

SMALL MOLECULES FOR TREATMENT OF HYPERCHOLESTEROLEMIA AND RELATED DISEASES

Cross-Reference to Related Application

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/578,228, filed June 9, 2004, which is incorporated herein by reference.

Background of the Invention

Field of the Invention

[0002] The invention relates to small molecule mediators of reverse cholesterol transport (RCT) for treating hypercholesterolemia and associated cardiovascular diseases and other diseases.

Description of the Related Art

[0003] It is now well-established that elevated serum cholesterol ("hypercholesterolemia") is a causal factor in the development of atherosclerosis, a progressive accumulation of cholesterol within the arterial walls. Hypercholesterolemia and atherosclerosis are leading causes of cardiovascular diseases, including hypertension, coronary artery disease, heart attack and stroke. About 1.1 million individuals suffer from heart attack each year in the United States alone, the costs of which are estimated to exceed \$117 billion. Although there are numerous pharmaceutical strategies for lowering cholesterol levels in the blood, many of these have undesirable side effects and have raised safety concerns. Moreover, none of the commercially available drug therapies adequately stimulate reverse cholesterol transport, an important metabolic pathway that removes cholesterol from the body.

[0004] Circulating cholesterol is carried by plasma lipoproteins – particles of complex lipid and protein composition that transport lipids in the blood. Low density lipoproteins (LDLs), and high density lipoproteins (HDLs) are the major cholesterol carriers. LDLs 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.

[0005] 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, plasma HDL levels correlate inversely with coronary heart disease – indeed, high plasma 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 Badimon *et al.*, 1992, *Circulation* 86 (Suppl. III) 86-94). Thus, HDLs have popularly become known as the “good” cholesterol.

[0006] The amount of intracellular cholesterol liberated from the LDLs controls cellular cholesterol metabolism. The accumulation of cellular cholesterol derived from LDLs controls three processes: (1) it reduces cellular cholesterol synthesis by turning off the synthesis of HMGCoA reductase, a key enzyme in the cholesterol biosynthetic pathway; (2) the incoming LDL-derived cholesterol promotes storage of cholesterol by activating LCAT, the cellular enzyme which converts cholesterol into cholesteryl esters that are deposited in storage droplets; and (3) 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).

[0007] Reverse cholesterol transport (RCT) is the pathway by which peripheral cell cholesterol can be returned to the liver for recycling to extrahepatic tissues, or excreted into the intestine as bile. The RCT pathway represents the only means of eliminating cholesterol from most extrahepatic tissues. The RCT consists mainly of three steps: (1) cholesterol efflux, the initial removal of cholesterol from peripheral cells; (2) cholesterol esterification by the action of lecithin:cholesterol acyltransferase (LCAT), preventing a re-entry of effluxed cholesterol into the peripheral cells; and (3) uptake/delivery of HDL cholesteryl ester to liver cells. LCAT is the key enzyme in the RCT pathway and 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. The RCT pathway is mediated by HDLs.

[0008] 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.

[0009] Each HDL particle contains at least one copy (and usually two to four copies) of apolipoprotein A-1 (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 (HDL₃ and HDL₂). Although most HDL in circulation contains both ApoA-I and ApoA-II, the fraction of HDL which contains only ApoA-I (AI-HDL) appears to be more effective in RCT. Epidemiologic studies support the hypothesis that AI-HDL is anti-atherogenic. (Parra *et al.*, 1992, *Arterioscler. Thromb.* **12**:701-707; Decossin *et al.*, 1997, *Eur. J. Clin. Invest.* **27**:299-307).

[0010] 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).

[0011] 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; Mezdoor *et al.*, 1995, *Atherosclerosis* **113**:237-246).

[0012] 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, *PNAS. 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).
Current Treatments for Hypercholesterolemia and other Dyslipidemias

[0013] In the past two decades or so, the segregation of cholesterolemic compounds into HDL and LDL regulators and recognition of the desirability of decreasing blood levels of LDL has led to the development of a number of drugs. However, many of these drugs have undesirable side effects and/or are contraindicated in certain patients, particularly when administered in combination with other drugs. These drugs and therapeutic strategies include:

- (1) bile-acid-binding resins, which interrupt the recycling of bile acids from the intestine to the liver [e.g., cholestyramine (QUESTRAN LIGHT, Bristol-Myers Squibb), and colestipol hydrochloride (COLESTID, Pharmacia & Upjohn Company)];

(2) statins, which inhibit cholesterol synthesis by blocking HMGCoA reductase— the key enzyme involved in cholesterol biosynthesis [e.g., lovastatin (MEVACOR, Merck & Co., Inc.), a natural product derived from a strain of *Aspergillus*, pravastatin (PRAVACHOL, Bristol-Myers Squibb Co.), and atorvastatin (LIPITOR, Warner Lambert)];

(3) niacin is a water-soluble vitamin B-complex which diminishes production of VLDL and is effective at lowering LDL;

(4) fibrates are used to lower serum triglycerides by reducing the VLDL fraction and may in some patient populations give rise to modest reductions of plasma cholesterol via the same mechanism [e.g., clofibrate (ATROMID-S, Wyeth-Ayerst Laboratories), and gemfibrozil (LOPID, Parke-Davis)];

(5) estrogen replacement therapy may lower cholesterol levels in post-menopausal women;

(6) long chain alpha,omega-dicarboxylic acids have been reported to lower serum triglyceride and cholesterol (See, e.g., Bisgaier et al., 1998, *J. Lipid Res.* **39**:17-30; WO 98/30530; U.S. Pat. No. 4,689,344; WO 99/00116; U.S. Pat. No. 5,756,344; U.S. Pat. No. 3,773,946; U.S. Pat. No. 4,689,344; U.S. Pat. No. 4,689,344; U.S. Pat. No. 4,689,344; and U.S. Pat. No. 3,930,024);

(7) other compounds including ethers (See, e.g., U.S. Pat. No. 4,711,896; U.S. Pat. No. 5,756,544; U.S. Pat. No. 6,506,799), phosphates of dolichol (U.S. Pat. No. 4,613,593), and azolidinedione derivatives (U.S. Pat. No. 4,287,200) are disclosed as lowering serum triglyceride and cholesterol levels.

[0014] None of these currently available drugs for lowering cholesterol safely elevate HDL levels and stimulate RCT. Indeed, most of these current treatment strategies appear to operate on the cholesterol transport pathway, modulating dietary intake, recycling, synthesis of cholesterol, and the VLDL population.

ApoA-I Agonists for Treatment of Hypercholesterolemia

[0015] 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 recommenced by UCB Belgium (Pharmaprojects, Oct. 27, 1995; IMS R&D Focus, Jun. 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 WO 94/13819) and were commenced and discontinued by Bio-Tech (Pharmaprojects, Apr. 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, Apr. 28-30, 1997, Washington, D.C.; Gouni *et al.*, 1993, *J. Lipid Res.* 94:139-146; Levine, WO 96/04916). 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.

[0016] 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; Segrest *et al.*, 1990, *PROTEINS: Structure, Function and Genetics* 8:103-117), 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 (See e.g., Background discussions in U.S. Pat. Nos. 6,376,464 and 6,506,799; incorporated herein in their entirety by reference thereto).

[0017] 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. The 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, *PNAS 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). 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*).

[0018] Another study involved model amphipathic peptides called "LAP peptides" (Pownall *et al.*, 1980, *PNAS 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.

[0019] 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).

[0020] 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. Pat. 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).

[0021] 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*).

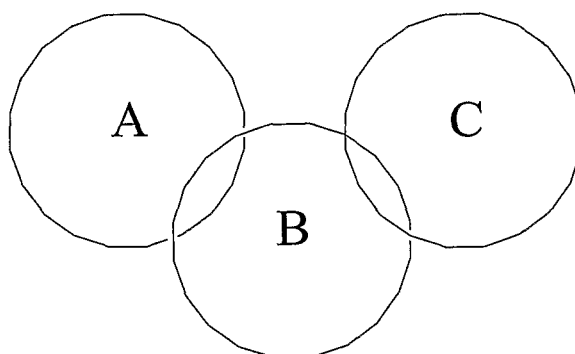
[0022] Based primarily on *in vitro* studies with the peptides described above, a set of "rules" has emerged for designing peptides which mimic the function of ApoA-I. Significantly, it is thought that an amphipathic α -helix having positively charged residues clustered at the hydrophilic-hydrophobic 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 (WO 93/25581).

[0023] However, despite the progress in elucidating “rules” for designing ApoA-I agonists, to date the best ApoA-I agonists are reported as having less than 40% of the activity of intact ApoA-I. None of the peptide agonists described in the literature have been demonstrated to be useful as a drug. Thus, there is a need for the development of a stable molecule that mimics the activity of ApoA-I and which is relatively simple and cost-effective to produce. Preferably, candidate molecules would mediate both indirect and direct RCT. Such molecules would be smaller than existing peptide agonists, and have broader functional spectra. However, the “rules” for designing efficacious mediators of RCT have not been fully elucidated and the principles for designing organic molecules with the function of ApoA-I are unknown.

Summary of the Invention

[0024] A mediator of reverse cholesterol transport is disclosed, comprising the structure:



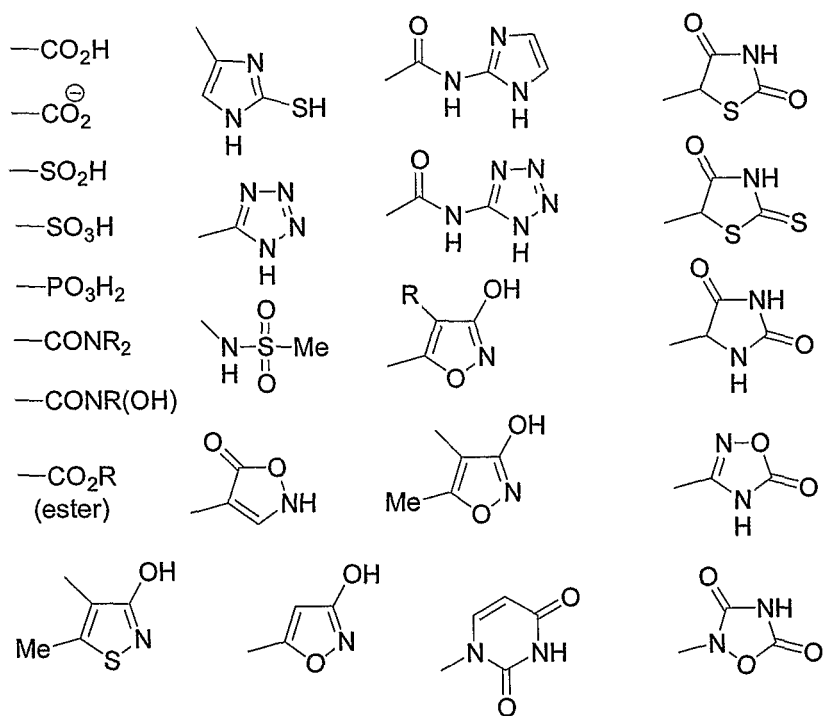
- [0025] wherein A, B, and C may be in any order, and wherein:
[0026] A comprises an acidic amino acid or bioisostere thereof;
[0027] B comprises an aromatic or lipophilic amino acid or analog thereof; and
[0028] C comprises a basic amino acid or bioisostere thereof,
[0029] wherein at least one of A or C comprises a bioisostere thereof.

[0030] In one embodiment, only one of A or C comprise a bioisostere. The alpha amino or alpha carboxy group may be removed from the underivatized amino or carboxy terminal amino acid.

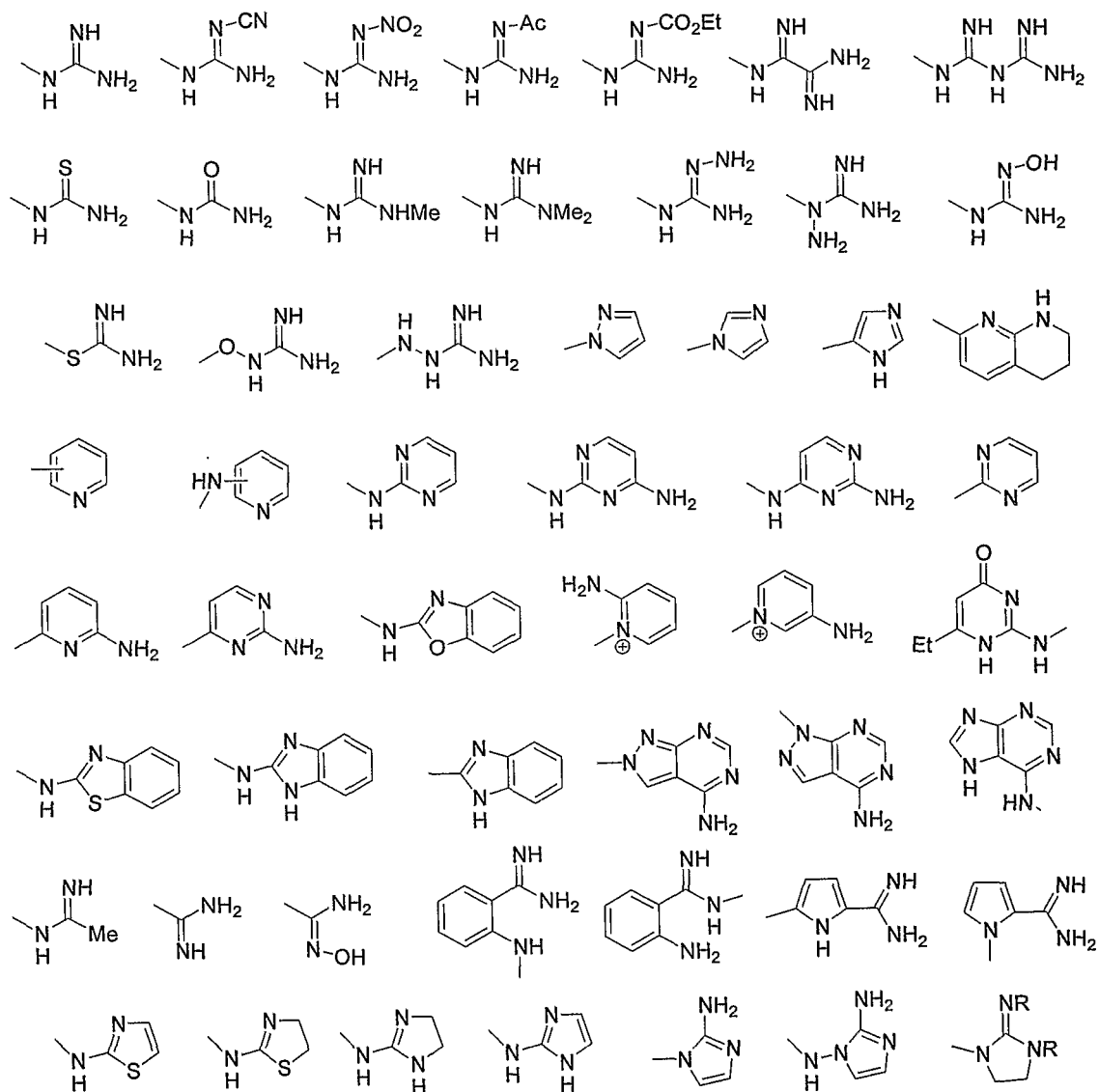
[0031] In another embodiment, if present, an alpha amino group from the amino terminal may be capped with a protecting group selected from the group consisting of formyl, acetyl, phenylacetyl, benzoyl, pivoyl, 9-fluorenylmethyloxycarbonyl, 2-naphthyl, nicotinic acid, a $\text{CH}_3-(\text{CH}_2)_n-\text{CO}-$ where n ranges from 1 to 20, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, and substituted saturated heteroaryl.

[0032] In another embodiment, if present, an alpha carboxy group from the carboxy terminal may be capped with a protecting group selected from the group consisting of an amine, such as RNH_2 where R = H, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, and substituted saturated heteroaryl.

