10-15 ml. The animals were slightly sedated before the manipulations. Blood samples (collected on EDTA) were taken before and 5, 15, 30, 60, 240 and 1440 minutes after injection. The hematocrit

(Hct) was determined for each sample. Samples were aliquoted and stored at -20°C before analysis.

11.4. ANALYSIS OF THE RABBIT SERA

Plasma Lipids. The total plasma cholesterol, plasma triglycerides and plasma phospholipids were determined enzymatically using commercially available assays according to the manufacturer's protocols (Boehringer Mannheim, Mannheim, Germany and Biomérieux, 69280, Marcy-L'étoile, France).

Lipoprotein Profiles. The plasma lipoprotein profiles of the fractions obtained after the separation of the plasma into its lipoprotein fractions were determined by spinning in a sucrose density gradient. The fractions were collected and in each individual fraction the phospholipid and cholesterol content was measured enzymatically.

11.5. RESULTS

The lipoprotein profile of rabbits injected with 10 mg/kg peptide 4 (SEQ ID NO:4) (in the form of peptide/DPPC complexes) as a function of time is shown in FIG. 10. A substantial increase in cholesterol of the HDL cholesterol fractions (fractions >1.06 mg/ml) is apparent at 5 min. following injection and lasts for approximately 1 hr.

The cholesterol of the combined HDL fractions obtained by density gradient ultracentrifugation is presented in TABLE XVI, below. The highest increase of HDL cholesterol (31.3%) occurred 15 min. after administration.

These data indicate that administration of peptide 4/DPPC complexes (10 mg/kg) induces rapid and efficient mobilization of peripheral cholesterol.

TABLE XVI

HDL CHOLESTEROL IN RABBITS FOLLOWING ADMINISTRATION OF 10 mg/kg PEPTIDE 4 (SEQ ID NO:4)

Time (min.)	HDL Cholesterol	Increase in HDL Cholesterol (%)
0	325	
	408	25.2
15	428	31.3
60	387	18.9
240	291	-10.7
1440	347	6.6

The dose-dependency of the peptide 4/DPPC complexes is shown in TABLE XVII, below. Based on these two time points, an approximately linear dose-dependency was observed.

TABLE XVII

DOSE-DEPENDENCY OF HDL CHOLESTEROL LEVELS FOLLOWING ADMINISTRATION OF PEPTIDE 4/DPPC COMPLEXES

Dose	HDL Cholesterol (5 min.)	HDL Cholesterol (15 min.)	HDL Cholesterol (60 min.)
5 mg/kg	33.7	28	60
5 mg/kg	49	40	20
Mean Increase (%)	41.4	35.0	40.0
10 mg/kg	60.7	75	121.3
10 mg/kg	35	42.5	35.6
Mean Increase (%)	47.9	58.8	78.5

The percent increase in HDL cholesterol following injection of 5 mg/kg peptide 1 (SEQ ID NO:1) (in the form of peptide/DPPC complexes) or 10 mg/kg peptide 3 (SEQ ID NO:3)

(in the form of peptide/DPPC complexes) is shown in TABLE XVIII, below.

TABLE XVIII

Time (min)	Increase (%) (Peptide 1, 5 mg/kg)	Increase (%) (Peptide 3, 10 mg/kg)
15	24.3	84
30	95	71
60	40.7	60
240	29	14.5

These experiments demonstrate the ability of the ApoA-I agonists of the invention to increase HDL-cholesterol. A substantial increase in HDL cholesterol is observed even 4 hr. following administration for peptide 1 (SEQ ID NO:1) and peptide 3 (SEQ ID NO:3).

12. EXAMPLE: PREPARATION OF PEPTIDE-LIPID COMPLEX BY CO-LYOPHILIZATION APPROACH

The following protocol was utilized to prepare peptide-lipid complexes.

One mg of peptide 4 (SEQ ID NO:4) peptide was dissolved in 250 µl HPLC grade methanol (Perkin Elmer) in a one ml clear glass vial with cap (Waters #WAT025054). Dissolving of the peptide was aided by occasional vortexing over a period of 10 minutes at room temperature. To this mixture an aliquot containing either 1, 2, 3, 4, 5, 7.5, 10 or 15 mg dipalmitoyl phosphatidylcholine (DPPC; Avanti Polar Lipids, 99% Purity, product #850355) from a 100 mg/ml stock solution in methanol was added. The volume of the mixture was brought to 400 µl by addition of methanol, and the mixture was further vortexed intermittently for a period of 10 minutes at room temperature. To each tube 200 µl of xylene (Sigma-Aldrich 99% pure, HPLC-grade) was added and the

tubes were vortexed for 10 seconds each. Two small holes were punched into the tops of each tube with a 20 gauge syringe needle, the tubes were frozen for 15 seconds each in liquid nitrogen, and the tubes were lyophilized overnight under vacuum. To each tube 200 ml of 0.9% NaCl solution was added. The tubes were vortexed for 20 seconds each. At this time the solutions in the tubes were milky in appearance. The tubes were then incubated in a water bath for 30 minutes at 41°C. The solutions in all of the tubes became clear (i.e., similar to water in appearance) except for the tube containing 15 mg DPPC, which remained cloudy.

The following protocol was used to prepare a greater amount of peptide-lipid complexes for *in vivo* experiments.

Peptide 4 (SEQ ID NO:4) (22.4 mg) was dissolved in methanol at a concentration of 3.5 mg/ml by incubation for several minutes and mixing by vortex intermittently. To this solution was added dipalmitoyl phosphatidylcholine (DPPC) in methanol (100 mg/ml stock solution) such that the final ratio of DPPC/peptide was 2.5:1 (weight/weight). This solution was mixed by vortexing. Xylene was added to this solution to a final concentration of 36%. Aliquots of the resulting solution were removed for later analysis by gel filtration chromatography. The solutions were frozen in liquid nitrogen and lyophilized to dryness by vacuum. An aliquot containing 20 mg peptide and 50 mg DPPC was rehydrated in sterile saline solution (0.9% NaCl), mixed, and heated to 41° C for several minutes until a clear solution of reconstituted peptide phospholipid complexes resulted.