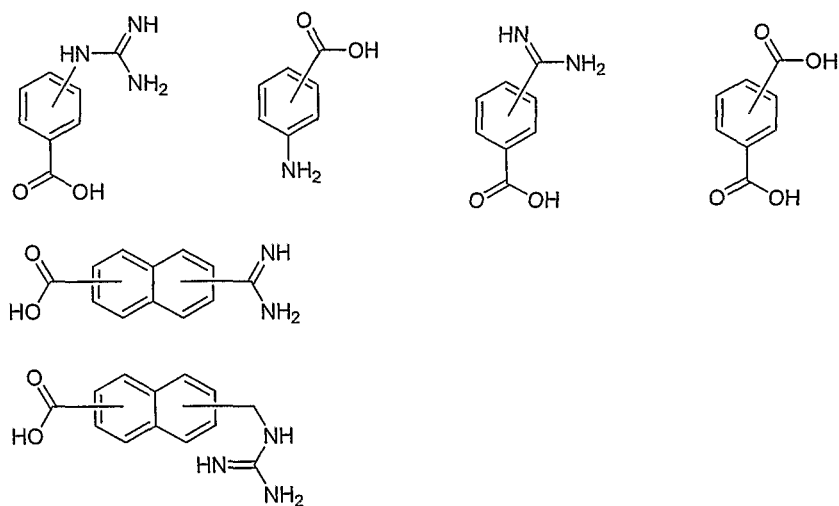
[0033] Bioisosteres of the acidic group may be selected from the group consisting of:



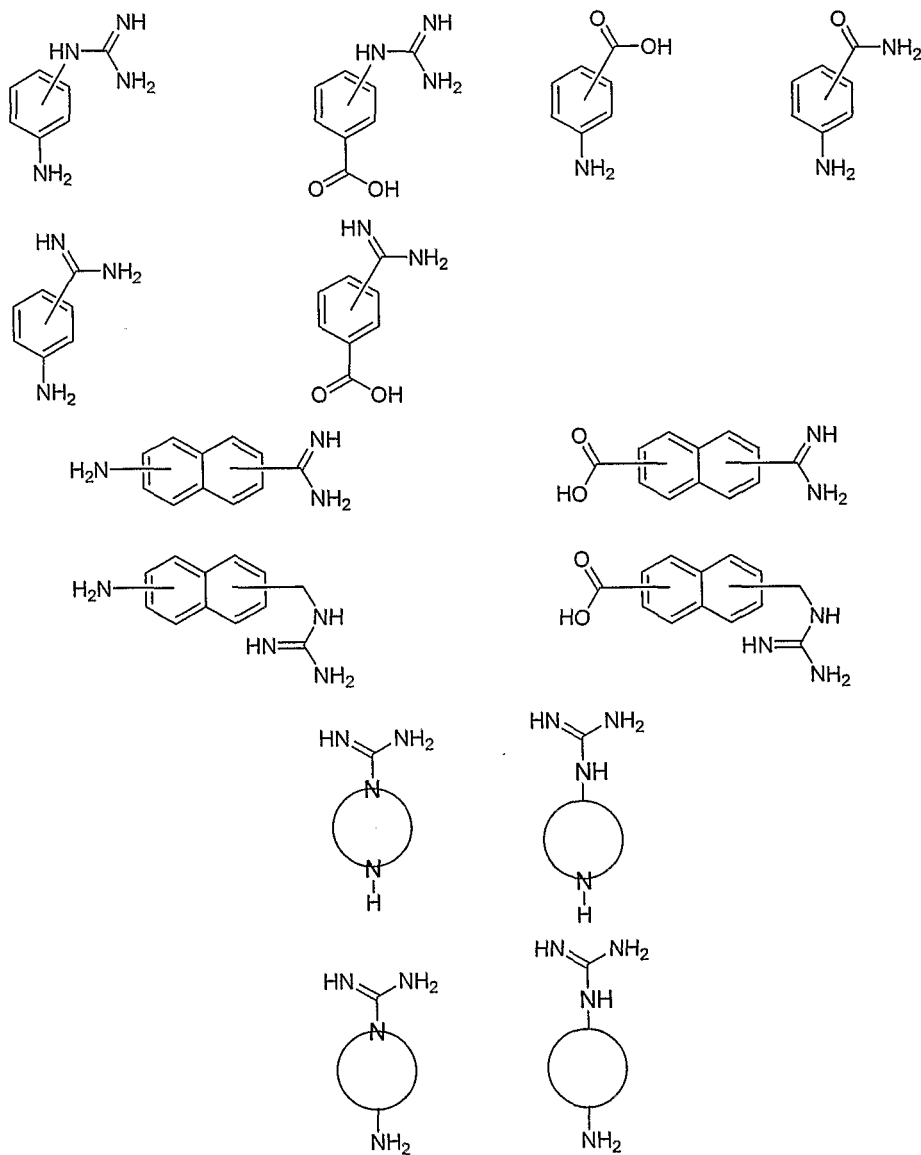
[0031] Bioisosteres of the basic group may be selected from the group consisting of:



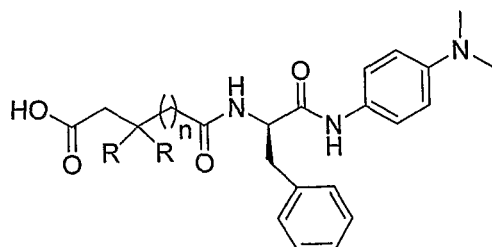
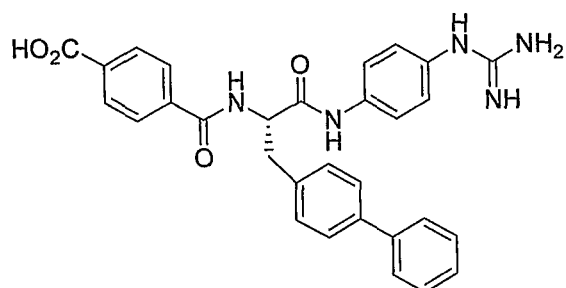
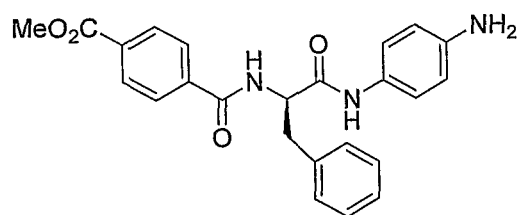
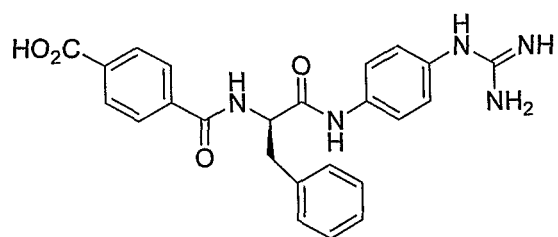
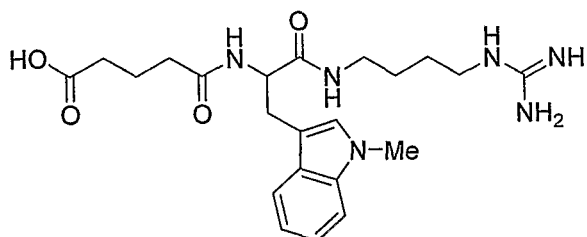
[0031] Bioisosteres of A may be selected from the group consisting of:



[0032] Bioisosteres of C may be selected from the group consisting of:

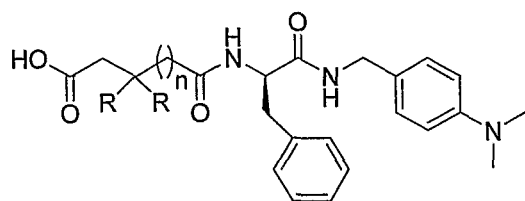


[0032] The mediator may be selected from the group consisting of:



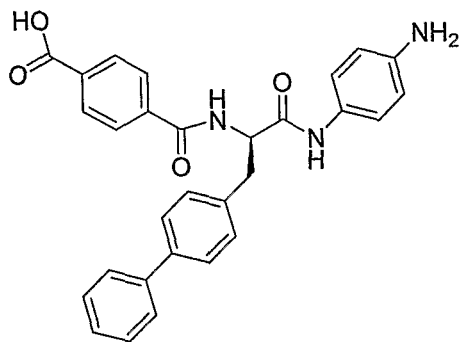
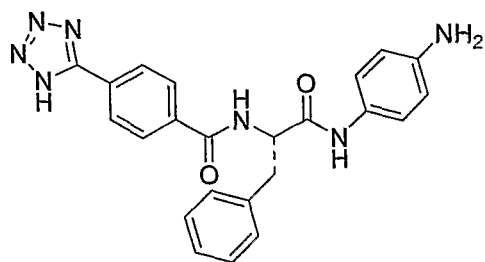
wherein R is H, methyl, cycloalkyl (C3-C7), and n =

1-10



= 1-10

wherein R is H, methyl, cycloalkyl (C3-C7), and n



[0034] In preferred embodiments, the mediator may be selected from the group consisting of BenOMe - bip - Aniline, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(pentamethylene)butanoic acid, 4-((S)-1-(4-guanidinophenylcarbamoyl)-2-(biphenyl)ethylcarbamoyl)benzoic acid, 3-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid, 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid, and 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid.

[0035] In other preferred embodiments, the mediator may be selected from 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid or 4-

((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid.

Detailed Description of the Preferred Embodiment

[0036] The mediators of RCT in preferred embodiments mimic ApoA-I function and activity. In a broad aspect, these mediators are molecules comprising three regions, an "acidic" region, a lipophilic (e.g., aromatic) region, and a basic region. The molecules preferably contain a positively charged region, a negatively charged region, and an uncharged, lipophilic region. The locations of the regions with respect to one another can vary between molecules; thus, in a preferred embodiment, the molecules mediate RCT regardless of the relative positions of the three regions within each molecule. Whereas in some preferred embodiments, the molecular template or model comprises an "acidic" amino acid-derived residue, a lipophilic amino acid-derived residue, and a basic amino acid-derived residue, linked in any order to form a mediator of RCT, in other preferred embodiments, the molecular model can be embodied by a single residue having acidic, lipophilic and basic regions, such as for example, the amino acid, phenylalanine.

[0037] In some preferred embodiments, the molecular mediators of RCT share the common aspect of reducing serum cholesterol through enhancing direct and/or indirect RCT pathways (i.e., increasing cholesterol efflux), ability to activate LCAT, and ability to increase serum HDL concentration.

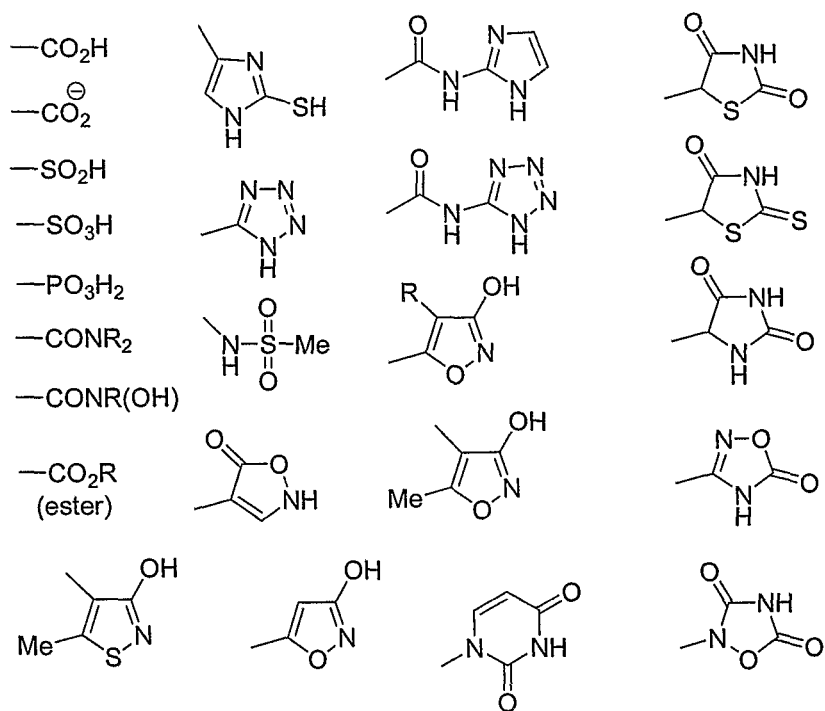
[0038] The mediator of reverse cholesterol transport preferably has up to 3 amino acid residues, bioisosteres thereof or any non-peptide compound containing a basic group, an acid group and a lipophilic group. The sequence may include: X1-X2-X3, X1-X2-Y3, Y1-X2-X3, or Y1-X2-Y3 wherein: X1 is an acidic amino acid or bioisostere thereof; X2 is an aromatic or a lipophilic amino acid or analog thereof; X3 is a basic amino acid or bioisostere thereof; Y1 is an amino acid residue or bioisostere thereof without the alpha amino group; and Y3 is a basic amino acid or bioisostere thereof without the alpha carboxy group. At least one of the amino or carboxy terminal groups comprise a bioisostere of an acidic or basic amino acid. When the alpha amino group on the amino terminal is present it may comprise a first protecting group, and when the alpha carboxy group on the carboxy terminal is present it may comprise a second protecting group. The first and second protecting groups are

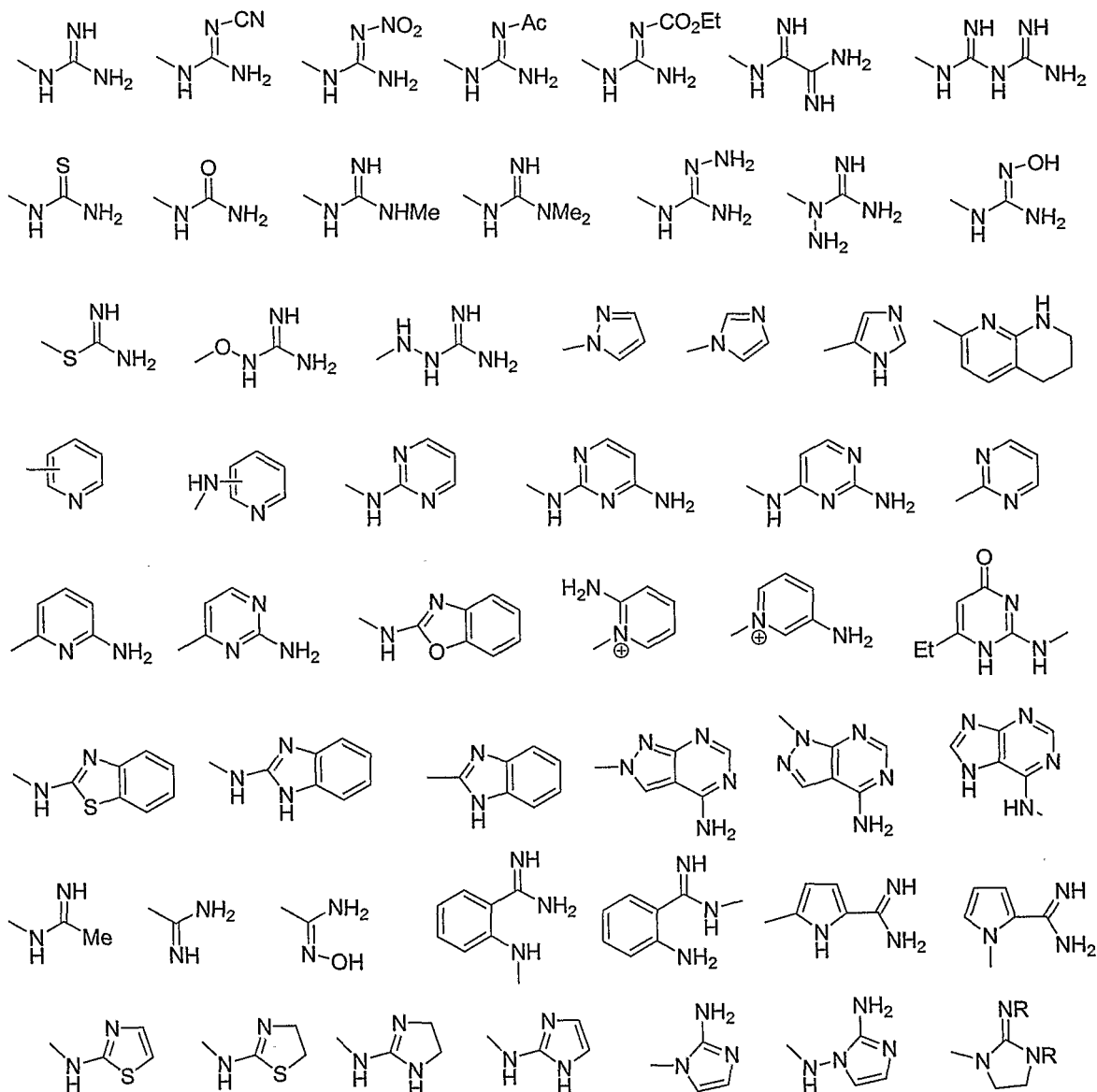
independently selected from the group consisting of a formyl, an acetyl, phenylacetyl, benzyl, pivoyl, 2-naphthyl, nicotinic acid, a $\text{CH}_3-(\text{CH}_2)_n-\text{CO}-$ where n ranges from 1 to 20, and an amide of acetyl, phenylacetyl, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl and the like. The C-terminal can be capped with an amine such as RNH_2 where $\text{R} = \text{H}$, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl and the like. The sequence could be scrambled in any and all possible orders to provide compounds that retain the basic features of the molecular model.

[0039] In another embodiment, the mediator can be incorporated into a larger entity, such as a peptide of about 1 to 10 amino acids, or a molecule.

[0040] The terms "bioisostere", "bioisosteric replacement", "bioisosterism" and closely related terms as used herein have the same meanings as those generally recognized in the art. Bioisosteres are atoms, ions, or molecules in which the peripheral layers of electrons can be considered identical. The term bioisostere is usually used to mean a portion of an overall molecule, as opposed to the entire molecule itself. Bioisosteric replacement involves using one bioisostere to replace another with the expectation of maintaining or slightly modifying the biological activity of the first bioisostere. The bioisosteres in this case are thus atoms or groups of atoms having similar size, shape and electron density. Bioisosterism arises from a reasonable expectation that a proposed bioisosteric replacement will result in maintenance of similar biological properties. Such a reasonable expectation may be based on structural similarity alone. This is especially true in those cases where a number of particulars are known regarding the characteristic domains of the receptor, etc. involved, to which the bioisosteres are bound or which works upon said bioisosteres in some manner.

[0041] Examples of bioisosteres for carboxylic acid and guanidine groups are shown below.

Carboxylic Acid Bioisosteres (R = H/alkyl)

Guanidine Bioisosteres (R = H/alkyl)

[0042] As used herein, the term “amino acid” can also refer to a molecule of the general formula $\text{NH}_2\text{-CHR-COOH}$ or the residue within a peptide bearing the parent amino acid, where “R” is one of a number of different side chains. “R” can be a substituent referring to one of the twenty genetically coded amino acids. “R” can also be a substituent referring to one that is not of the twenty genetically coded amino acids. As used herein, the term “amino acid residue” refers to the portion of the amino acid which remains after losing a water

molecule when it is joined to another amino acid. As used herein, the term “amino acid analog” refers to a structural derivative of an amino acid parent compound that often differs from it by a single element. The term “modified amino acid” refers to an amino acid bearing an “R” substituent that does not correspond to one of the twenty genetically coded amino acids.

[0043] The protecting groups on the amino terminal and carboxy terminal are independently selected from the group consisting of a formyl, acetyl, phenylacetyl, pivoyl, 2-naphthyl, nicotinic acid, a $\text{CH}_3\text{---}(\text{CH}_2)_n\text{---CO---}$ where n ranges from 1 to 20, and an amide of acetyl, phenylacetyl, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl and the like. The C-terminal can be capped with an amine such as RNH_2 where R = H, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl and the like.

[0044] Certain compounds can exist in tautomeric forms. All such isomers including diastereomers and enantiomers are covered by the embodiments. It is assumed that the certain compounds are present in either of the tautomeric forms or mixture thereof.

[0045] Certain compounds can exist in polymorphic forms. Polymorphism results from crystallization of a compound in at least two distinct forms. All such polymorphs are covered by the embodiments. It is assumed that the certain compounds are present in a certain polymorph or mixture thereof.

RCT Mediation

[0046] To date, efforts at designing ApoA-I agonists have focused on the 22-mer unit structures, e.g., the “consensus 22-mer” of Anantharamaiah *et al.*, 1990, *Arteriosclerosis* 10(1):95-105; Venkatachalapathi *et al.*, 1991, *Mol. Conformation and Biol. Interactions, Indian Acad. Sci.* B:585-596, which are capable of forming amphipathic α -helices in the presence of lipids. (See e.g., U.S. Pat. No. 6,376,464 directed at peptide mimetics derived from modifications of the consensus 22-mer). There are several advantages of using such relatively short peptides compared to longer 22-mers. For example, the shorter mediators of

RCT are easier and less costly to produce, they are chemically and conformationally more stable, the preferred conformations remain relatively rigid, there is little or no intra-molecular interactions within the peptide chain, and the shorter peptides exhibit a higher degree of oral availability. Multiple copies of these shorter peptides might bind to the HDL or LDL producing the same effect of a more restrained large peptide. Although ApoA-I multifunctionality may be based on the contributions of its multiple α -helical domains, it is also possible that even a single function of ApoA-I, e.g., LCAT activation, can be mediated in a redundant manner by more than one of the α -helical domains. Thus, in a preferred aspect of the embodiments, multiple functions of ApoA-I may be mimicked by the disclosed mediators of RCT which are directed to a single sub-domain.

[0047] Three functional features of ApoA-I are widely accepted as major criteria for ApoA-I agonist design: (1) ability to associate with phospholipids; (2) ability to activate LCAT; and (3) ability to promote efflux of cholesterol from the cells. The molecular mediators of RCT in accordance with some modes of the preferred embodiments may exhibit only the last functional feature—ability to increase RCT. However, quite a few other properties of ApoA-I, which are often overlooked, make ApoA-I a particularly attractive target for therapeutic intervention. For example, ApoA-I directs the cholesterol flux into the liver via a receptor-mediated process and modulates pre- β -HDL (primary acceptor of cholesterol from peripheral tissues) production via a PLTP driven reaction. However, these features allow broadening of the potential usefulness of ApoA-I mimetic molecules. This, entirely novel approach to viewing ApoA-I mimetic function, will allow use of the peptides or amino acid-derived small molecules, which are disclosed herein, to facilitate direct RCT (via HDL pathway) as well as indirect RCT (i.e., to intercept and clear the LDLs from circulation, by redirecting their flux to the liver). To be capable of enhancing indirect RCT, the molecular mediators of the preferred embodiments will preferably be able to associate with phospholipids and bind to the liver (i.e., to serve as ligand for liver lipoprotein binding sites).

[0048] Thus, a goal of the research efforts which led to the preferred embodiments was to identify, design, and synthesize the stable small molecule mediators of RCT that exhibit preferential lipid binding conformation, increase cholesterol flux to the liver

by facilitating direct and/or indirect reverse cholesterol transport, improve the plasma lipoprotein profile, and subsequently prevent the progression or/and even promote the regression of atherosclerotic lesions.

[0049] The mediators of RCT of the preferred embodiments 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 preferred embodiments include the pharmaceutical formulations and the use of such preparations in the treatment of hyperlipidemia, hypercholesterolemia, coronary heart disease, atherosclerosis, diabetes, obesity, Alzheimer's Disease, multiple sclerosis, conditions related to hyperlipidemia, such as inflammation, and other conditions such as endotoxemia causing septic shock.

[0050] The preferred embodiments are illustrated by working examples which demonstrate that the mediators of RCT of the preferred embodiments associate with the HDL and LDL component of plasma, and can increase the concentration of HDL and pre- β -HDL particles, and lower plasma levels of LDL. Thus promote direct and indirect RCT. The mediators of RCT of the preferred embodiments increase human LDL mediated cholesterol accumulation in human hepatocytes (HepG2 cells). The mediators of RCT are also efficient at activating PLTP and thus promote the formation of pre- β -HDL particles. Increase of HDL cholesterol served as indirect evidence of LCAT involvement (LCAT activation was not shown directly (*in vitro*)) in the RCT. Use of the mediators of RCT of the preferred embodiments *in vivo* in animal models results in an increase in serum HDL concentration.

[0051] The preferred embodiments are set forth in more detail in the subsections below, which describe composition and structure of the mediators of RCT, including bioisosteres that can be used within the structures of the mediators of RCT, and protected versions, half denuded versions, and denuded versions thereof; structural and functional characterization; methods of preparation of bulk and unit dosage formulations; and methods of use.

Structure and Function

[0052] The mediators of RCT of the preferred embodiments are generally peptides, or analogues thereof, which mimic the activity of ApoA-I. In some embodiments, at least one amide linkage in the peptide is replaced with a substituted amide, an isostere of

an amide or an amide mimetic. 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.

[0053] As used herein, the abbreviations for the genetically encoded L-enantiomeric amino acids are conventional and are as follows: The D-amino acids are designated by lower case, e.g. D-alanine = a, etc.

Table 1

Amino Acids	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
Lysine	K	Lys
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

[0054] Certain amino acid residues in the peptide mediators of RCT 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 preferred embodiments are altered or mutated forms of the peptide mediators of RCT wherein at least one defined amino acid residue in the structure is substituted with another amino acid residue or derivative and/or analog thereof. It will be recognized that in preferred embodiments, 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.

[0055] 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 nonpolar and aromatic amino acids. The definitions of the various categories of amino acids that define ApoA-I are as follows:

[0056] The term "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).

[0057] The term "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**:1.25-142. 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).

[0058] The term "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).

[0059] The term "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).

[0060] The term "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).

[0061] The term "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 nonpolar amino acids include Leu (L), Val (V), Ile (I), Met (M), Gly (G) and Ala (A).

[0062] The term "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, —NO₂, —NO, —NH₂, —NHR, —NRR, —C(O)R, —C(O)OH, —C(O)OR, —C(O)NH₂, —C(O)NHR, —C(O)NRR and the like where each R is independently (C₁ — C₆) alkyl, substituted (C₁ — C₆) alkyl, (C₁ — C₆) alkenyl, substituted (C₁ — C₆) alkenyl, (C₁ — C₆) alkynyl, substituted (C₁ — C₆) alkynyl, (C₅ — C₂₀) aryl, substituted (C₅ — C₂₀) aryl, (C₆ — C₂₆) alkaryl, substituted (C₆ — C₂₆) 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).

[0063] The term "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).

[0064] 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 preferred

embodiments Cys (C) is categorized as a polar hydrophilic amino acid, notwithstanding the general classifications defined above.

[0065] 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.

[0066] 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 peptide mediators of RCT contain genetically non-encoded amino acids. Thus, in addition to the naturally occurring genetically encoded amino acids, amino acid residues in the peptide mediators of RCT may be substituted with naturally occurring non-encoded amino acids and synthetic amino acids.

[0067] Certain commonly encountered amino acids which provide useful substitutions for the peptide mediators of RCT 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-phenylphenylalanine, 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 (pNH₂)); N-methyl valine (MeVal); homocysteine (hCys), homophenylalanine (hPhe) and homoserine (hSer);

hydroxyproline (Hyp), homoproline (hPro), N-methylated amino acids and peptoids (N-substituted glycines).

[0068] 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.

[0069] The classifications of the genetically encoded and common non-encoded amino acids according to the categories defined above are summarized in Table 2, below. It is to be understood that Table 2 is for illustrative purposes only and does not purport to be an exhaustive list of amino acid residues and derivatives that can be used to substitute the peptide mediators of RCT described herein.

Table 2. CLASSIFICATIONS OF COMMONLY ENCOUNTERED AMINO ACIDS

Classification	Genetically Encoded	Non-Genetically Encoded
<u>Hydrophobic</u>		
Aromatic	F, Y, W	Phg, Nal, Thi, Tic, Phe (4-Cl), Phe (2-F), Phe (3-F), Phe (4-F), hPhe
Nonpolar	L, V, I, M, G, A, P	t-BuA, t-BuG, Melle, Nle, MeVal, Cha, McGly, Aib
Aliphatic	A, V, L, I	b-Ala, Dpr, Aib, Aha, McGly, t-BuA, t-BuG, Melle, Cha, Nle, MeVal
<u>Hydrophilic</u>		
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)

[0070] 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.

[0071] While in most instances, the amino acids of the peptide mediators of RCT will be substituted with D-enantiomeric amino acids, the substitutions are not limited to D-enantiomeric amino acids. Thus, also included in the definition of "mutated" or "altered" forms are those situations where an D-amino acid is replaced with an identical L-amino acid (e.g., D-Arg→L-Arg) or with a L-amino acid of the same category or subcategory (e.g., D-

Arg D-Lys), and vice versa. 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.

Linkers

[0072] The peptide mediators of RCT can be connected or linked 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. The linker 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.

[0073] Linkers of sufficient length and flexibility include, but are not limited to, Pro (P), Gly (G), Cys-Cys, Gly-Gly, $\text{H}_2\text{N}-(\text{CH}_2)_n-\text{COOH}$ where n is 1 to 12, preferably 4 to 6; H_2N -aryl-COOH and carbohydrates. However, in some embodiments, no separate linkers per se are used at all. Instead, the acidic, lipophilic and basic moieties are all part of a single molecule.

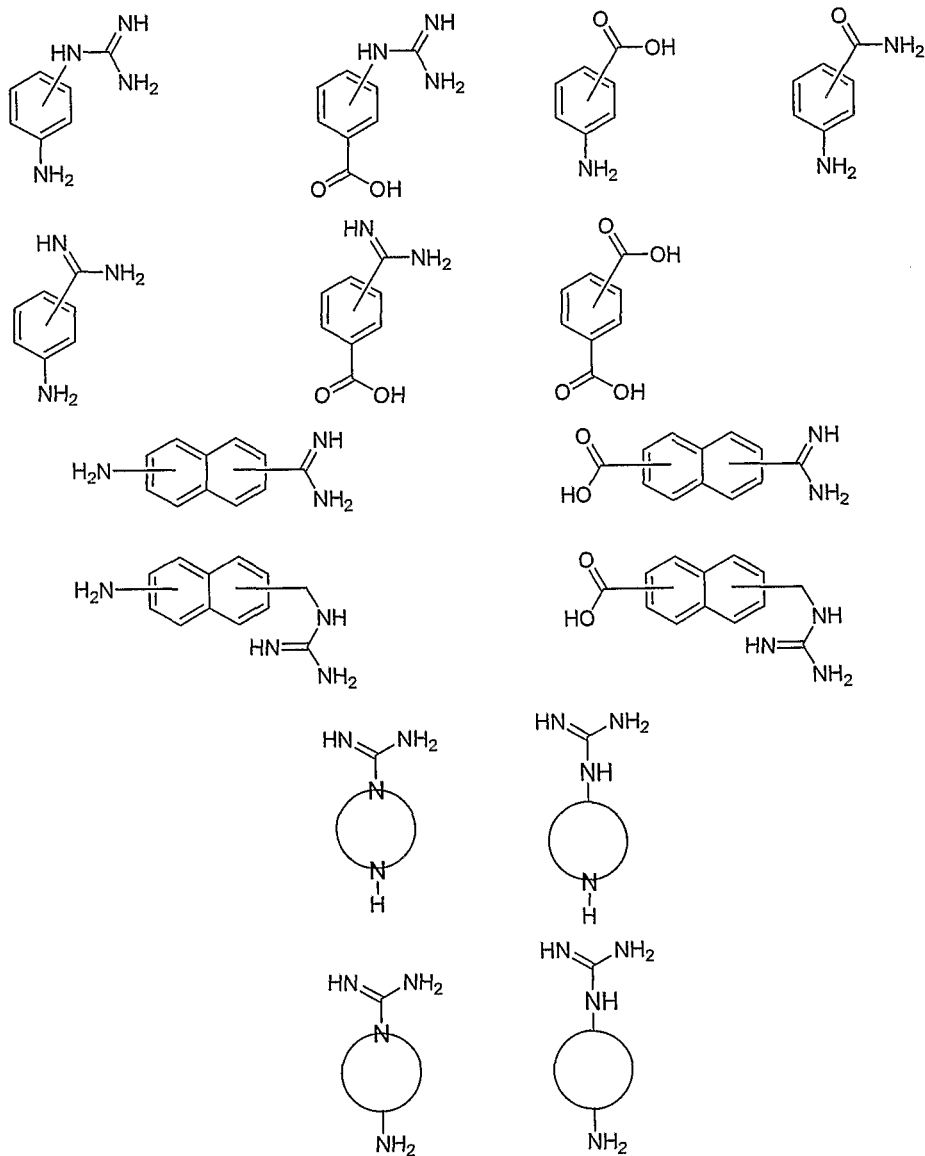
[0074] In an embodiment, there is a molecule comprising an amino acid-based composition having three independent regions: an acidic region, an aromatic or lipophilic region, and a basic region. The relative locations of the regions with respect to one another can vary between molecular mediators; the molecules mediate RCT regardless of the position of the three regions within each molecule. The trimeric region peptide may consist of natural D- or L- amino acids, amino acid analogs, and amino acid derivatives.

[0075] In another preferred variation, the molecular mediators comprising an amino acid-based trimeric structure can be capped by a lipophilic group(s) on the amino or carboxyl terminal at either end to improve the physicochemical properties of the molecular mediators of RCT and take advantage of the natural or active transport (absorption) system of fat or lipophilic materials into the body. The capping groups may be D or L enantiomers or non-enantiomeric molecules or groups. In preferred embodiments, the N-terminal capping

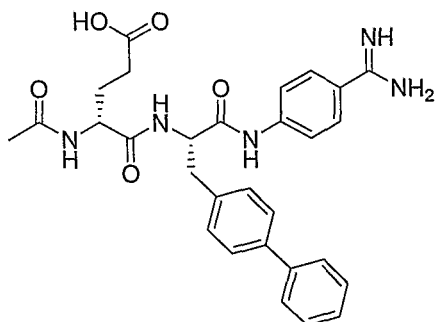
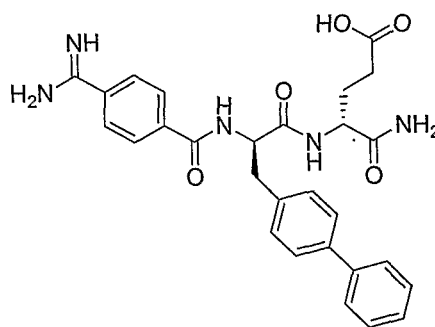
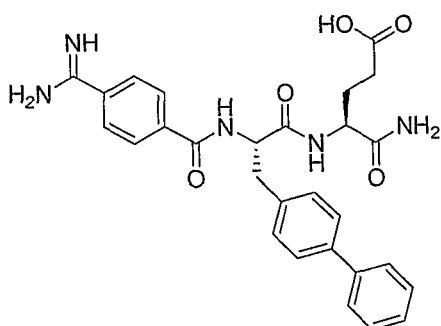
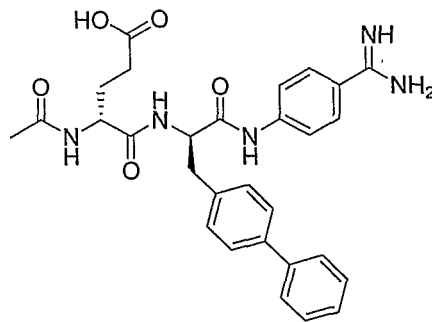
groups are selected from the group consisting of formyl, acetyl, phenylacetyl, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl and the like. The C-terminal is preferably capped with an amine such as RNH_2 where $\text{R} = \text{H}$, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, substituted saturated heteroaryl, and the like.

Bioisosteres Used Within the Structures of the Mediators of RCT

[0076] Examples of preferred molecular bioisosteres that can be used within preferred RCT mediators are shown below. Bioisosteres containing a guanidium or amidino group serve to substitute amino acids, such as arginine. Bioisosteres containing a carboxylic acid serve to substitute amino acids, such as glutamate. Any other bioisostere that can serve to substitute the basic amino acids, arginine, lysine, or histidine, and the acidic amino acids, glutamate and aspartate are contemplated. Circles represent acyclic or cyclic structures, including non-aromatic and aromatic structures.