12.1. CHARACTERIZATION OF COMPLEXES BY SUPEROSE 6 GEL FILTRATION CHROMATOGRAPHY

Peptide-phospholipid complexes containing ^{14}C-labeled peptide 4 (SEQ ID NO:4) (specific radioactivity 159,000 DPM/mg peptide by weight, assuming 50% peptide content) were prepared by colyophilization as described in

the text. The preparation contained 1 mg peptide and 4 mg DPPC by weight. After reconstituting the complexes in 200 µl 0.9% NaCl, 20 µl (100 µg) of the complexes were applied to a Pharmacia Superose 6 column using 0.9% NaCl as the liquid phase at a flow rate of 0.5 ml/minute. After a 5 ml delay (column void volume = 7.7 ml), 1 ml fractions were collected. Aliquots containing 20 µl of the fractions were assayed for phospholipid content using the bioMerieux Phospholipides Enzymatique PAP 150 kit (#61491) according to the instructions supplied by the manufacturer. The remainders of each fraction were counted for 3 minutes in a Wallach 1410 liquid scintillation counter (Pharmacia) using the Easy Count program. The vast majority of both phospholipid and peptide were recovered together in a few fractions with peaks at approximately 16 ml. The UV absorbance profile for this sample (not shown) indicates that the complexes elute from the column at a volume of 14.7 ml. The discrepancy between the elution volume as measured by radioactivity/phospholipid assay and UV absorbance is due to the 1.3 ml dead volume of tubing between the UV absorbance flow cell and the fraction collector outlet. This elution volume corresponds to a Stokes' diameters of 114 Angstroms. Elution volumes of about 15-19 ml correspond to particles of Stokes' diameters of 50-120 Angstroms, which is the size range of human HDL.

12.2. SUPEROSE 6 GEL FILTRATION CHROMATOGRAPHY OF HUMAN HDL

Human HDL_2 was prepared as follows: 300 ml frozen human plasma (Mannheim Blutspendzentrale #1185190) was thawed, adjusted to density 1.25 with solid potassium bromide, and centrifuged for 45 hours at 40,000 PRM using a Ti45 rotor (Beckman) at 20°C. The floating layer was collected, dialyzed against distilled water, adjusted to density 1.07 with solid potassium bromide, and centrifuged as described above for 70 hours. The bottom layer (at a level of one cm above the tube bottom) was collected, brought to 0.01% sodium azide, and stored at 4°C for 4 days until

chromatography. 20 μl of the HDL_2 was loaded onto a Pharmacia Superose 6 FPLC gel filtration chromatography system using 0.9% NaCl as column eluate. The column flow rate was 0.5 ml/min. The column eluate was monitored by absorbance or scattering of light of wavelength 254 nm. A series of proteins of known molecular weight and Stokes' diameter were used as standards to calibrate the column for the calculation of Stokes' diameters of the particles (Pharmacia Gel Filtration Calibration Kit Instruction Manual, Pharmacia Laboratory Separation, Piscataway, NJ, revised April 1985). The HDL eluted with a retention volume of 14.8 ml, corresponding to a Stokes' diameter of 108 nm.

13. EXAMPLE: PREPARATION OF ANTIBODIES

Peptides 4 or 8 were conjugated to keyhole limpet hemocyanine (KLH; 1 mg peptide to 10 mg KLH). The KLH conjugate (LMG) was suspended in complete Freund's adjuvant and injected into rabbits at time 0, and boosted with 0.25 mg KLH conjugate at 4 weeks and again at 5 weeks. Pre-bleeds and six week post-bleeds were tested for antibody titer against authentic antigen by ELISA.

The production bleeds were pooled from 2 rabbits each. Antibodies directed exclusively against the peptide antigens were isolated as follows:

1. Free peptide was attached to cyanogen bromide activated Sepharose 4B (Pharmacia) according to the manufacturer's protocol.

2. The antisera was preabsorbed on a column of irrelevant peptides and on columns of irrelevant human and mouse serum proteins.

3. The pre-absorbed antisera was passed through the corresponding peptide column (see point 1).

4. The columns were washed with 0.1 M borate buffered saline (pH 8.2) and the bound antibodies were eluted using a low pH gradient step from pH 4.0 to pH 3.0 to pH 2.0 (0.1 M glycine buffer) and finally with 0.1 M HCl.

5. The eluted material was neutralized with excess borate saline, concentrated by ultrafiltration (Amicon, YM30) and dialyzed against borate saline.

6. The protein concentration was determined by absorbance at 280 nm.

The resulting antibodies were tested for species specificity using purified human ApoA-I or purified mouse ApoA-I in a direct ELISA binding assay. The human and murine antibodies were specific for human ApoA-I, and demonstrated minimal cross-activity.

The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference for all purposes.

1. An ApoA-I agonist compound comprising:

(i) a 22 to 29-residue peptide or peptide analogue which forms an amphipathic ∀-helix in the presence of lipids and which comprises formula (I):

(I) Z_1-X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-X_{18}-X_{19}-X_{20}-X_{21}-X_{22}-X_{23}-Z_2

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is Pro (P), Ala (A), Gly (G), Gln (Q), Asn (N), Asp (D) or D-Pro (p);

X_2 is an aliphatic residue;

X_3 is Leu (L) or Phe (F);

X_4 is an acidic residue;

X_5 is Leu (L) or Phe (F);

X_6 is Leu (L) or Phe (F);

X_7 is a hydrophilic residue;

X_8 is an acidic or a basic residue;

X_9 is Leu (L) or Gly (G);

X_{10} is Leu (L), Trp (W) or Gly (G);

X_{11} is a hydrophilic residue;

X_{12} is a hydrophilic residue;

X_{13} is Gly (G) or an aliphatic residue;

X_{14} is Leu (L), Trp (W), Gly (G) or Nal;

X_{15} is a hydrophilic residue;

X_{16} is a hydrophobic residue;

X_{17} is a hydrophobic residue;

X_{18} is Gln (Q), Asn (N) or a basic residue;

X_{19} is Gln (Q), Asn (N) or a basic residue;

X_{20} is a basic residue;

X_{21} is an aliphatic residue;

X_{22} is a basic residue;

X_{23} is absent or a basic residue;

Z_1 is R_2N- or RC(O)NR-;

Z_2 is -C(O)NRR, -C(O)OR or -C(O)OH or a salt thereof;

each R is independently -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl, 6-26 membered alkheteroaryl or a 1 to 7-residue peptide or peptide analogue;

each " - " between residues X_1 through X_{23} independently designates an amide linkage, a substituted amide linkage, an isostere of an amide or an amide mimetic; or

(ii) a 15 to 28-residue deleted peptide or peptide analogue according to formula (I) in which at least one and up to eight of residues X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, X_{21} and X_{22} are optionally deleted; or

(iii) a 22 to 29-residue altered peptide or peptide analogue according to formula (I) in which at least one of residues X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, X_{21}, X_{22} or X_{23} is conservatively substituted with another residue.

2. The ApoA-I agonist compound of Claim 1 which exhibits at least about 38% LCAT-activation activity as compared with human ApoA-I.

3. The ApoA-I agonist compound of Claim 1 which is the altered peptide or peptide analogue according to formula (I).

4. The ApoA-I agonist compound of Claim 3 in which the hydrophobic residues are fixed according to formula (I) and at least one non-fixed residue is conservatively substituted with another residue.

5. The ApoA-I agonist compound of Claim 4 in which:

X_1 is Pro (P), D-Pro (p), Gly (G) or Ala (A);

X_2 is Ala (A), Leu (L) or Val (V);

X_3 is Leu (L) or Phe (F);

X_5 is Leu (L) or Phe (F);

X_6 is Leu (L) or Phe (F);

X_9 is Leu (L) or Gly (G);

X_{10} is Leu (L), Trp (W) or Gly (G);

X_{13} is Leu (L), Gly (G) or Aib;

X_{14} is Leu (L), Nal, Trp (W) or Gly (G);

X_{16} is Ala (A), Nal, Trp (W), Gly (G), Leu (L) or Phe (F);

X_{17} is Leu (L), Gly (G) or Nal;

X_{21} is Leu (L); and

at least one of X_4, X_7, X_8, X_{11}, X_{12}, X_{15}, X_{18}, X_{19}, X_{20}, X_{22} and X_{23} is conservatively substituted with another residue.

6. The ApoA-I agonist compound of Claim 3 in which the hydrophilic residues are fixed according to formula (I) and at least one non-fixed residue is conservatively substituted with another residue.

7. The ApoA-I agonist compound of Claim 6 in which:

X_4 is Asp (D) or Glu (E);

X_7 is Lys (K), Arg (R) or Orn;

X_8 is Asp (D) or Glu (E);

X_{11} is Asn (N) or Gln (Q);

X_{12} is Glu (E) or Asp (D);

X_{15} is Asp (D) or Glu (E);

X_{18} is Gln (Q), Asn (N), Lys (K) or Orn;

X_{19} is Gln (Q), Asn (N), Lys (K) or Orn;

X_{20} is Lys (K) or Orn;

X_{22} is Lys (K) or Orn;

X_{23} is absent or Lys (K); and

at least one of X_1, X_2, X_3, X_5, X_6, X_9, X_{10}, X_{13}, X_{14}, X_{16}, X_{17} and X_{21} is conservatively substituted with another residue.

8. The ApoA-I agonist compound of Claim 7 in which X_3 is Leu (L) or Phe (F), X_6 is Phe (F), X_9 is Leu (L) or Gly (G), X_{10} is Leu (L) or Trp (W) or Gly (G) and at least one of X_1, X_2, X_5, X_{13}, X_{14}, X_{16}, X_{17} and X_{21} is conservatively substituted with another residue.

9. The ApoA-I agonist compound of Claim 5 or 7 in which the substituting residue is classified within the same sub-category as the substituted residue.

10. The ApoA-I agonist compound of Claim 1 which is the deleted peptide or peptide analogue according to formula (I).

11. The ApoA-I agonist compound of Claim 10 in which one or two helical turns of the peptide or peptide analogue are optionally deleted.

12. The ApoA-I agonist compound of Claim 1 which is a 22-23 residue peptide or peptide analogue according to formula (I).

13. The ApoA-I agonist compound of Claim 12 in which:
the "-" between residues designates -C(O)NH-;
Z_1 is H_2N-; and
Z_2 is -C(O)OH or a salt thereof.

14. The ApoA-I agonist compound of Claim 13, in which:
X_1 is Pro (P), Ala (A), Gly (G), Asn (N), Gln (Q), Asp (D) or D-Pro (p);
X_2 is Ala (A), Val (V) or Leu (L);
X_3 is Leu (L) or Phe (F);
X_4 is Asp (D) or Glu (E);
X_5 is Leu (L) or Phe (F);
X_6 is Leu (L) or Phe (F);
X_7 is Lys (K), Arg (R) or Orn;
X_8 is Asp (D) or Glu (E);
X_9 is Leu (L) or Gly (G);
X_{10} is Leu (L), Trp (W) or Gly (G);
X_{11} is Asn (N) or Gln (Q);
X_{12} is Glu (E) or Asp (D);
X_{13} is Gly (G), Leu (L) or Aib;
X_{14} is Leu (L), Nal, Trp (W) or Gly (G);
X_{15} is Asp (D) or Glu (E);

X_{16} is Ala (A), Nal, Trp (W), Leu (L), Phe (F) or Gly (G);

X_{17} is Gly (G), Leu (L) or Nal;

X_{18} is Gln (Q), Asn (N), Lys (K) or Orn;

X_{19} is Gln (Q), Asn (N), Lys (K) or Orn;

X_{20} is Lys (K) or Orn;

X_{21} is Leu (L);

X_{22} is Lys (K) or Orn; and

X_{23} is absent or Lys (K).

15. The ApoA-I agonist compound of Claim 14, in which X_{23} is absent.

16. The ApoA-I agonist compound of Claim 13 or 14, in which one of X_{18} or X_{19} is Gln (Q) or Asn (N) and the other of X_{18} or X_{19} is Lys (K) or Orn.

17. The ApoA-I agonist compound of Claim 14 in which each of X_9, X_{10}, X_{13}, X_{14}, X_{15} and X_{17} is other than Gly (G).

18. The ApoA-I agonist compound of Claim 14 in which one of X_9, X_{10}, X_{13}, X_{14}, X_{15} and X_{17} is Gly (G) and the others are other than Gly (G).