[0077] Examples of preferred molecular bioisosteric versions of RCT mediators are shown below.

BIOISOSTERE SERIES:**L-AMINO ACIDS SEQUENCE****D-AMINO ACIDS SEQUENCE****Analysis of Structure and Function**

[0078] The structure and function of the mediators of RCT of the preferred embodiments, including the multimeric forms described above, can be assayed in order to select active compounds. For example, the peptides or peptide analogues can be assayed for their ability to bind lipids, to form complexes with lipids, to activate LCAT, and to promote cholesterol efflux, etc.

[0079] 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 nuclear magnetic resonance (NMR) assays described, *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, *infra*. The ability of the peptides and/or peptide analogues to activate LCAT can be readily determined using the LCAT activation described, *infra*. The *in vitro* and *in vivo* assays described, *infra*, can be used to evaluate the half-life, distribution, cholesterol efflux and effects on RCT.

[0080] In one preferred embodiment, there is a molecule comprising an amino acid-based composition having three independent regions: an acidic region, an aromatic or lipophilic region, and a basic region. The relative locations of the regions with respect to one another can vary between molecular mediators; the molecules mediate RCT regardless of the position of the three regions within each molecule.

[0081] In another preferred embodiment, the aromatic region of the trimer may consist of nicotinic acid with an acidic or basic side chain(s).

[0082] In another preferred embodiment, the aromatic region of the trimer may consist of 4-phenyl phenylalanine.

[0083] The abbreviations used for the D-enantiomers of the genetically encoded amino acids are lower-case equivalents of the one-letter symbols shown in Table 1. For example, "R" designates L-arginine and "r" designates D-arginine. Unless otherwise specified (eg. "OH"), the N-terminus is acetylated and the C-terminus is amidated. PhAc denotes phenylacetylated, and BIP denotes biphenylalanine.

[0084] Amino acid substitutions need not be, and in certain embodiments preferably are not, restricted to the genetically encoded amino acids. Thus, in addition to the naturally occurring genetically encoded amino acids, amino acid residues in the peptide mediators of RCT may be substituted with naturally occurring non-encoded amino acids and synthetic amino acids.

Preferred Mediators

[0085] In preferred embodiments, the mediator may be selected from the group consisting of BenOMe - bip - Aniline, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid, 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(pentamethylene)butanoic

acid, 4-((S)-1-(4-guanidinophenylcarbamoyl)-2-(biphenyl)ethylcarbamoyl)benzoic acid, 3-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid, 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid, and 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid.

Synthetic Methods

[0086] The mediators of the preferred embodiments 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.

[0087] The mediators of RCT 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 Fla., and references cited therein; *Solid Phase Peptide Synthesis: A Practical Approach*, Atherton & Sheppard, Eds., 1989, IRL Press, Oxford, England, and references cited therein).

[0088] In conventional solid-phase synthesis, attachment of the first amino acid entails chemically reacting its carboxyl-terminal (C-terminal) end with derivatized resin to form the carboxyl-terminal end of the oligopeptide. The alpha-amino end of the amino acid is typically blocked with a t-butoxy-carbonyl group (Boc) or with a 9-fluorenylmethyloxycarbonyl (Fmoc) group to prevent the amino group which could otherwise react from participating in the coupling reaction. The side chain groups of the amino acids, if reactive, are also blocked (or protected) by various benzyl-derived protecting groups in the form of ethers, thioethers, esters, and carbamates.

[0089] The next step and subsequent repetitive cycles involve deblocking the amino-terminal (N-terminal) resin-bound amino acid (or terminal residue of the peptide chain) to remove the alpha-amino blocking group, followed by chemical addition (coupling) of the next blocked amino acid. This process is repeated for however many cycles are necessary to synthesize the entire peptide chain of interest. After each of the coupling and deblocking steps, the resin-bound peptide is thoroughly washed to remove any residual reactants before proceeding to the next. The solid support particles facilitate removal of reagents at any given step as the resin and resin-bound peptide can be readily filtered and washed while being held in a column or device with porous openings.

[0090] Synthesized peptides may be released from the resin by acid catalysis (typically with hydrofluoric acid or trifluoroacetic acid), which cleaves the peptide from the resin leaving an amide or carboxyl group on its C-terminal amino acid. Acidolytic cleavage also serves to remove the protecting groups from the side chains of the amino acids in the synthesized peptide. Finished peptides can then be purified by any one of a variety of chromatography methods.

[0091] In accordance with a preferred embodiment, the peptides and peptide derivative mediators of RCT were synthesized by solid-phase synthesis methods with N^a-Fmoc chemistry. N^a-Fmoc protected amino acids and Rink amide MBHA resin from Novabiochem (San Diego, CA) or Chem-Impex Intl (Wood Dale, IL) and Sasrin resin purchased from Aldrich (Milwaukee, WI). Other chemicals and solvents were purchased from the following sources: trifluoroacetic acid (TFA), anisole, 1,2-ethanedithiol, thioanisole, piperidine, acetic anhydride, 2-Naphthoic acid and Pivaloic acid (Aldrich, Milwaukee, WI), HOBt and NMP (Chem-Impex Intl, Wood Dale, IL), dichloromethane, methanol and HPLC grade solvents from Fischer Scientific, Pittsburgh, PA. The purity of the peptides was checked by LC/MS. The purification of the peptides was achieved using Preparative HPLC system (Agilent technologies, 1100 Series) on a C₁₈-bonded silica column (Tosoh Biospec preparative column, ODS-80TM, Dim: 21.5 mm x 30cm). The peptides were eluted with a gradient system [50% to 90% of B solvent (acetonitrile:water 60:40 with 0.1% TFA)].

[0092] All peptides were synthesized in a stepwise fashion via the solid-phase method, using Rink amide MBHA resin (0.5-0.66 mmol/g) or Sasrin resin (0.6-1.1 mmol/g). The side chain's protecting groups were Arg (Pbf), Glu (OtBu) and Asp (OtBu). Each Fmoc-protected amino acid was coupled to this resin using a 1.5 to 3-fold excess of the protected amino acids. The coupling reagents were N-hydroxybenzotriazole (HOBt) and diisopropyl carbodiimide (DIC), and the coupling was monitored by Ninhydrin test. The Fmoc group were removed with 20% piperidine in NMP 30-60 minutes treatment and then successive washes with CH₂Cl₂, 10%TEA in CH₂Cl₂, Methanol and CH₂Cl₂. Coupling steps were followed by acetylation or with other capping groups as necessary.

[0093] A mixture of TFA, thioanisole, ethanedithiol and anisole (90:5:3:2, v/v) was used (4-5 hours at room temperature) to cleave the peptide from the peptide-resin and

remove all of the side chain protecting groups. The crude peptide mixture was filtered from the sintered funnel, which was washed with TFA (2-3 times). The filtrate was concentrated into thick syrup and added into cold ether. The peptide precipitated as a white solid after keeping overnight in the freezer and centrifugation. The solution was decanted and the solid was washed thoroughly with ether. The resulting crude peptide was dissolved in buffer (acetonitrile:water 60:40 with 0.1% TFA) and dried. The crude peptide was purified by HPLC using preparative C-18 column (reverse phase) with a gradient system 50 – 90% B in 40 minutes [Buffer A: water containing 0.1% (v/v) TFA, Buffer B: Acetonitrile:water (60:40) containing 0.1% (v/v) TFA]. The pure fractions were concentrated over Speedvac. The yields varied from 5% to 20%.

[0094] Alternatively, the peptides of the preferred embodiments may be prepared by way of segment condensation, i.e., the joining together of small constituent peptide chains to form a larger peptide chain, 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; Schnolzer and Kent, 1992, *Science* **256**:221-225; Liu and Tam, 1994, *J. Am. Chem. Soc.* **116**(10):4149-4153; Liu and Tam, 1994, *PNAS. USA* **91**:6584-6588; Yamashiro and Li, 1988, *Int. J. Peptide Protein Res.* **31**:322-334; 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; the disclosures of which are incorporated herein in their entirety by reference thereto). Other methods useful for synthesizing the peptides of the preferred embodiments are described in Nakagawa et al., 1985, *J. Am. Chem. Soc.* **107**:7087-7092.

[0095] For peptides produced by segment condensation, the coupling efficiency of the condensation step can be significantly increased by increasing the coupling time. Typically, increasing the coupling time results in increased racemization of the product (Sieber et al., 1970, *Helv. Chim. Acta* **53**:2135-2150). Mediators of RCT 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.

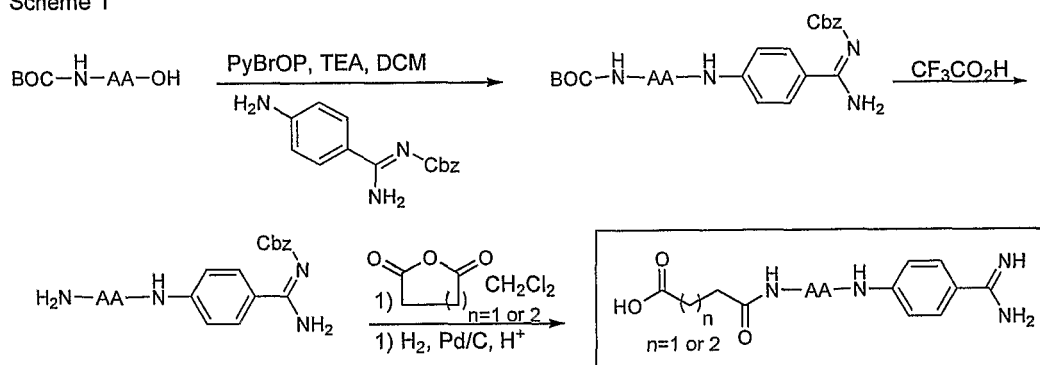
[0096] Likewise, for example, methods for deprotection of a protecting group on the N-terminus of a peptide or the C-terminus of a peptide are well-known in the art. Modes of carrying other modifications at the N- and/or C-terminus will be apparent to those of skill in the art, as will modes of deprotecting any side-chain functionalities as may be necessary to remove terminal blocking groups.

[0097] Pharmaceutically acceptable salts (counter ions) can be conveniently prepared by ion-exchange chromatography or other methods as are well known in the art.

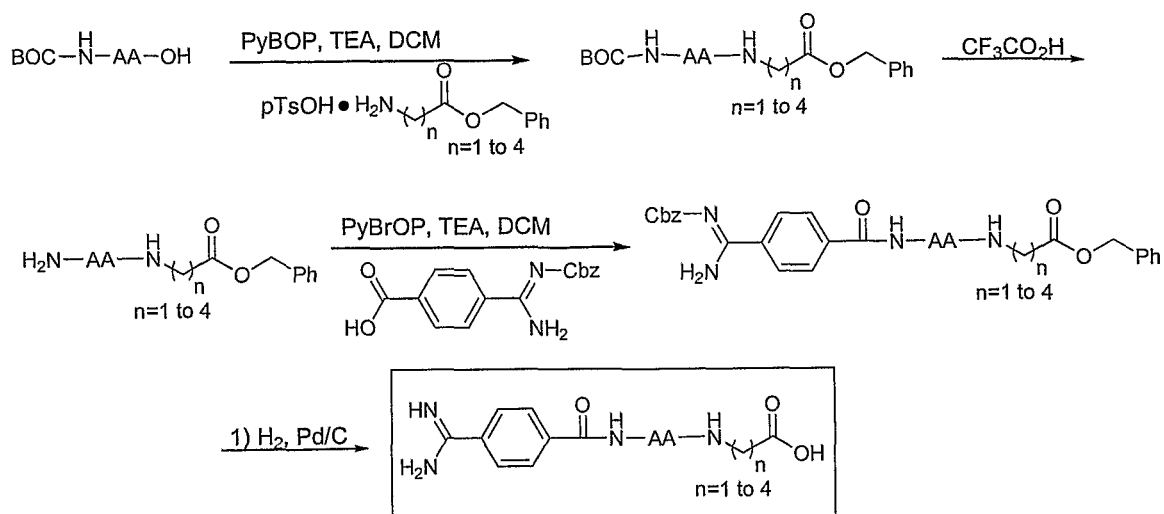
Bioisosteres Used Within the Structures of the Mediators of RCT

[0098] The synthetic schemes below show examples of methods that can be used to synthesize RCT mediators bearing bioisosteres.

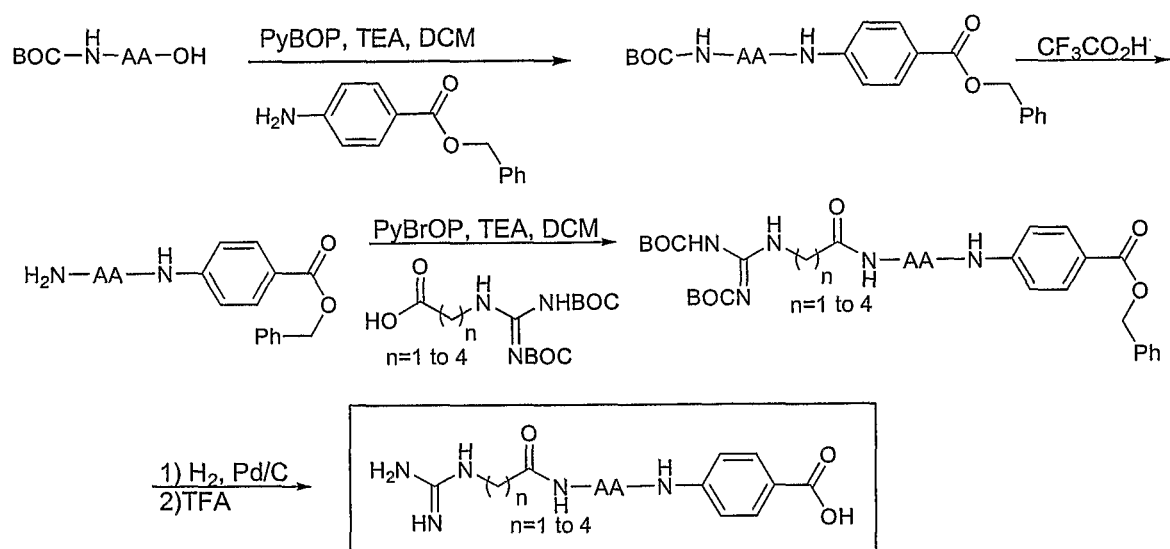
Scheme 1



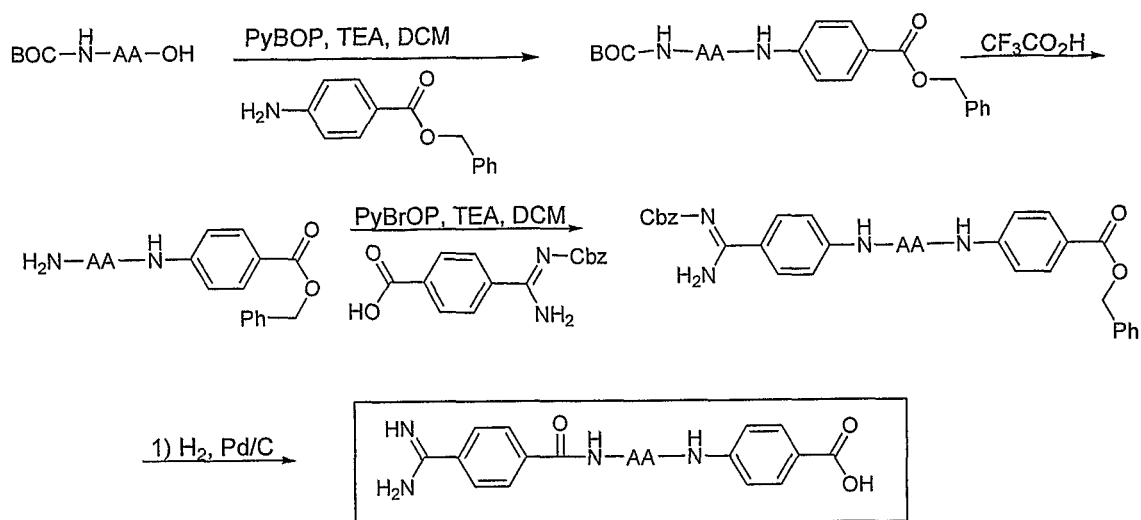
Scheme 2



Scheme 3



Scheme 4



Pharmaceutical Formulations and Methods of Treatment

[0099] The mediators of RCT of the preferred embodiments can be used to treat any disorder in animals, especially mammals including humans, for which lowering serum cholesterol is beneficial, including without limitation conditions in 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) and coronary artery disease; 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 ischemia, and endotoxemia, which often results in septic shock. The mediators of RCT 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.

[0100] For example, in the treatment of hypercholesterolemia or atherosclerosis, the formulations of molecular mediators of RCT 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 treatment 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 mediators of RCT affect RCT, increase HDL, increase LCAT activity and promote cholesterol efflux.

[0101] The mediators of RCT may be used in conjunction with fibrates to treat hyperlipidemia, hypercholesterolemia and/or cardiovascular disease such as atherosclerosis.

[0102] The mediators of RCT can be used in combination with the anti-microbials and anti-inflammatory agents currently used to treat septic shock induced by endotoxin.

[0103] The mediators of RCT can be formulated as molecule-based compositions or as molecule-lipid complexes which can be administered to subjects in a variety of ways, preferably via oral administration, to deliver the mediators of RCT to the circulation. Exemplary formulations and treatment regimens are described below.