19. The ApoA-I agonist compound of Claim 1 which is selected from the group consisting of:

peptide **1**	PVLDLFRELLNELLEZLKQKLK	(SEQ ID NO:1)
peptide **2**	GVLDLFRELLNELLEALKQKLKK	(SEQ ID NO:2)
peptide **3**	PVLDLFRELLNELLEWLKQKLK	(SEQ ID NO:3)
peptide **4**	PVLDLFRELLNELLEALKQKLK	(SEQ ID NO:4)
peptide **5**	pVLDLFRELLNELLEALKQKLKK	(SEQ ID NO:5)
peptide **6**	PVLDLFRELLNEXLEALKQKLK	(SEQ ID NO:6)
peptide **7**	PVLDLFKELLNELLEALKQKLK	(SEQ ID NO:7)
peptide **8**	PVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:8)
peptide **9**	PVLDLFRELGNELLEALKQKLK	(SEQ ID NO:9)
peptide **10**	PVLDLFRELLNELLEAZKQKLK	(SEQ ID NO:10)
peptide **11**	PVLDLFKELLQELLEALKQKLK	(SEQ ID NO:11)
peptide **12**	PVLDLFRELLNELLEAGKQKLK	(SEQ ID NO:12)
peptide **13**	GVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:13)

peptide 14	PVLDLFRELLNELLEALOQOLO	(SEQ ID NO:14)
peptide 15	PVLDLFRELWNELLEALKQKLK	(SEQ ID NO:15)
peptide 16	PVLDLLRELLNELLEALKQKLK	(SEQ ID NO:16)
peptide 17	PVLELFKELLQELLEALKQKLK	(SEQ ID NO:17)
peptide 18	GVLDLFRELLNELLEALKQKLK	(SEQ ID NO:18)
peptide 19	pVLDLFRELLNEGLEALKQKLK	(SEQ ID NO:19)
peptide 20	PVLDLFREGLNELLEALKQKLK	(SEQ ID NO:20)
peptide 21	pVLDLFRELLNELLEALKQKLK	(SEQ ID NO:21)
peptide 22	PVLDLFRELLNELLEGLKQKLK	(SEQ ID NO:22)
peptide 23	PLLELFKELLQELLEALKQKLK	(SEQ ID NO:23)
peptide 24	PVLDLFRELLNELLEALQKKLK	(SEQ ID NO:24)
peptide 25	PVLDFFRELLNEXLEALKQKLK	(SEQ ID NO:25)
peptide 26	PVLDLFRELLNELLELLKQKLK	(SEQ ID NO:26)
peptide 27	PVLDLFRELLNELZEALKQKLK	(SEQ ID NO:27)
peptide 28	PVLDLFRELLNELWEALKQKLK	(SEQ ID NO:28)
peptide 29	AVLDLFRELLNELLEALKQKLK	(SEQ ID NO:29)
peptide 123	QVLDLFRELLNELLEALKQKLK	(SEQ ID NO:123)
peptide 124	PVLDLFOELLNELLEALOQOLO	(SEQ ID NO:124)
peptide 125	NVLDLFRELLNELLEALKQKLK	(SEQ ID NO:125)
peptide 126	PVLDLFRELLNELGEALKQKLK	(SEQ ID NO:126)
peptide 127	PVLDLFRELLNELLELLKQKLK	(SEQ ID NO:127)
peptide 128	PVLDLFRELLNELLEFLKQKLK	(SEQ ID NO:128)
peptide 129	PVLELFNDLLRELLEALQKKLK	(SEQ ID NO:129)
peptide 130	PVLELFNDLLRELLEALKQKLK	(SEQ ID NO:130)
peptide 131	PVLELFKELLNELLDALRQKLK	(SEQ ID NO:131)
peptide 132	PVLDLFRELLENLLEALQKKLK	(SEQ ID NO:132)
peptide 133	PVLELFERLLEDLLQALNKKLK	(SEQ ID NO:133)
peptide 134	PVLELFERLLEDLLKALNQKLK	(SEQ ID NO:134)
peptide 135	DVLDLFRELLNELLEALKQKLK	(SEQ ID NO:135)
peptide 136	PALELFKDLLQELLEALKQKLK	(SEQ ID NO:136)
peptide 137	PVLDLFRELLNEGLEAZKQKLK	(SEQ ID NO:137)
peptide 138	PVLDLFRELLNEGLEWLKQKLK	(SEQ ID NO:138)
peptide 139	PVLDLFRELWNEGLEALKQKLK	(SEQ ID NO:139)
peptide 140	PVLDLFRELLNEGLEALOQOLO	(SEQ ID NO:140)
peptide 141	PVLDFFRELLNEGLEALKQKLK	(SEQ ID NO:141)
peptide 142	PVLELFRELLNEGLEALKQKLK	(SEQ ID NO:142)

and the N-terminal acylated and/or C-terminal amidated or esterified forms thereof, wherein X is Aib; Z is Nal; and O is Orn.

20. A multimeric ApoA-I agonist compound which exhibits at least about 38% LCAT activation activity as compared with human ApoA-I, comprising formula (II):

(II) $HH\text{-}\left(LL_m\text{-}HH\right)_n LL_m\text{-}HH$

or a pharmaceutically acceptable salt thereof, wherein:

each m is independently an integer from 0 to 1;

n is an integer from 0 to 10;

each "HH" is independently a peptide or peptide analogue according to Claim 1;

each "LL" is independently a bifunctional linker; and

each " - " independently designates a covalent linkage.