[0104] In another preferred embodiment, methods are provided for ameliorating and/or preventing one or more symptoms of hypercholesterolemia and/or atherosclerosis. The methods preferably involve administering to an organism, preferably a mammal, more preferably a human one or more of the compounds of the preferred embodiments (or mimetics of such compounds). The compound(s) can be administered, as described herein, according to any of a number of standard methods including, but not limited to injection, suppository, nasal spray, time-release implant, transdermal patch, and the like. In one particularly preferred embodiment, the compound(s) are administered orally (e.g. as a syrup, capsule, or tablet).

[0105] The methods involve the administration of a single compound of the preferred embodiments or the administration of two or more different compounds. The compounds can be provided as monomers or in dimeric, oligomeric or polymeric forms. In certain embodiments, the multimeric forms may comprise associated monomers (e.g. ionically or hydrophobically linked) while certain other multimeric forms comprise covalently linked monomers (directly linked or through a linker).

[0106] While the preferred embodiments are described with respect to use in humans, it is also suitable for animal, e.g. veterinary use. Thus preferred organisms include,

but are not limited to humans, non-human primates, canines, equines, felines, porcines, ungulates, largomorphs, and the like.

[0107] The methods of the preferred embodiments are not limited to humans or non-human animals showing one or more symptom(s) of hypercholesterolemia and/or atherosclerosis (e.g., hypertension, plaque formation and rupture, reduction in clinical events such as heart attack, angina, or stroke, high levels of low density lipoprotein, high levels of very low density lipoprotein, or inflammatory proteins, etc.), but are useful in a prophylactic context. Thus, the compounds of the preferred embodiments (or mimetics thereof) may be administered to organisms to prevent the onset/development of one or more symptoms of hypercholesterolemia and/or atherosclerosis. Particularly preferred subjects in this context are subjects showing one or more risk factors for atherosclerosis (e.g., family history, hypertension, obesity, high alcohol consumption, smoking, high blood cholesterol, high blood triglycerides, elevated blood LDL, VLDL, IDL, or low HDL, diabetes, or a family history of diabetes, high blood lipids, heart attack, angina or stroke, etc.). The preferred embodiments include the pharmaceutical formulations and the use of such preparations in the treatment of hyperlipidemia, hypercholesterolemia, coronary heart disease, atherosclerosis, diabetes, obesity, Alzheimer's Disease, multiple sclerosis, conditions related to hyperlipidemia, such as inflammation, and other conditions such as endotoxemia causing septic shock.

[0108] In one preferred embodiment, the molecular mediators of RCT can be synthesized or manufactured using any technique described in earlier sections pertaining to synthesis and purification of the mediators of RCT. Stable preparations which have a long shelf life may be made by lyophilizing the compounds—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.

[0109] In another preferred embodiment, the mediators of RCT may be formulated and administered in a molecule-lipid complex. This approach has some 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 molecule-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 molecule-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.

[0110] A variety of methods well known to those skilled in the art can be used to prepare the molecule-lipid vesicles or complexes. To this end, a number of available techniques for preparing liposomes or proteoliposomes may be used. For example, the compound can be cosonicated (using a bath or probe sonicator) with appropriate lipids to form complexes. Alternatively the compound can be combined with preformed lipid vesicles resulting in the spontaneous formation of molecule-lipid complexes. In yet another alternative, the molecule-lipid complexes can be formed by a detergent dialysis method; e.g., a mixture of the compound, lipid and detergent is dialyzed to remove the detergent and reconstitute or form molecule-lipid complexes (e.g., see Jonas et al., 1986, *Methods in Enzymol.* **128**:553-582).

[0111] While the foregoing approaches are feasible, each method presents its own peculiar production problems in terms of cost, yield, reproducibility and safety. In accordance with one preferred method, the compound 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 should be carefully selected to ensure co-solubility of both the amphipathic compound and the lipid. In one embodiment, compound(s) or derivatives/analogs thereof, 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 compound and lipid can be incorporated into a co-solvent system; i.e., a mixture of the miscible solvents. A suitable proportion of compound 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.

[0112] The lyophilized product can be reconstituted in order to obtain a solution or suspension of the molecule-lipid complex. To this end, the lyophilized powder may be rehydrated with an aqueous solution to a suitable volume (often 5 mgs compound/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.

[0113] 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. For example, a Pharmacia Superose 6 FPLC gel filtration chromatography system can be 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 compound/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 preferably 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.

[0114] The mediators of RCT of the preferred embodiments 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, cerebroside, dilaurylphosphatidylcholine, (1,3)-D-mannosyl-(1,3)diglyceride, aminophenylglycoside, 3-cholesteryl-6'-(glycosylthio)hexyl ether glycolipids, and cholesterol and its derivatives.

[0115] The pharmaceutical formulation of the preferred embodiments contain the molecular mediators of RCT or the molecule-lipid complex as the active ingredient in a pharmaceutically acceptable carrier suitable for administration and delivery *in vivo*. As the compounds may contain acidic and/or basic termini and/or side chains, the compounds can be included in the formulations in either the form of free acids or bases, or in the form of pharmaceutically acceptable salts.

[0116] 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.

[0117] 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 mediators of RCT may be lyophilized, or the co-lyophilized molecule-lipid complex may be prepared. The stored preparations can be supplied in unit dosage forms and reconstituted prior to use *in vivo*.

[0118] 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 mediators of RCT.

[0119] 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 mediators of RCT of the preferred embodiments or the molecule-lipid complex into a nitroglycerin patch for use in patients with ischemic heart disease and hypercholesterolemia.

[0120] 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.

[0121] 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.

[0122] 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.

[0123] 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.

[0124] The molecule mediators of RCT and/or molecule-lipid complexes of the preferred embodiments may be administered by any suitable route that ensures bioavailability in the circulation. This can 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. Oral administration has the advantage of easy of use and therefore enhanced compliance. 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 preferred embodiments 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.

[0125] The actual dose of molecular mediators of RCT or molecule-lipid complex used will vary with the route of administration, and should be adjusted to achieve circulating plasma concentrations of 1.0 mg/l to 2 g/l. Data obtained in animal model systems described herein show that the ApoA-I agonists of the preferred embodiments associate with the HDL component, and have a projected half-life in humans of about five days. Thus, in one embodiment, the mediators of RCT 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 0.1 mg/kg/hr to 100 mg/kg/hr.

[0126] Toxicity and therapeutic efficacy of the various mediators of RCT can be determined using standard pharmaceutical procedures in cell culture or experimental animals for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (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 LD₅₀ /ED₅₀. ApoA-I molecular agonists which exhibit large therapeutic indices are preferred.

Other Uses

[0127] The mediators of RCT agonists of the preferred embodiments can be used in assays in vitro to measure serum HDL, e.g., for diagnostic purposes. Because the mediators of RCT associate with the HDL and LDL component of serum, the agonists can be used as "markers" for the HDL and LDL 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 mediators of RCT. 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.

[0128] 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).

Assays For Analysis of Mediators of Reverse Cholesterol Transport

LCAT Activation Assay

[0129] The mediators of RCT in accordance with preferred embodiments can be evaluated for potential clinical efficacy by various *in vitro* assays, for example, by their ability to activate LCAT *in vitro*. In the LCAT assay, substrate vesicles (small unilamellar vesicles or "SUVs") composed of egg phosphatidylcholine (EPC) or 1-palmitoyl-2-oleyl-phosphatidyl-choline (POPC) and radiolabelled cholesterol are preincubated with equivalent masses either of compound 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 molecular mediators can be calculated as the concentration of mediator that achieves maximum LCAT activation. For example, a series of concentrations of the compound (e.g., a limiting dilution) can be assayed to determine the "specific activity" for the compound--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 compound used, the "specific activity" can be identified as the concentration of compound that achieves a plateau on the plotted curve.

Preparation of Substrate Vesicles

[0130] 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 μ mol), 78 μ g (0.2 μ mol) 4-¹⁴C-cholesterol, 116 μ g cholesterol (0.3 μ mol) 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, 6×5 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 (16,000×g) to remove titanium particles. The resulting clear solution is used for the enzyme assay.

Purification of LCAT

[0131] 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.

Preparation of LPDS

[0132] To prepare LPDS, 500 ml plasma is added to 50 ml dextran sulfate (MW=500,000) solution. Stir 20 minutes. Centrifuge for 30 minutes at 3000 rpm (16,000×g) at 4°C. Use supernatant (LPDS) for further purification (ca. 500 ml).

Phenylsepharose Chromatography

[0133] The following materials and conditions were used for the phenylsepharose chromatography. Solid phase: phenylsepharose fast flow, high subst. grade, Pharmacia: 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 pH 7.4, 0.01% sodium azide.

[0134] 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.

Affigelblue Chromatography

[0135] The phenylsepharose pool is dialyzed overnight at 4°C against 20 mM Tris-HCl, pH 7.4, 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.

ConA Chromatography

[0136] The Affigelblue pool was reduced via Amicon (YM30) to 30-40 ml and dialyzed against ConA starting buffer (1 mM Tris HCl pH7.4; 1 mM MgCl₂, 1 mM MnCl₂, 1 mM CaCl₂, 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 1 mM 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.

Anti-ApoA-I Affinity Chromatography

[0137] 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.

Pharmacokinetics of the Mediators of RCT

[0138] The following experimental protocols can be used to demonstrate that the mediators of RCT are stable in the circulation and associate with the HDL component of plasma.

Synthesis and/or Radiolabeling of Compound Agonists

[0139] The ¹²⁵I-labeled LDL was prepared by the iodine monochloride procedure to a specific activity of 500-900 cpm/ng (Goldstein and Brown 1974 *J. Biol. Chem.* **249**:5153-5162). Binding and degradation of low density lipoproteins by cultured human fibroblasts were determined at final specific activities of 500-900 cpm/ng as described (Goldstein and Brown 1974 *J. Biol. Chem.* **249**:5153-5162). In every case, >99% radioactivity was precipitable by incubation of the lipoproteins at 4°C with 10% (wt/vol) trichloroacetic acid (TCA). The Tyr residue was attached to N-Terminus of each compound to enable its radioiodination. The compounds were radioiodinated with Na¹²⁵I (ICN), using

Iodo-Beads (Pierce Chemicals) and following the manufacturer's protocol, to a specific activity of 800-1000 cpm/ng. After dialysis, the precipitable radioactivity (10% TCA) of the compounds was always >97%.

[0140] Alternatively, radiolabeled compounds could be synthesized by coupling ^{14}C -labeled Fmoc-Pro as the N-terminal amino acid. L-[U- ^{14}C]X, specific activity 9.25 GBq/mmol, can be used for the synthesis of labeled agonists containing X. The synthesis may be carried out according to Lapatsanis, *Synthesis*, 1983, 671-173. Briefly, 250 μM (29.6 mg) of unlabeled L-X is 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-X. The liquid is 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 is extracted with Diethylether (2×5ml) and chloroform (1×5ml), the remaining aqueous phase is acidified with 30% HCl and extracted with chloroform (5×8 ml). The organic phase is dried over Na_2SO_4 filtered off and the volume is 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) with UV detection, e.g., radiochemical purity:Linear Analyzer, Berthold, Germany; reaction yields may be approximately 90% (as determined by LSC).

[0141] The chloroform solution containing ^{14}C -compound X is used directly for synthesis. A resin containing amino acids 2-22, can be synthesized automatically as described above and used for the synthesis. The sequence of the peptide is determined by Edman degradation. The coupling is performed as previously described except that HATU (O-(7-azabenzotriazol-1-yl)-1, 1,3,3-tetramethyluroniumhexafluorophosphate) is preferably used instead of TBTU. A second coupling with unlabeled Fmoc-L-X is carried out manually.

Pharmacokinetics in Mice

[0142] In each experiment, 300 – 500 $\mu\text{g/kg}$ (0.3 – 0.5 mg/kg) [or more such as 2.5 mg/kg] radiolabeled compound may be 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 are taken at multiple time intervals for assessment of radioactivity in plasma.

Stability in Human Serum

[0143] 100 μg of labeled compound may be 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 is carried out by extracting the lipids with an equal volume of 2:1 (v/v) chloroform:methanol. The samples are 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% w TFA). Elution profiles are followed by absorbance (220 nm) and radioactivity.

Formation of Pre- β Like Particles

[0144] Human HDL may be isolated by KBr density ultra centrifugation at density $d = 1.21 \text{ g/ml}$ to obtain top fraction followed by Superose 6 gel filtration chromatography to separate HDL from other lipoproteins. Isolated HDL is 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 is removed from the isolated HDL preparation and incubated with 100 μl labeled compound (0.2-1.0 $\mu\text{g}/\mu\text{l}$) for two hours at 37°C. Multiple separate incubations are analyzed including a blank containing 100 μl physiological saline and four dilutions of labeled compound. For example: (i) 0.20 $\mu\text{g}/\mu\text{l}$ compound:HDL ratio = 1:15; (ii) 0.30 $\mu\text{g}/\mu\text{l}$ compound:HDL ratio = 1:10; (iii) 0.60 $\mu\text{g}/\mu\text{l}$ compound:HDL ratio = 1:5; and (iv) 1.00 $\mu\text{g}/\mu\text{l}$ compound:HDL ratio = 1:3. Following the two hour incubation, a 200 μl aliquot of the sample (total volume = 400 μl) is loaded onto a Superose 6 gel filtration column for lipoprotein separation and analysis and 100 μl is used to determine total radioactivity loaded.

Association of Mediators With Human Lipoproteins

[0145] The association of molecular mediators with human lipoprotein fractions can be determined by incubating labeled compound with each lipoprotein class (HDL, LDL and VLDL) and a mixture of the different lipoprotein classes. HDL, LDL and VLDL are isolated by KBr density gradient ultracentrifugation at $d = 1.21 \text{ g/ml}$ and purified by FPLC on a Superose 6B column size exclusion column (chromatography is carried out with a flow rate of 0.7 ml/min and a running buffer of 1 mM Tris (pH 8), 115 mM NaCl, 2 mM EDTA and 0.0% NaN_3). Labeled compound is incubated with HDL, LDL and VLDL at a compound: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) is mixed with 0.2 ml of compound stock solution (1 mg/ml) and the solution is brought up to 2.2 ml using 0.9% of NaCl.

[0146] After incubating for 2 hr at 37°C, an aliquot (0.1 ml) is removed for determination of the total radioactivity (e.g., by liquid scintillation counting or gamma counting depending on labeling isotope), the density of the remaining incubation mixture is 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 is 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 is used for counting. The top two fractions contain the floating lipoproteins, the other fractions (3-5) correspond to compound in solution.

Selective Binding to HDL Lipids

[0147] Human plasma (2 ml) is incubated with 20, 40, 60, 80, and 100 µg of labeled compound for 2 hr at 37°C. The lipoproteins are 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) is taken for the analysis. 50 µl from each 300 µl fraction is counted for radioactivity and 200 µl from each fraction is analyzed by FPLC (Superose 6/Superose 12 combination column).

Use of the Mediators of Reverse Cholesterol Transport in Animal Model Systems

[0148] The efficacy of the mediators of RCT of the preferred embodiments can be demonstrated in rabbits or other suitable animal models.

Preparation of the Phospholipid/Compound Complexes

[0149] Small discoidal particles consisting of phospholipid (DPPC) and compound are prepared following the cholate dialysis method. The phospholipid is dissolved in chloroform and dried under a stream of nitrogen. The compound is dissolved in buffer (saline) at a concentration of 1-2 mg/ml. The lipid film is redissolved in buffer containing cholate (43°C) and the compound solution is added at a 3:1 phospholipid/compound weight ratio. The mixture is incubated overnight at 43° C and 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 may be filter sterilized (0.22 μ m) for injection and storage at 4°C.

Isolation and Characterization of the Compound/Phospholipid Particles

[0150] The particles may be separated on a gel filtration column (Superose 6 HR). The position of the peak containing the particles is identified by measuring the phospholipid concentration in each fraction. From the elution volume, the Stokes radius can be determined. The concentration of compound in the complex is determined by measuring the phenylalanine content (by HPLC) following a 16 hr acid hydrolysis.

Injection in the Rabbit

[0151] Male New Zealand White rabbits (2.5-3 kg) are injected intravenously with a dose of phospholipid/compound complex (5 or 10 mg/kg bodyweight, expressed as compound) in a single bolus injection not exceeding 10-15 ml. The animals are slightly sedated before the manipulations. Blood samples (collected on EDTA) are taken before and 5, 15, 30, 60, 240 and 1440 minutes after injection. The hematocrit (Hct) is determined for each sample. Samples are aliquoted and stored at -20°C before analysis.