21. A multimeric ApoA-I agonist compound which exhibits at least about 38% LCAT activation activity as compared with human ApoA-I, comprising formula (III):

(III) $X\text{—}N_{ya}\text{—}X_{(ya-1)}\text{—}\left(N_{yb}\text{—}X_{(yb-1)}\right)_p$

or a pharmaceutically acceptable salt thereof, wherein:

each X is independently $HH\text{-}\left(LL_m\text{—}HH\right)_n LL_m\text{—}HH$;

each HH is independently a peptide or peptide analogue according to Claim 1;

each LL is independently a bifunctional linker;

each m is independently an integer from 0 to 1;

each n is independently an integer from 0 to 8;

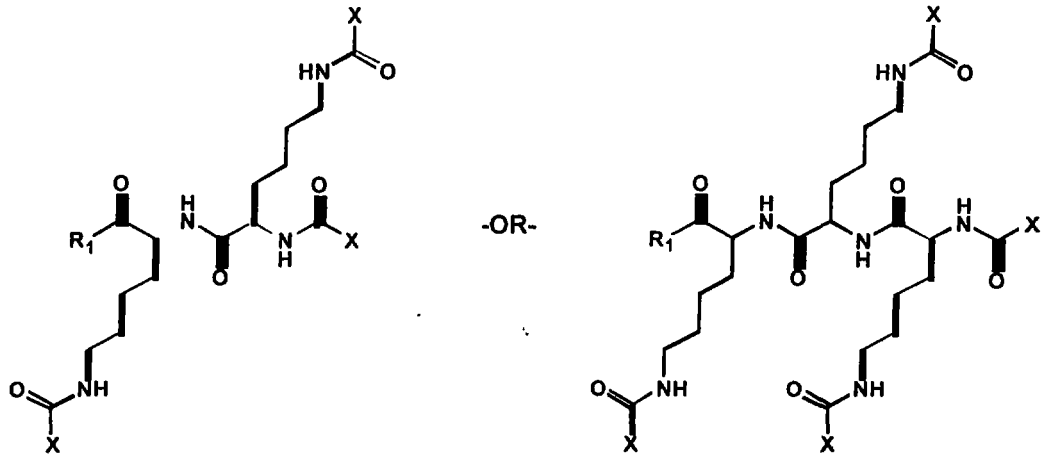
N_{ya} and N_{yb} are each independently a multifunctional linking moiety where y_a and y_b represent the number of functional groups on N_{ya} and N_{yb}, respectively;

each y_a or y_b is independently an integer from 3 to 8;

p is an integer from 0 to 7; and

each "—" independently designates a covalent bond.

22. A multimeric ApoA-I agonist compound which exhibits at least about 38% LCAT



(IV) (V)

activation activity as compared with human ApoA-I, comprising formula (IV) or (V):

or a pharmaceutically acceptable salt thereof, wherein:

each X is independently $HH\text{-}(LL_m\text{-}HH\text{-})_nLL_m\text{-}HH$;

each HH is independently a peptide or peptide analogue according to Claim 1;

each LL is independently a bifunctional linker;

each n is independently an integer from 0 to 1;

each m is independently an integer from 0 to 8;

R_1 is -OR or -NRR; and

each R is independently -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl, 5-20 membered heteroaryl or 6-26 membered alkheteroaryl.

23. The multimeric ApoA-I agonist compound of Claim 20, 21 or 22 in which the bifunctional linker is cleavable.

24. The multimeric ApoA-I agonist compound of Claim 20, 21 or 22 in which n is 0.

25. The multimeric ApoA-I agonist compound of Claim 24 in which m is 0.

26. The multimeric ApoA-I agonist compound of Claim 20, 21 or 22 in which each HH is independently a peptide or peptide analogue according to Claim 13.

27. The multimeric ApoA-I agonist compound of Claim 20, 21 or 22 in which each HH is independently a peptide or peptide analogue according to Claim 14.

28. The multimeric ApoA-I agonist compound of Claim 20, 21 or 22 in which each HH is independently a peptide or peptide analogue according to Claim 19.

29. An ApoA-I agonist-lipid complex comprising an ApoA-I agonist and a lipid, wherein the ApoA-I agonist is a peptide or peptide analogue according to Claim 1, a multimeric ApoA-I agonist compound according to Claim 20, a multimeric ApoA-I agonist compound according to Claim 21, or a multimeric ApoA-I agonist compound according to Claim 22.

30. The ApoA-I agonist-lipid complex of Claim 29 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 12.

31. The ApoA-I agonist-lipid complex of Claim 29 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 13.

32. The ApoA-I agonist-lipid complex of Claim 29 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 14.

33. The ApoA-I agonist-lipid complex of Claim 29 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 19.

34. The ApoA-I agonist-lipid complex of Claim 29 in which the lipid is sphingomyelin.

35. The ApoA-I agonist-lipid complex of Claim 29 which is in the form of a lyophilized powder.

36. The ApoA-I agonist-lipid complex of Claim 29 which is in the form of a solution.

37. A pharmaceutical composition comprising an ApoA-I agonist and a pharmaceutically acceptable carrier, excipient or diluent, wherein the ApoA-I agonist is a peptide or peptide analogue according to Claim 1, a multimeric ApoA-I agonist compound according to Claim 20, a multimeric ApoA-I agonist compound according to Claim 21, or a multimeric ApoA-I agonist compound according to Claim 22.

38. The pharmaceutical composition of Claim 37 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 12.

39. The pharmaceutical composition of Claim 37 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 13.

40. The pharmaceutical composition of Claim 37 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 14.

41. The pharmaceutical composition of Claim 37 in which the ApoA-I agonist is a peptide or peptide analogue according to Claim 19.

42. The pharmaceutical composition of Claim 37, 38, 39, 40 or 41, in which the ApoA-I agonist is in the form of an ApoA-I agonist-lipid complex, said complex comprising the ApoA-I agonist compound and a lipid.

43. The pharmaceutical composition of Claim 42 in which the ApoA-I agonist-lipid complex is in the form of a lyophilized powder.

44. A method of treating a subject suffering from a disorder associated with dyslipidemia, said method comprising the step of administering to the subject an effective amount of the ApoA-I agonist compound of Claim 1.

45. The method of Claim 44 in which the ApoA-I agonist is in the form of a pharmaceutical composition, said composition comprising the ApoA-I agonist compound and a pharmaceutically acceptable carrier, excipient or diluent.

46. The method of Claim 44 in which the ApoA-I agonist is in the form of an ApoA-I agonist-lipid complex, said complex comprising the ApoA-I agonist compound and a lipid.

47. The method of Claim 44 in which the disorder associated with dyslipidemia is hypercholesterolemia.

48. The method of Claim 44 in which the disorder associated with dyslipidemia is cardiovascular disease.

49. The method of Claim 44 in which the disorder associated with dyslipidemia is atherosclerosis.

50. The method of Claim 44 in which the disorder associated with dyslipidemia is restenosis.

51. The method of Claim 44, in which the disorder associated with dyslipidemia is HDL or ApoA-I deficiency.

52. The method of Claim 44, in which the disorder associated with dyslipidemia is hypertriglyceridemia.

53. The method of Claim 44, in which the disorder associated with dyslipidemia is metabolic syndrome.

54. A method of treating a subject suffering from septic shock, said method comprising the step of administering to the subject an effective amount of the ApoA-I agonist compound of Claim 1.

55. The method of Claim 44 or 54 in which said subject is a human.

56. The method of Claim 44 or 54 in which about 0.5 mg/kg to about 100 mg/kg ApoA-I agonist compound is administered to said subject.

SEQUENCE LISTING

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(ii) TITLE OF THE INVENTION: APOLIPOPROTEIN A-I AGONISTS AND THEIR USE TO TREAT DYSLIPIDEMIC DISORDERS

(iii) NUMBER OF SEQUENCES: 254

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(v) COMPUTER READABLE FORM:
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(D) SOFTWARE: FastSEQ Version 2.0

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 16
(D) OTHER INFORMATION: Xaa = Naphthylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Xaa
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys Lys
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Trp
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = D-Pro

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Xaa Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Pro Val Leu Asp Leu Phe Lys Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Pro Val Leu Asp Leu Phe Arg Glu Leu Gly Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 17
(D) OTHER INFORMATION: Xaa = Naphthylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Xaa Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Pro Val Leu Asp Leu Phe Lys Glu Leu Leu Gln Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Gly Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gly Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 18
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 20
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 22
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Xaa Gln Xaa Leu Xaa
            20
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Pro Val Leu Asp Leu Phe Arg Glu Leu Trp Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Pro Val Leu Asp Leu Leu Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro Val Leu Glu Leu Phe Lys Glu Leu Leu Gln Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gly Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = D-Pro

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Pro Val Leu Asp Leu Phe Arg Glu Gly Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = D-Pro

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Xaa Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Gly
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Pro Leu Leu Glu Leu Phe Lys Glu Leu Leu Gln Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Pro Val Leu Asp Phe Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Leu
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 14
(D) OTHER INFORMATION: Xaa = Naphthylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Xaa Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Trp Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Ala Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: N-terminal dansylated peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Pro Val Leu Asp Leu Phe Leu Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Xaa Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Pro Val Leu Asp Leu Phe Arg Glu Lys Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 5
(D) OTHER INFORMATION: Xaa = Naphthylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Pro Val Leu Asp Xaa Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Pro Val Leu Asp Trp Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Pro Leu Leu Glu Leu Leu Lys Glu Leu Leu Gln Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Pro Val Leu Asp Leu Phe Arg Glu Trp Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Trp Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Leu Lys Ala
1 5 10 15
Leu Lys Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Pro Val Leu Asp Leu Phe Asn Glu Leu Leu Arg Glu Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Pro Val Leu Asp Leu Trp Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Trp Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Trp Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All genetically encoded amino acids are in the D-configuration

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Pro Val Leu Asp Leu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala Leu
1 5 10 15
Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = D-Pro

(A) NAME/KEY: Other
(B) LOCATION: 2
(D) OTHER INFORMATION: Xaa = D-Val

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Xaa Xaa Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Pro Val Leu Asp Leu Phe Arg Asn Leu Leu Glu Lys Leu Leu Glu Ala
1 5 10 15
Leu Glu Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Trp Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Pro Val Leu Asp Leu Phe Trp Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Pro Val Trp Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Val Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Trp Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Pro Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala Leu Lys Gln
1 5 10 15
Lys Leu Lys

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Lys Lys
20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Pro Val Leu Asp Leu Phe Arg Asn Leu Leu Glu Glu Leu Leu Lys Ala
1 5 10 15
Leu Glu Gln Lys Leu Lys

20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu
20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Leu Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15

Leu Lys Gln

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Pro Val Leu Asp Glu Phe Arg Trp Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Pro Val Leu Asp Glu Trp Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Pro Val Leu Asp Phe Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Trp Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 12
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala Leu
1 5 10 15
Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Pro Val Leu Asp Leu Phe Arg Asn Leu Leu Glu Glu Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala Leu
1 5 10 15
Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Lys Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Pro Val Leu Asp Glu Phe Arg Lys Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Tyr Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 14
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Leu Xaa Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Xaa Leu Trp Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Pro Val Leu Asp Glu Phe Trp Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Pro Val Leu Asp Lys Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Phe Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Lys Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Pro Val Leu Asp Glu Phe Arg Asp Lys Leu Asn Glu Xaa Leu Glu Ala

1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Pro Val Leu Asp Leu Phe Glu Arg Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Trp Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 11
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala Leu Lys
1 5 10 15
Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Trp Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Pro Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala Leu
1 5 10 15
Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Pro Val Leu Glu Leu Phe Glu Arg Leu Leu Asp Glu Leu Leu Asn Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All amino acids are in the D-configuration

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Pro Leu Leu Glu Leu Leu Lys Glu Leu Leu Gln Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Pro Val Leu Asp Lys Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Trp Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys

20

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 10
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala Leu Lys Gln
1 5 10 15
Lys Leu Lys

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Pro Val Leu Asp Glu Phe Arg Glu Leu Tyr Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Lys Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Ala Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Leu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All genetically encoded amino acids are in the D-configuration

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Glu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Lys Leu Lys Gln Lys Leu Ala Glu Leu Leu Glu Asn Leu Leu Glu Arg
1 5 10 15
Phe Leu Asp Leu Val Pro
20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All amino acids are in the D-configuration

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Trp Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Leu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Glu Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Pro Val Leu Asp Glu Phe Arg Glu Leu Leu Asn Glu Glu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Pro Leu Leu Asn Glu Leu Leu Glu Ala Leu Lys Gln Lys Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Pro Ala Ala Asp Ala Phe Arg Glu Ala Ala Asn Glu Ala Ala Glu Ala
1 5 10 15
Ala Lys Gln Lys Ala Lys
20

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Pro Val Leu Asp Leu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All amino acids are in the D-configuration

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Lys Leu Lys Gln Lys Leu Ala Glu Leu Leu Glu Asn Leu Leu Glu Arg
1 5 10 15
Phe Leu Asp Leu Val Pro
20