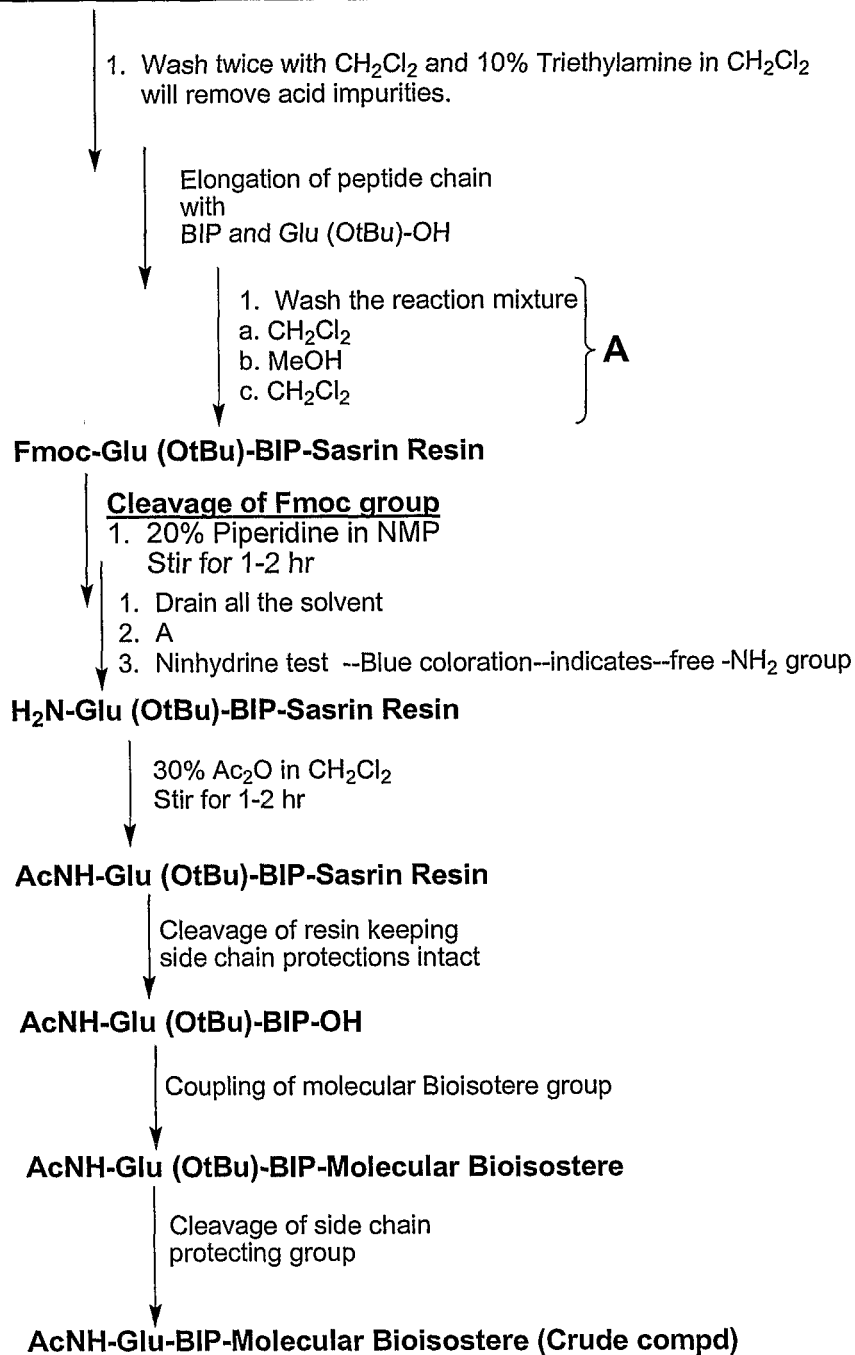
Analysis of the Rabbit Sera

[0152] The total plasma cholesterol, plasma triglycerides and plasma phospholipids are determined enzymatically using commercially available assays, for example, according to the manufacturer's protocols (Boehringer Mannheim, Mannheim, Germany and Biomerieux, 69280, Marcy-L'etoile, France).

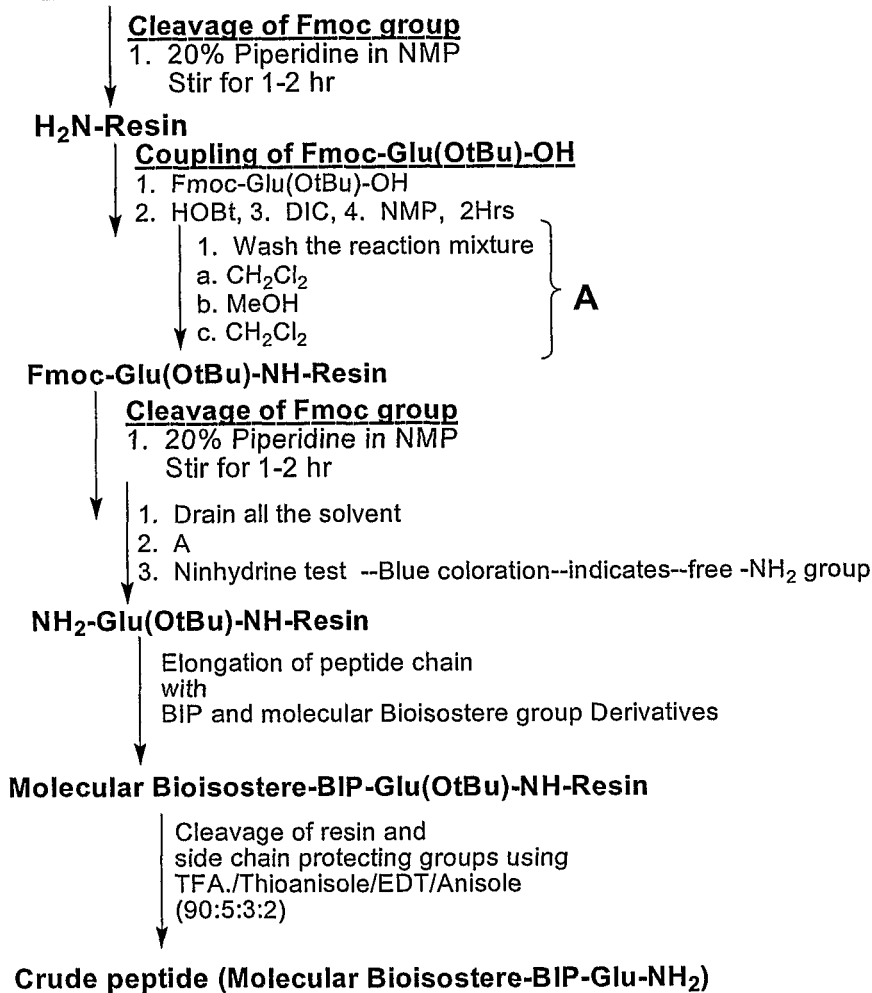
[0153] The plasma lipoprotein profiles of the fractions obtained after the separation of the plasma into its lipoprotein fractions may be determined by spinning in a sucrose density gradient. For example, fractions are collected and the levels of phospholipid and cholesterol can be measured by conventional enzymatic analysis in the fractions corresponding to the VLDL, ILDL, LDL and HDL lipoprotein densities.

Synthesis of RCT Mediators Bearing Bioisosteres

[0154] These compounds have been prepared by using standard SPPS protocol using Sasrin Resin (4-hydroxy-2-methoxybenzyl alcohol, Aldrich) and Rink amide MBHA resin.

SASRIN RESIN (100-200 MESH) (1G) (Substitution 0.6-1.1 mmol/g)

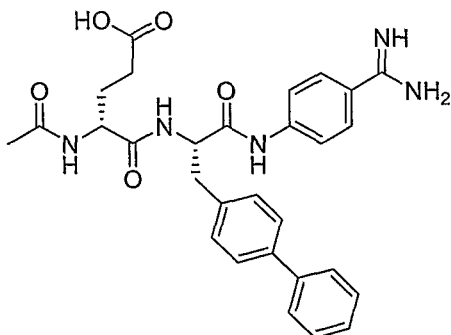
Rink amide MBHA Resin (1G) (Substitution 0.66 mmol/g)
Fmoc-HN-Resin



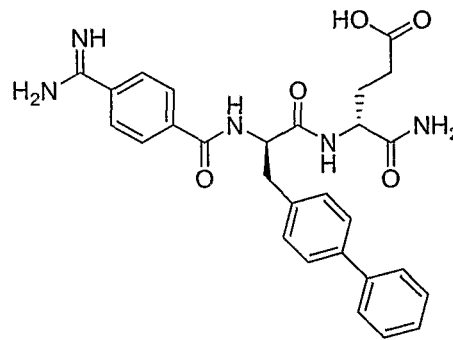
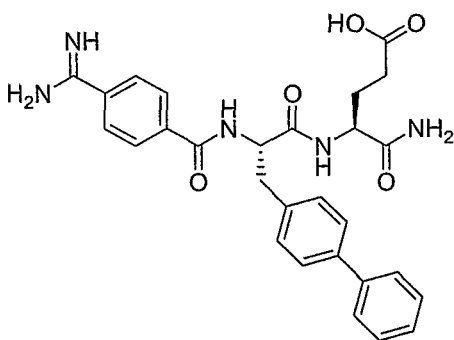
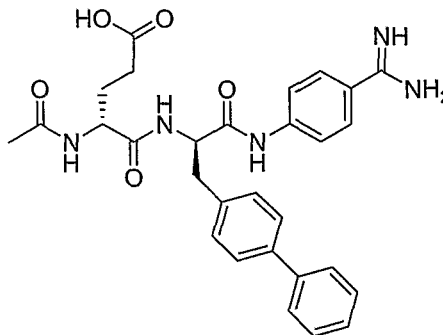
[0155] Examples of synthesized compounds include the following:

Bioisostere sequence:

L-AMINO ACIDS SEQUENCE



D-AMINO ACIDS SEQUENCE



General Analytical Methods.

[0156] All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from commercial sources. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F₂₅₄, Merck, and preparative TLC was performed on 20 cm × 20 cm glass plates coated with a 2 mm layer of silica gel PF₂₅₄, Merck. Silica gel 60 (230-400 mesh), Merck, was used for flash chromatography. Melting points were taken on a micro-hot-stage apparatus and are uncorrected. ¹H NMR spectra were recorded with Bruker 400 spectrometer, operating at 400 MHz, using TMS or solvent as reference. Elemental analyses were carried out at

NuMega Resonance Laboratories, San Diego. Preparative reverse-phase HPLC (Glison) of the final products was performed on a Phenomenex Luna 5 μ C₁₈ (2) (60 mm $\times$ 21.2 mm) column with a flow rate of 15 mL/min, using a tunable UV detector set at 254 nm. Mixtures of CH₃CN and H₂O were used as mobile phases in gradient mode (CH₃CN = 5 % - 95 %). Analysis by LC/UV/ELSD/MS was performed using an API 150 EX instrument from PE Sciex. ESI-MS experiments were performed, in positive mode.

General Procedure A (Amide Coupling)

[0157] To a mixture of acid (1.05 equiv.), amine (1.00 equiv.), and HOBt (1.05 equiv.) in anhydrous CH₂Cl₂ (20 mL) was added Et₃N (1.5 equiv.). EDCI (1.05 equiv.) was added and stirred under nitrogen at rt overnight (16 h). Then water (15 mL) was added and stirred at rt for 5 min. The layers were separated and the aq. layer was extracted with CH₂Cl₂ (2 x 15 mL). The combined organic layers were washed successively with water (15 mL), brine (15 mL) and dried (Na₂SO₄). After filtration the solvent was removed in a rotary evaporator and dried *in vacuo* to obtain the expected product.

[0158] In a few cases, the volatiles were removed in a rotary evaporator after the reaction was complete, and the residue was stirred with water (25 mL) at rt for 15 min. The heterogeneous mixture was filtered, washed with water (3 x 25 mL) and dried to afford the desired amide.

General Procedure B (Fmoc Deprotection)

[0159] To N-Fmoc derivative (1.0 mmol) in CH₂Cl₂ (20 mL) 4-aminomethylpiperidine (4-AMP) (5 mL) was added and stirred at rt for 16 h. The volatiles were removed in a rotary evaporator. The crude was retaken in CH₂Cl₂ (50 mL) and successively washed with phosphate buffer pH 5.5 (4 x 25 mL), water (25 mL), brine (25 mL), and dried (Na₂SO₄). After filtration the solvent was removed in a rotary evaporator and dried *in vacuo* to obtain the expected amine product.

General Procedure C (Reaction of Amine with Acid Anhydride)

[0160] To the amine (1.0 mmol) in THF (25 mL) acid anhydride (1.5 to 2.0 mmol) was added and stirred under nitrogen at rt for 24 h. The volatiles were removed in a rotary evaporator. Either the crude was used in the subsequent reaction or purified from reverse-phase HPLC column.

General Procedure D (Deprotection of N-Boc Group)

[0161] The N-Boc derivative (1.0 mmol) was stirred in 1: 1 trifluoroacetic acid (TFA)/CH₂Cl₂ (10 mL) under nitrogen at rt for 4 h. The volatiles were removed in a rotary evaporator. The crude was stirred with aq. NaHCO₃ (15 mL) (Caution! CO₂ gas evolution) for 1h. In a few cases, to obtain fine solids, longer stirring time may be needed. The solids were filtered, washed with water (3 x 25 mL) and dried to furnish the free amine.

[0162] When solids were not formed or the material became gummy upon stirring with NaHCO₃, the product was extracted with CH₂Cl₂ (2 x 25 mL) and the combined organics sequentially were washed with water (25 mL), brine (25 mL), and dried (Na₂SO₄). After filtration the solvent was removed in a rotary evaporator and dried *in vacuo* to obtain the expected amine product.

General Procedure E (Ester Hydrolysis)

[0163] To a stirred solution of the ester (1.0 mmol) in MeOH-THF (3:2, 15 mL) aq. 1 N NaOH (4.0 mL, 4.0 mmol) was added and stirred at rt under nitrogen overnight (16 h). The volatiles then were removed in a rotary evaporator and 2 M NaHSO₄ (2 mL) was added to neutralize the base. The acid was extracted with EtOAc (3 x 15 mL). The combined organic extracts successively were washed with water (20 mL), brine (20 mL), and dried (Na₂SO₄). After filtration the solvent was removed in a rotary evaporator and dried *in vacuo* to obtain the acid product.

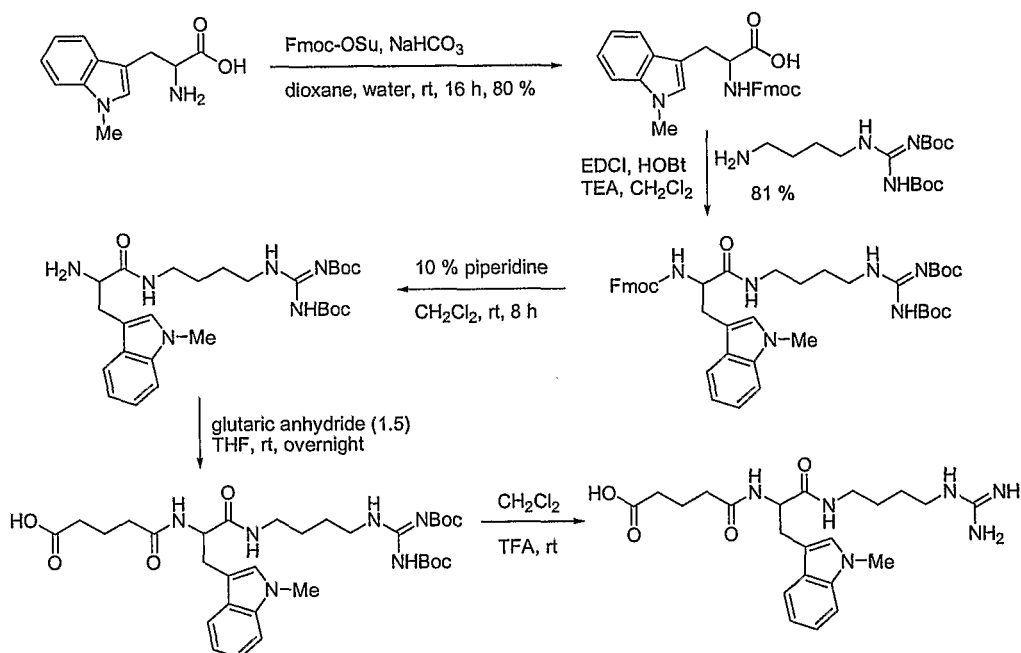
General Procedure F (Guanidinylation of Amine)

[0164] To a solution of amine (1.0 mmol) in CHCl₃ (15 mL), Et₃N (1.5 mmol) was added followed by addition of 1,3-*di*-Boc-2-(trifluoromethylsulfonyl)guanidine (Goodman's reagent) (1.5 mmol). The homogeneous reaction was stirred under nitrogen at rt for 3 days, then additional amounts of Et₃N (1.5 mmol) and 1,3-*di*-Boc-2-(trifluoromethylsulfonyl)guanidine (1.5 mmol) were added and stirred for 3 more days. The reaction contents successively were washed with 2 M NaHSO₄ (10 mL), aq. NaHCO₃ (10 mL) and brine (10 mL). The solvent was evaporated to dryness to furnish *bis*-Boc-guanidine derivative.

General Procedure G (Reduction of Nitro Group)

[0165] To a solution of nitro compound (1.0 mmol) in MeOH (25 mL) or MeOH-THF (2:1, 25 mL), 10 % Pd/C (0.075 g) was added under argon. The reaction was stirred with a hydrogen balloon overnight (16 h). Then the reaction was degassed, purged with argon, and filtered through a pad of Celite® 545. The reaction flask was rinsed with MeOH and passed through the filter cake. The combined washings were concentrated to produce the amine product.