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Pro Val Leu Asp Leu Phe Arg Trp Leu Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Arg Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(A) NAME/KEY: Other
(B) LOCATION: 14
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Xaa Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Trp Glu Xaa Trp Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Ser Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Pro Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Met Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Pro Lys Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

Pro His Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Pro Glu Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 13
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Xaa Leu Glu Ala
1 5 10 15
Leu Glu Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 17
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

```
Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Ala
 1               5                  10                  15
Xaa Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 16
(D) OTHER INFORMATION: Xaa = Aib

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

```
Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Xaa
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

```
Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Ala
 1               5                  10                  15
Leu Trp Gln Lys Leu Lys
```

20

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Trp
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Gln Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 18
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 20
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 22
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Pro Val Leu Asp Leu Phe Xaa Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Xaa Gln Xaa Leu Xaa
20

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Asn Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Gly Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Leu
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Phe
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

Pro Val Leu Glu Leu Phe Asn Asp Leu Leu Arg Glu Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Pro Val Leu Glu Leu Phe Asn Asp Leu Leu Arg Glu Leu Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys

20

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Pro Val Leu Glu Leu Phe Lys Glu Leu Leu Asn Glu Leu Leu Asp Ala
1 5 10 15
Leu Arg Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Asn Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Pro Val Leu Glu Leu Phe Glu Arg Leu Leu Glu Asp Leu Leu Gln Ala
1 5 10 15
Leu Asn Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

```
Pro Val Leu Glu Leu Phe Glu Arg Leu Leu Glu Asp Leu Leu Lys Ala
 1               5                  10                  15
Leu Asn Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

```
Asp Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

```
Pro Ala Leu Glu Leu Phe Lys Asp Leu Leu Gln Glu Leu Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 17
(D) OTHER INFORMATION: Xaa = Naphthylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
Xaa Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Trp
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Trp Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 18
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 20
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 22
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

```
Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
Leu Xaa Gln Xaa Leu Xaa
            20
```

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

```
Pro Val Leu Asp Phe Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
Leu Lys Gln Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

```
Pro Val Leu Glu Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
 1               5                  10                  15
```

Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Gly Leu Glu Ala
1 5 10 15
Leu Lys Gln Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: Xaa = D-Pro

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

Xaa Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

Gly Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Phe Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Gly Asp Ala

1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

Pro Val Leu Glu Leu Phe Glu Asn Leu Trp Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

Pro Leu Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

Pro Val Leu Glu Leu Phe Glu Asn Leu Gly Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

Pro Val Phe Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Ala Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Gly Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:155:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

Pro Val Leu Glu Leu Phe Leu Asn Leu Trp Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:156:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

Pro Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:157:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

Pro Val Leu Glu Phe Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:158:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

Pro Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Arg Leu Leu Asp Trp

1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:159:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

Pro Val Leu Asp Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:160:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Trp
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:161:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:162:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

```
Pro Val Leu Glu Leu Phe Glu Asn Trp Leu Glu Arg Leu Leu Asp Ala
 1               5                  10                  15
Leu Gln Lys Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:163:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

```
Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Trp Asp Ala
 1               5                  10                  15
Leu Gln Lys Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:164:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

```
Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
 1               5                  10                  15
Trp Gln Lys Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Leu
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

Pro Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Lys Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

Pro Val Leu Glu Leu Phe Glu Asn Gly Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

Pro Val Leu Glu Leu Phe Glu Gln Leu Leu Glu Lys Leu Leu Asp Ala

1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Lys Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:170:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 12
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 19
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 20
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 22
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Xaa Leu Leu Asp Ala
1 5 10 15

Leu Gln Xaa Xaa Leu Xaa
20

(2) INFORMATION FOR SEQ ID NO:171:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Lys Leu Leu Asp Leu
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:172:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

Pro Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Arg Leu Gly Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:173:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

Pro Val Leu Asp Leu Phe Asp Asn Leu Leu Asp Arg Leu Leu Asp Leu
1 5 10 15
Leu Asn Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:174:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...22
(D) OTHER INFORMATION: All amino acids are in the D-configuration

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:175:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Glu Leu
1 5 10 15
Leu Asn Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:176:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

Pro Val Leu Glu Leu Trp Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:177:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

Gly Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:178:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

Pro Val Leu Glu Leu Phe Asp Asn Leu Leu Glu Lys Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Arg
20

(2) INFORMATION FOR SEQ ID NO:179:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:

Pro Val Leu Glu Leu Phe Asp Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:180:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:

Pro Val Leu Glu Leu Phe Asp Asn Leu Leu Asp Lys Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Arg
20

(2) INFORMATION FOR SEQ ID NO:181:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Trp Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:182:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Lys Leu Leu Glu Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:183:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

Pro Leu Leu Glu Leu Phe Glu Asn Leu Leu Glu Lys Leu Leu Asp Ala

```
 1               5                  10                  15
Leu Gln Lys Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:184:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

```
Pro Val Leu Glu Leu Phe Leu Asn Leu Leu Glu Arg Leu Leu Asp Ala
 1               5                  10                  15
Trp Gln Lys Lys Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:185:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 19
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 20
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 22
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