SCHEME-5

4-(1-(4-Guanidinobutylcarbamoyl)-2-(1-methyl-1H-indol-3-yl)ethylcarbamoyl)butanoic acid

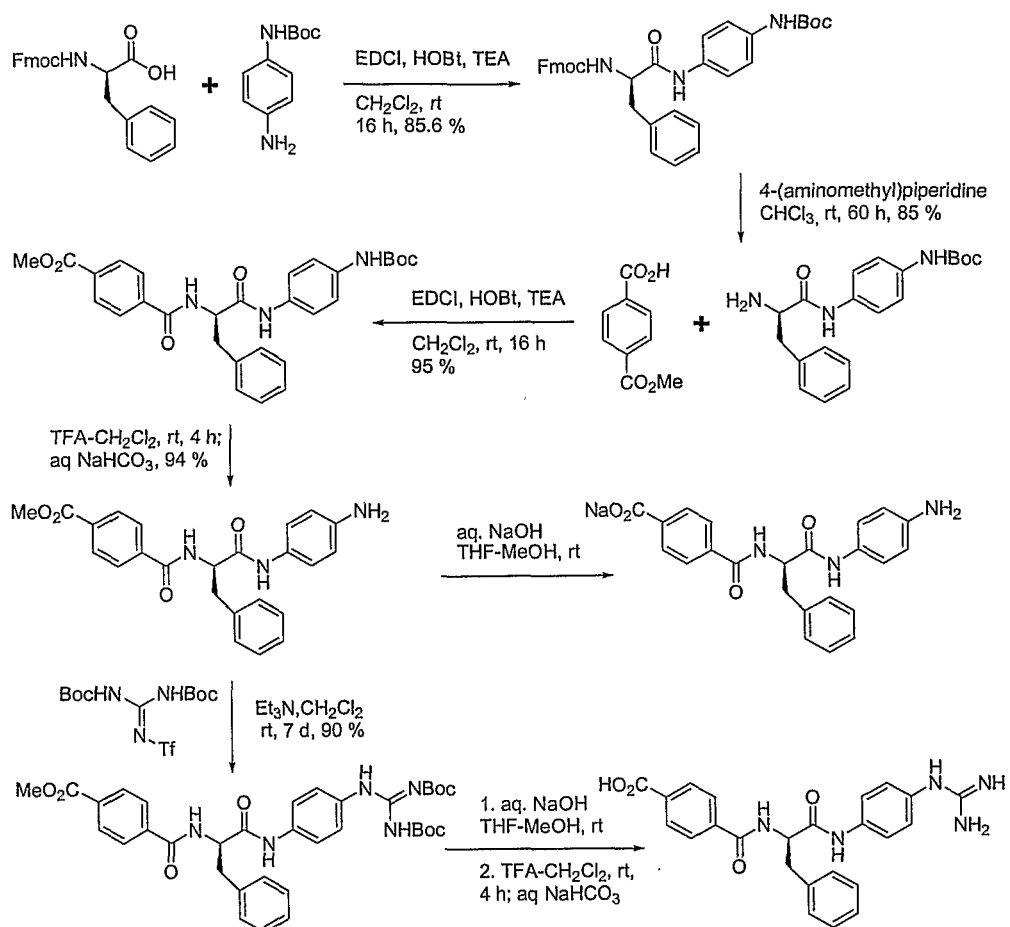
[0166] To a stirred suspension of the racemic 2-amino-3-(1-methyl-1H-indol-3-yl)propanoic acid (2.10 g, 9.62 mmol) and NaHCO₃ in 175 mL H₂O-dioxane (1:2) was added Fmoc-OSu and stirred at rt (Scheme 5). The volatiles were removed in a rotary evaporator and the residue was taken up in 100 mL ice-water and acidified with 5N HCl (aq.) to pH ~ 3.0. The precipitate was filtered, washed with water (3 x 50 mL), dried and triturated with ether (30 mL) to furnish 2-(Fmoc-amino)-3-(1-methyl-1H-indol-3-yl)propanoic acid as off-white fluffy solids (3.40 g, 80 %).

[0167] The 2-(Fmoc-amino)-3-(1-methyl-1H-indol-3-yl)-propanoic acid (0.463 g, 1.05 mmol) was reacted with (N^{2,3}-di-*tert*-butoxycarbonyl)agmatine (0.331 g, 1.00 mmol), EDCI (0.23 g, 1.20 mmol), HOBt (0.142 g, 1.05 mmol) and Et₃N (0.152 g, 1.50 mmol) according to the General Procedure A. N-Fmoc-(1-(4-(N^{2,3}-di-*tert*-butoxycarbonyl)guanidinobutylcarbamoyl)-2-(1-methyl-1H-indol-3-yl)ethylamine) was isolated in 81 % yield (0.64 g).

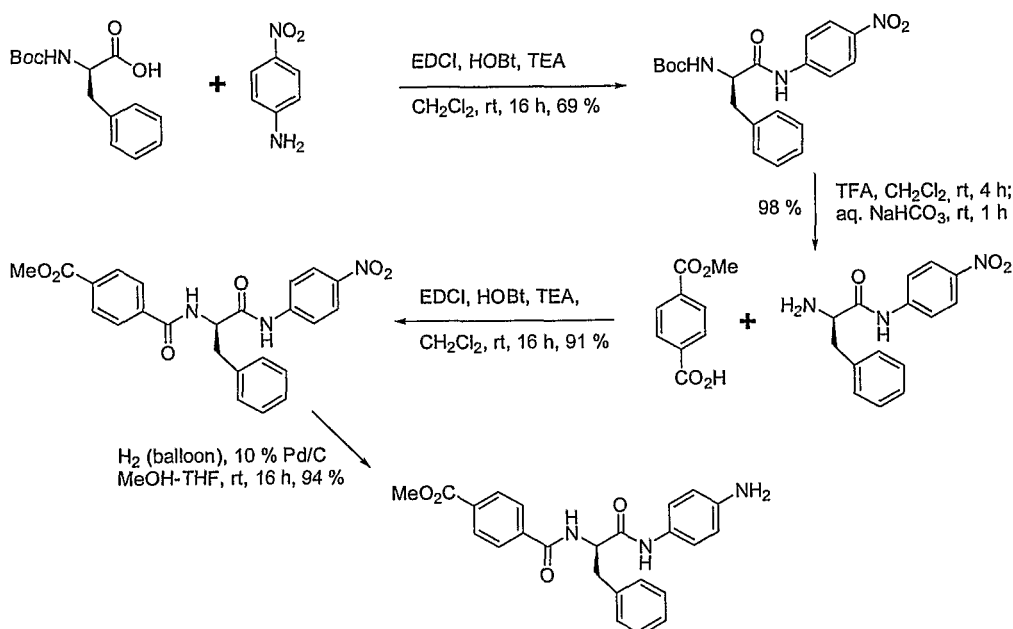
[0168] The above N-Fmoc derivative (1.10 g, 1.46 mmol) upon treatment with piperidine (5 mL) according to the General Procedure B yielded 1.28 g of the crude. It contaminated with the Fmoc-derived byproduct and was used in the following step without further purification.

[0169] The above crude amine [1-(4-(N^{2,3}-di-*tert*-butoxycarbonyl)guanidinobutylcarbamoyl)-2-(1-methyl-1H-indol-3-yl)ethylamine] (0.36 g, 0.68 mmol) in THF (10 mL) was reacted with glutaric anhydride (0.116 g, 1.02 mmol) according to the General Procedure C. The crude (*bis*-Boc derivative) was treated with TFA, according to the General Procedure D. The volatiles were removed in a rotary evaporator. The crude was taken in DMSO and treated with NaHCO₃ (0.15 g) for 1 h. Then minimal amounts of water (0.7 mL) was added and passed through a syringe filter-disc (Whatman, PTFE, 0.45 µm, 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.03 g of the 4-(1-(4-guanidinobutylcarbamoyl)-2-(1-methyl-1H-indol-3-yl)ethylcarbamoyl)butanoic acid as white solid. Mp 291°C. HPLC [Phenomenex Luna 5µ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 7.53 min (CH₃CN-H₂O = 45:55). ¹H NMR (DMSO-d₆, δ in ppm): 9.97 (s, 1 H), 8.12 (d, J = 4.0 Hz, 1 H), 8.02 (d, J = 8.8 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.35 (d, J = 7.2 Hz, 1 H), 7.04 (t, J = 7.4 Hz, 1 H), 7.00 (s, 1 H), 6.92 (d, J = 7.4 Hz, 2 H), 4.37 (dt, J = 8.8, 4.0 Hz, 1 H), 3.63 (s, 3 H), 3.30 - 3.00 (m, 3 H), 2.95 - 2.85 (m, 3 H), 2.15 - 2.08 (m, 1 H), 1.94 - 1.85 (m, 2 H), 1.81 - 1.74 (m, 1 H), 1.68 - 1.52 (m, 3 H), 1.42 - 1.34 (m, 3 H). MS: [EI] m/e 445.5 [M+H]⁺. Anal: (C₂₂H₃₂N₆O₄ + 1.89 H₂O + 0.18 CF₃CO₂H) C, H, N.

SCHEME-6



SCHEME-7



(R)-{N-[1-(4-Amino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester}

[0170] The Fmoc-D-Phe-OH (3.50 g, 9.0 mmol) was allowed to react with N-Boc-*p*-phenylenediamine (1.79 g, 8.6 mmol), HOBt (1.22 g, 9.0 mmol), Et₃N (1.04 g, 10.3 mmol), and EDCI (1.73 g, 9.0 mmol) in anhydrous CH₂Cl₂ (50 mL) according to the General Procedure A (Scheme 6). The volatile materials were removed in a rotary evaporator and the residue was stirred with water (25 mL) at rt for 15 min. The off-white solids were filtered, washed with water (3 x 25 mL) and dried to afford (R)-{2-benzyl-N-(4-tert-butoxycarbonylamino-phenyl)-malonamic acid 9H-fluoren-9-ylmethyl ester} in 85.6 % (4.47 g) yield.

[0171] The above Fmoc-derivative (2.1 g, 3.63 mmol) was treated with 4-AMP (2.07 g, 18.2 mmol) for 60 h, according to the General Procedure B. Upon extractive work-up and evaporation, (R)-{[4-(2-amino-3-phenyl-propionylamino)-phenyl]-carbamic acid tert-butyl ester} was obtained as pale-yellow solid (1.1 g, 85 %).

[0172] Then *mono*-methyl terephthalate (0.51 g, 2.8 mmol) was allowed to react with (R)-{[4-(2-amino-3-phenyl-propionylamino)-phenyl]-carbamic acid tert-butyl ester}

(1.00 g, 2.8 mmol), HOBt (0.38 g, 2.8 mmol), Et₃N (0.34 g, 3.4 mmol), and EDCI (0.54 g, 2.8 mmol) in anhydrous CH₂Cl₂ (20 mL) according to the General Procedure A. The volatile materials were removed in a rotary evaporator and the residue was stirred with water (15 mL) at rt for 15 min. The heterogeneous mixture were filtered, washed with water (3 x 25 mL) and dried to afford the desired amide (R)-{N-[1-(4-tert-butoxycarbonylamino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} (1.38 g, 94.8 %) as pale-yellow solid.

[0173] The N-Boc group in (R)-{N-[1-(4-tert-butoxycarbonylamino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} (1.0 g, 1.93 mmol) was deprotected according to the General Procedure D for 3 h. The solids were filtered, washed with water (3 x 25 mL) and dried to furnish the free aniline (0.76 g, 94 %). This amine also was prepared by reduction of a nitro compound, as shown in Scheme 7. 0.075 g of the crude was dissolved in minimal volume of DMSO (1.2 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 μm, 13 mm), then purified from reverse-phase HPLC column. HPLC [Phenomenex Luna 5μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.10 min (CH₃CN-H₂O = 55:45). The fraction containing the pure material were combined and lyophilized to obtain 0.048 g of (R)-{N-[1-(4-Amino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} as pale-yellow solid. Mp 240-2 °C. ¹H NMR (CDCl₃, δ in ppm): 8.03 (d, J = 8.4 Hz, 2 H), 7.72 (d, J = 8.0 Hz, 2 H), 7.28 - 7.15 (m, 7 H), 6.93 (d, J = 8.8 Hz, 2 H), 4.91 - 4.82 (m, 1 H), 3.88 (s, 3 H), 3.29 (dd, J = 14.0, 6.4 Hz, 1 H), 3.14 (dd, J = 14.0, 8.0 Hz, 1 H). MS: [EI] m/e 418.5 [M+H]⁺. Anal: (C₂₄H₂₃N₃O₄ + 0.25 H₂O) C, H, N.

(R)-{N-[1-(4-Amino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid}

[0174] Employing the General Procedure E, the ester in methyl (R)-{N-[1-(4-amino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} (0.25 g, 0.60 mmol) was hydrolyzed (Scheme 6). The crude reaction mixture was dissolved in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 μm, 13 mm), then purified from reverse-phase HPLC column. HPLC [Phenomenex Luna 5μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 3.47 min (CH₃CN-H₂O = 25:75). The fraction containing the pure material were combined and lyophilized to obtain 0.06 g of the Na-salt of the titled compound. Mp 220°C (decomposed). ¹H NMR (DMSO-d₆, δ in ppm): 9.88 (s, 1 H), 8.61 (d,

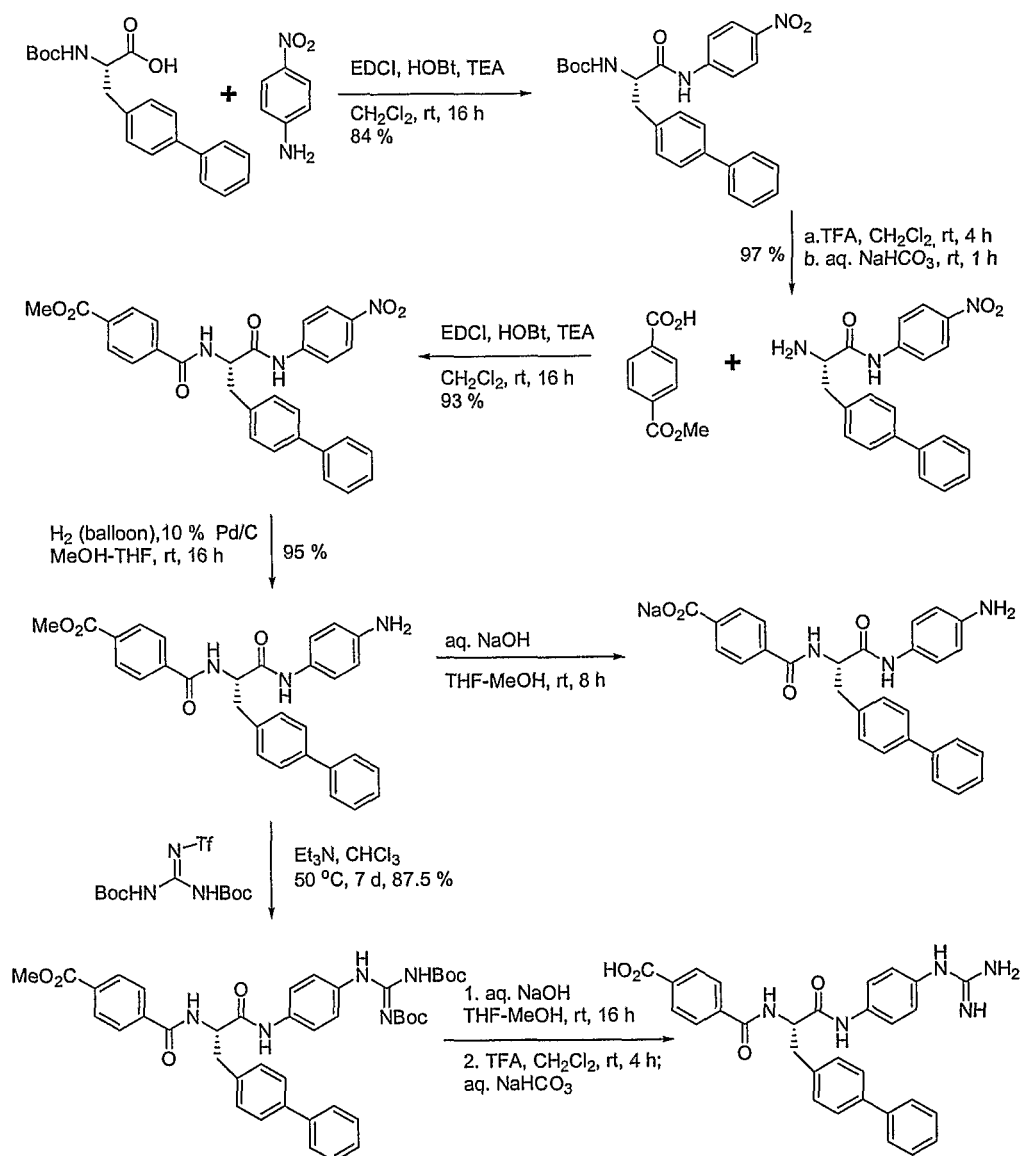
$J = 8.4$ Hz, 1 H), 7.85 (d, $J = 9.2$ Hz, 2 H), 7.73 (d, $J = 8.8$ Hz, 2 H), 7.44 (d, $J = 7.6$ Hz, 2 H), 7.26 - 7.18 (m, 4 H), 7.11 (d, $J = 7.4$ Hz, 2 H), 6.54 (d, $J = 7.6$ Hz, 2 H), 4.82 (br. s, 1 H), 4.73 - 4.68 (m, 1 H), 3.07 - 3.01 (m, 2 H). MS: [EI] m/e 404.5 $[M(\text{corresponding acid})+H]^+$. Anal: ($C_{23}H_{20}N_3NaO_4 + 2.4 H_2O + 0.06 CF_3CO_2Na$) C, H, N.