```
Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
 1               5                  10                  15
Leu Gln Xaa Xaa Leu Xaa
            20
```

(2) INFORMATION FOR SEQ ID NO:186:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

Pro Val Leu Glu Leu Phe Glu Gln Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:187:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Asn Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:188:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

Pro Val Leu Glu Leu Phe Glu Asn Leu Leu Asp Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

Asp Val Leu Glu Leu Phe Glu Asn Leu Leu Glu Arg Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

Pro Val Leu Glu Phe Trp Asp Asn Leu Leu Asp Lys Leu Leu Asp Ala
1 5 10 15
Leu Gln Lys Lys Leu Arg
20

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

Pro Val Leu Asp Leu Leu Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

Pro Val Leu Asp Leu Phe Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:193:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:194:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

Pro Val Leu Glu Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:195:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

Pro Val Leu Glu Leu Phe Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:196:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Asn Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:197:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

Pro Leu Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:198:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

Gly Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:199:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

Pro Val Leu Asp Leu Phe Arg Glu Leu Trp Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:200:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

Asn Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:201:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

Pro Leu Leu Asp Leu Phe Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:202:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

Pro Ala Leu Glu Leu Phe Lys Asp Leu Leu Glu Glu Leu Arg Gln Lys
1 5 10 15
Leu Arg

(2) INFORMATION FOR SEQ ID NO:203:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

Ala Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:204:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18

(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

Pro Val Leu Asp Phe Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:205:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

Pro Val Leu Asp Leu Phe Arg Glu Trp Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:206:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:

Pro Leu Leu Glu Leu Leu Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:207:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

Pro Val Leu Glu Leu Leu Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:208:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

Pro Ala Leu Glu Leu Phe Lys Asp Leu Leu Glu Glu Leu Arg Gln Arg
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:209:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:210:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:211:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(A) NAME/KEY: Other
(B) LOCATION: 14
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 16
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 18
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Xaa Gln Xaa
1 5 10 15
Leu Xaa

(2) INFORMATION FOR SEQ ID NO:212:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(A) NAME/KEY: Other
(B) LOCATION: 7
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 14
(D) OTHER INFORMATION: Xaa = Orn

(A) NAME/KEY: Other
(B) LOCATION: 16
(D) OTHER INFORMATION: Xaa = Orn

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

Pro Val Leu Asp Leu Phe Xaa Glu Leu Leu Glu Glu Leu Xaa Gln Xaa
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18

(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

Pro Ala Leu Glu Leu Phe Lys Asp Leu Leu Glu Glu Phe Arg Gln Arg
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(A) NAME/KEY: Other
(B) LOCATION: 1
(D) OTHER INFORMATION: D-configuration of Pro.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:215:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Trp Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:216:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

Pro Val Leu Glu Leu Phe Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:217:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Leu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:218:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Leu Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:219:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Trp Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:220:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Gln Lys Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:221:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

Asp Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:222:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

Pro Val Leu Asp Ala Phe Arg Glu Leu Leu Glu Ala Leu Leu Gln Leu
1 5 10 15
Lys Lys

(2) INFORMATION FOR SEQ ID NO:223:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

Pro Val Leu Asp Ala Phe Arg Glu Leu Leu Glu Ala Leu Ala Gln Leu
1 5 10 15
Lys Lys

(2) INFORMATION FOR SEQ ID NO:224:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

Pro Val Leu Asp Leu Phe Arg Glu Gly Trp Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:225:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

Pro Val Leu Asp Ala Phe Arg Glu Leu Ala Glu Ala Leu Ala Gln Leu
1 5 10 15
Lys Lys

(2) INFORMATION FOR SEQ ID NO:226:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

Pro Val Leu Asp Ala Phe Arg Glu Leu Gly Glu Ala Leu Leu Gln Leu
1 5 10 15
Lys Lys

(2) INFORMATION FOR SEQ ID NO:227:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

Pro Val Leu Asp Leu Phe Arg Glu Leu Gly Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:228:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

Pro Val Leu Asp Leu Phe Arg Glu Gly Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:229:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Gly Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:230:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

Pro Val Leu Glu Leu Phe Glu Arg Leu Leu Glu Asp Leu Gln Lys Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:231:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Lys Leu Glu Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:232:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:

Pro Leu Leu Glu Leu Phe Lys Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:233:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:

This sequence has been intentionally skipped

(2) INFORMATION FOR SEQ ID NO:234:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:

This sequence has been intentionally skipped

(2) INFORMATION FOR SEQ ID NO:235:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:

This sequence has been intentionally skipped

(2) INFORMATION FOR SEQ ID NO:236:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:

This sequence has been intentionally skipped

(2) INFORMATION FOR SEQ ID NO:237:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:

Leu Asp Asp Leu Leu Gln Lys Trp Ala Glu Ala Phe Asn Gln Leu Leu
1 5 10 15
Lys Lys

(2) INFORMATION FOR SEQ ID NO:238:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:

Glu Trp Leu Lys Ala Phe Tyr Glu Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:239:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:

Glu Trp Leu Glu Ala Phe Tyr Lys Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:240:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys Glu
1 5 10 15
Ala Phe

(2) INFORMATION FOR SEQ ID NO:241:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:241:

Asp Trp Phe Lys Ala Phe Tyr Asp Lys Val Phe Glu Lys Phe Lys Glu
1 5 10 15
Phe Phe

(2) INFORMATION FOR SEQ ID NO:242:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:242:

Gly Ile Lys Lys Phe Leu Gly Ser Ile Trp Lys Phe Ile Lys Ala Phe
1 5 10 15
Val Gly

(2) INFORMATION FOR SEQ ID NO:243:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:243:

Asp Trp Phe Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Phe Lys Glu
1 5 10 15
Ala Phe

(2) INFORMATION FOR SEQ ID NO:244:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:244:

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys Glu
1 5 10 15
Ala Phe

(2) INFORMATION FOR SEQ ID NO:245:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Phe Glu Lys Phe Lys Glu
1 5 10 15
Phe Phe

(2) INFORMATION FOR SEQ ID NO:246:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:246:

Glu Trp Leu Glu Ala Phe Tyr Lys Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:247:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:247:

Asp Trp Phe Lys Ala Phe Tyr Asp Lys Phe Phe Glu Lys Phe Lys Glu
1 5 10 15
Phe Phe

(2) INFORMATION FOR SEQ ID NO:248:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:248:

Glu Trp Leu Lys Ala Phe Tyr Glu Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:249:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:249:

Glu Trp Leu Lys Ala Glu Tyr Glu Lys Val Glu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:250:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:250:

Glu Trp Leu Lys Ala Glu Tyr Glu Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:251:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:251:

Glu Trp Leu Lys Ala Phe Tyr Lys Lys Val Leu Glu Lys Leu Lys Glu
1 5 10 15
Leu Phe

(2) INFORMATION FOR SEQ ID NO:252:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...15
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:252:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Gln Lys Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:253:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...16
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:253:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Glu Leu Lys Gln Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:254:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other

(B) LOCATION: 1...16
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:254:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Lys Leu Lys Gln Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:255:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...15
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:255:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Lys Leu Gln Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:256:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...16
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:256:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Glu Ala Leu Lys Gln Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:257:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...16
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:257:

Pro Val Leu Asp Leu Phe Glu Asn Leu Leu Glu Arg Leu Lys Gln Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:258:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 1...16
(D) OTHER INFORMATION: N-terminal acetylated and C-terminal amidated

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:258:

Pro Val Leu Asp Leu Phe Arg Glu Leu Leu Asn Glu Leu Lys Gln Lys
1 5 10 15