(R)-{N-[1-(4-Guanidino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid}

[0175] According to the General Procedure F, the amine in (R)-{N-[1-(4-amino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} (0.30 g, 0.72 mmol) was allowed to react with 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine and Et_3N to yield (R)-{N-[1-(4-($N^{2,3}$ -di-*tert*-butoxycarbonyl)guanidino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} in 90% yield (0.427 g). It was used in the subsequent reaction (Scheme 6).

[0176] The ester group in (R)-{N-[1-(4-($N^{2,3}$ -di-*tert*-butoxycarbonyl)guanidino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid methyl ester} (0.40 g, 0.61 mmol) was hydrolyzed, employing the General Procedure E. The crude reaction mixture (0.45 g), containing $N^{2,3}$ -di-*tert*-butoxycarbonyl groups, was submitted under General Procedure D for 5h. The concentrated crude material was dissolved in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 μm , 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.095 g of (R)-{N-[1-(4-guanidino-phenylcarbamoyl)-2-phenyl-ethyl]-terephthalamic acid} as white solid. HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), $\phi = 15$ mL/min] $t_R = 6.20$ min (CH₃CN-H₂O = 25:75). Mp 242 °C (decomposed). ¹H NMR (DMSO- d_6 , δ in ppm): 10.92 (br. s, 1 H), 10.39 (s, 1 H), 8.55 (d, $J = 7.6$ Hz, 1 H), 7.91 (d, $J = 8.4$ Hz, 2 H), 7.79 (d, $J = 8.4$ Hz, 2 H), 7.77 (br. s, 3 H), 7.67 (d, $J = 8.8$ Hz, 2 H), 7.39 (d, $J = 7.2$ Hz, 2 H), 7.27 (t, $J = 7.6$ Hz, 2 H), 7.18 (d, $J = 8.8$ Hz, 2 H), 7.17 (t, $J = 7.2$ Hz, 1 H), 4.82 (apparent q, $J = 6.0$ Hz, 1 H), 3.15 - 3.04 (m, 2 H). MS: [EI] m/e 446.4 $[M+H]^+$. Anal: ($C_{24.6}H_{27.6}F_{0.9}N_5Na_{0.3}O_{6.9}$) C, H, N.

SCHEME-8



(S)-{N-[1-(4-Amino-phenyl)carbamoyl]-2-biphenyl-4-yl-ethyl]-terephthalamic acid}

[0177] The Boc-L-Bip-OH (3.00 g, 8.8 mmol) was allowed to react with p-nitroaniline (1.34 g, 9.7 mmol), HOBT (1.19 g, 8.8 mmol), Et₃N (1.07 g, 10.5 mmol), and EDCI (1.68 g, 8.8 mmol) in anhydrous CH₂Cl₂ (50 mL) according to the General Procedure A (Scheme 8). The volatile materials were removed in a rotary evaporator and the residue was stirred with water (50 mL) at rt for 15 min. The solids were filtered, washed with water

(2 x 25 mL) and dried to afford (S)-{[1-(4-nitro-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-carbamic acid tert-butyl ester} in 84 % (4.4 g) yield.

[0178] The above Boc-derivative (2.1 g, 3.63 mmol) was treated with TFA, according to the General Procedure D. Upon filtration, water wash and drying, (S)-{2-amino-3-biphenyl-4-yl-N-(4-nitro-phenyl)-propionamide} was obtained as light-yellow solid (1.75 g, 97 %).

[0179] Then *mono*-methyl terephthalate (0.90 g, 5.02 mmol) was allowed to react with the above amine (1.65 g, 4.56 mmol), HOBt (0.68 g, 5.02 mmol), Et₃N (0.55 g, 5.48 mmol), and EDCI (0.96 g, 5.02 mmol) in anhydrous CH₂Cl₂ (20 mL) according to the General Procedure A. The volatile materials were removed in a rotary evaporator and the residue was stirred with water (15 mL) at rt for 15 min. The heterogeneous mixture were filtered, washed with water (3 x 25 mL) and dried to afford (S)-{N-[1-(4-nitro-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} (2.23 g, 93 %) as light-yellow solid.

[0180] The above nitro compound (0.90 g, 1.72 mmol) was reduced in MeOH-THF (2:1, 25 mL), following the General Procedure G, to furnish (S)-{N-[1-(4-amino-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} (0.81 g, 95 %).

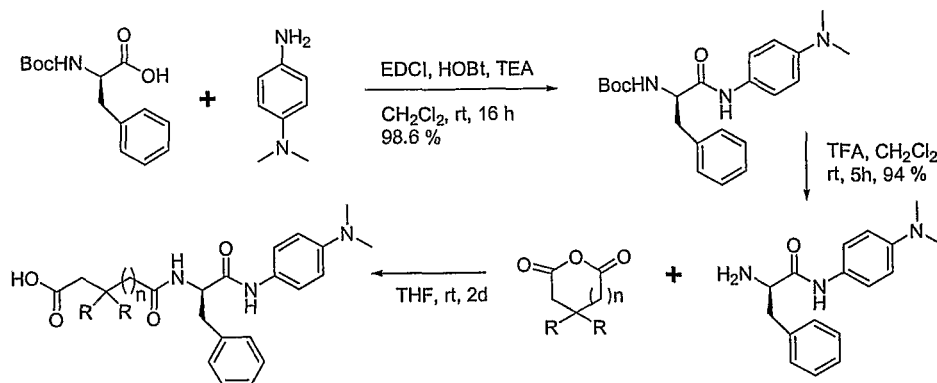
[0181] Employing the General Procedure E, the ester in (S)-{N-[1-(4-amino-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} (0.12 g, 0.24 mmol) was hydrolyzed. The crude reaction mixture was dissolved in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 µm, 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.03 g of the Na-salt of the titled compound as off-white solid. HPLC [Phenomenex Luna 5µ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 8.92 min (CH₃CN-H₂O = 55:45). Mp 251°C (decomposed). ¹H NMR (DMSO-d₆, δ in ppm): 9.98 (s, 1 H), 8.97 (d, J = 8.0 Hz, 1 H), 8.00 (d, J = 8.8 Hz, 2 H), 7.97 (d, J = 8.4 Hz, 2 H), 7.61 (d, J = 8.8 Hz, 2 H), 7.58 (d, J = 7.2 Hz, 2 H), 7.48 (d, J = 8.0 Hz, 2 H), 7.40 (d, J = 8.2 Hz, 2 H), 7.32 (t, J = 7.6 Hz, 1 H), 7.30 (d, J = 7.0 Hz, 2 H), 6.68 (d, J = 8.4 Hz, 2 H), 4.89 - 4.83 (m, 1 H), 3.16 - 3.06 (m, 2 H). MS: [EI] m/e 480.4 [M(corresponding acid)+H]⁺. Anal: (C₂₉H_{27.2}N₃NaO_{5.6}) C, H, N.

(S)-{N-[2-Biphenyl-4-yl-1-(4-guanidino-phenylcarbamoyl)-ethyl]-terephthalamic acid}

[0182] According to the General Procedure F, (S)-{N-[1-(4-amino-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} (0.23 g, 0.466 mmol) was allowed to react with 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine [2 x (0.54 g, 1.4 mmol)] and Et₃N [2 x (0.14 g, 1.4 mmol)] to yield (S)-{N-[1-(4-(N^{2,3}-di-*tert*-butoxycarbonyl)guanidino-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} in 87.5 % yield (0.30 g). It was used in the subsequent reaction (Scheme 8).

[0183] The ester group in (S)-{N-[1-(4-(N^{2,3}-di-*tert*-butoxycarbonyl)guanidino-phenylcarbamoyl)-2-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester} (0.29 g, 0.39 mmol) was hydrolyzed, employing the General Procedure E. The crude reaction mixture containing N-Boc groups was submitted subsequently under General Procedure D for 5 h. The concentrated crude material was dissolved in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 µm, 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.06 g of the titled acid as white solid. HPLC [Phenomenex Luna 5µ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 6.92 min (CH₃CN-H₂O = 40:60). Mp 238-40 °C (decomposed). ¹H NMR (DMSO-d₆, δ in ppm): 10.85 (br. s, 1 H), 10.47 (s, 1 H), 9.05 (d, J = 6.4 Hz, 1 H), 8.02 (d, J = 7.2 Hz, 2 H), 7.94 (d, J = 7.6 Hz, 2 H), 7.79 (s, 3 H), 7.78 (d, J = 7.2 Hz, 2 H), 7.70 (t, J = 8.4 Hz, 4 H), 7.58 (d, J = 7.2 Hz, 2 H), 7.50 (t, J = 7.2 Hz, 2 H), 7.39 (t, J = 8.0 Hz, 1 H), 7.27 (d, J = 7.6 Hz, 2 H), 4.96 (q, J = 6.8 Hz, 1 H), 3.24 (d, J = 5.6 Hz, 2 H). MS: [EI] m/e 522.8 [M+H]⁺. Anal: (C₃₀H_{35.2}N₅O_{8.1}) C, H, N.

SCHEME-9



3-((R)-1-(4-(Dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid

[0184] The Boc-D-Phe-OH (2.00 g, 7.54 mmol) was allowed to react with N,N-dimethyl-*p*-phenylenediamine (0.98 g, 7.18 mmol), HOBt (1.02 g, 7.54 mmol), Et₃N (1.09 g, 10.77 mmol), and EDCI (1.44 g, 7.54 mmol) in anhydrous CH₂Cl₂ (50 mL) according to the General Procedure A to afford *tert*-butyl (R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamate (2.85 g, 98.6 %) as gray-colored solids (Scheme 9).

[0185] The above Boc-derivative (1.85 g, 4.82 mmol) was treated with TFA, according to the General Procedure D. Upon filtration, water wash and drying (R)-2-amino-N-(4-(dimethylamino)phenyl)-3-phenylpropanamide was obtained as gray solid (1.29 g, 94 %).

[0186] The above amine (0.12 g, 0.42 mmol) in THF (5 mL) was reacted with succinic anhydride (0.051 g, 0.51 mmol), according to the General Procedure C. The crude was taken in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 μm, 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.105 g of 3-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid as light gray solid. HPLC [Phenomenex Luna 5μ C₁₈ (2) (gradient, CH₃CN/H₂O), φ = 15 mL/min] *t*_R = 8.32 min (CH₃CN-H₂O = 45:55). Mp 197-8°C. ¹H NMR (DMSO-*d*₆, δ in ppm): 12.21 (br. s, 1 H), 9.86 (s, 1 H), 8.42 - 8.25 (m, 1 H), 7.50 - 7.25 (m, 9 H), 6.89 (br. s, 1 H), 4.69 - 4.61 (m, 1 H), 3.16-3.04 (m, 2 H), 2.95 (s, 6 H), 2.45 - 2.33 (m, 4 H). MS: [EI] *m/e* 384.4 [M+H]⁺. Anal: (C₂₁H_{25.5}N₃O_{4.25}) C, H, N.

4-((R)-1-(4-(Dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid

[0187] It was prepared according to the General Procedure C. From 0.12 g (0.42 mmol) of (R)-2-amino-N-(4-(dimethylamino)phenyl)-3-phenylpropanamide and glutaric anhydride (0.058 g, 0.51 mmol), 0.089 g of 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid was obtained as light gray solid (Scheme 9). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 8.81 min (CH₃CN-H₂O = 48:52). Mp 205-7°C. ¹H NMR (DMSO-d₆, δ in ppm): 12.21 (br. s, 1 H), 9.86 (s, 1 H), 8.42 - 8.25 (m, 1 H), 7.50 - 7.25 (m, 9 H), 6.84 (br. t, J = 8.2 Hz, 1 H), 4.69 - 4.61 (m, 1 H), 3.16-2.95 (m, 2 H), 2.93 (s, 6 H), 2.15 (t, J = 6.6 Hz, 4 H), 1.68 (t, J = 6.6 Hz, 2 H). MS: [EI] m/e 398.5 [M+H]⁺. Anal: (C₂₂H_{27.5}N₃O_{4.25}) C, H, N.

4-((R)-1-(4-(Dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid

[0188] It was prepared according to the General Procedure C. From 0.12 g of (R)-2-amino-N-(4-(dimethylamino)phenyl)-3-phenylpropanamide and 3,3-(dimethyl)glutaric anhydride (0.072 g, 0.51 mmol), 0.10 g of desired acid was obtained as light gray solid (Scheme 9). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.60 min (CH₃CN-H₂O = 55:45). Mp 85-6 °C. ¹H NMR (DMSO-d₆, δ in ppm): 12.21 (br. s, 1 H), 9.86 (s, 1 H), 8.42 - 8.25 (m, 1 H), 7.50 - 7.25 (m, 9 H), 6.84 (br. t, J = 8.2 Hz, 1 H), 4.69 - 4.61 (m, 1 H), 3.16-2.95 (m, 2 H), 2.93 (s, 6 H), 2.26 - 2.14 (m, 4 H), 0.92 (s, 6 H). MS: [EI] m/e 426.5 [M+H]⁺. Anal: (C_{24.2}H_{32.3}N_{3.1}O_{4.5}) C, H, N.

Synthesis of 4-((R)-1-(4-(Dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(tetramethylene)butanoic acid

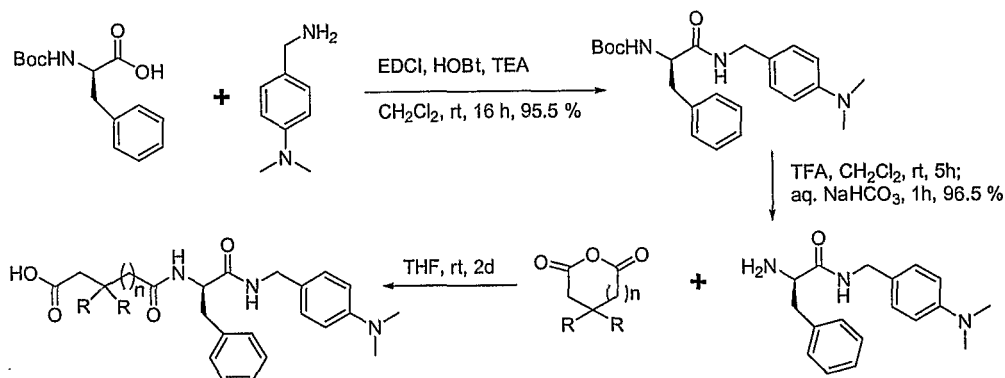
[0189] It was prepared according to the General Procedure C. From 0.12 g of (R)-2-amino-N-(4-(dimethylamino)phenyl)-3-phenylpropanamide and 3,3-(tetramethylene)glutaric anhydride (0.085 g, 0.51 mmol), 0.076 g of the desired acid was obtained as light gray solid (Scheme 9). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.54 min (CH₃CN-H₂O = 62:38). Mp 91-2 °C. ¹H NMR (DMSO-d₆, δ in ppm): 12.21 (br. s, 1 H), 9.86 (s, 1 H), 8.42 - 8.25 (m, 1 H), 7.50 - 7.25 (m, 9 H), 6.84 (br. s, 1 H), 4.83 (apparent q, J = 7.4 Hz, 1 H), 3.20-3.02 (m, 2 H), 2.91 (s, 6 H),

2.27 (s, 2 H), 2.23 (d, $J = 13.2$ Hz, 1 H), 2.09 (d, $J = 13.2$ Hz, 1 H), 1.55 - 1.30 (m, 8 H). MS: [EI] m/e 452.4 $[M+H]^+$. Anal: (C₂₆H_{33.5}N₃O_{4.25}) C, H, N.

4-((R)-1-(4-(Dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(pentamethylene)butanoic acid

[0190] It was prepared according to the General Procedure C. From 0.12 g of (R)-2-amino-N-(4-(dimethylamino)phenyl)-3-phenylpropanamide and 3,3-(pentamethylene)glutaric anhydride (0.092 g, 0.51 mmol), 0.119 g of the desired acid was obtained as light gray solid (Scheme 9). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), $\phi = 15$ mL/min] $t_R = 11.02$ min (CH₃CN-H₂O = 66:34). Mp 110-1°C. ¹H NMR (CDCl₃+DMSO-d₆, δ in ppm): 12.21 (br. s, 1 H), 9.86 (s, 1 H), 8.42 - 8.25 (m, 1 H), 7.50 - 7.25 (m, 9 H), 6.84 (br. s, 1 H), 4.83 (apparent q, $J = 7.4$ Hz, 1 H), 3.20-3.02 (m, 2 H), 2.83 (s, 6 H), 2.27 (s, 2 H), 2.32 - 2.11 (m, 4 H), 1.32 (br. s, 6 H), 1.21 (br. s, 2 H), 1.11 (br. s, 2 H). MS: [EI] m/e 466.6 $[M+H]^+$. Anal: (C_{27.12}H_{35.4}F_{0.18}N₃Na_{0.06}O_{4.32}) C, H, N.

SCHEME-10



3-((R)-1-(4-(Dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid

[0191] The Boc-D-Phe-OH (2.00 g, 7.54 mmol) was allowed to react with 4-N,N-dimethylaminobenzylamine dihydrochloride (1.60 g, 7.18 mmol), HOBt (1.02 g, 7.54 mmol), Et₃N (2.54 g, 25.1 mmol), and EDCI (1.44 g, 7.54 mmol) in anhydrous CH₂Cl₂ (50 mL), according to the General Procedure A, to afford *tert*-butyl (R)-1-(4-

(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamate (2.79 g, 95.5 %) as off-white solids (Scheme 10).

[0192] The above tert-butyl (R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamate (1.80 g, 4.53 mmol) was treated with TFA, according to the General Procedure D. Upon filtration, water wash and drying the free amine [(R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide] was obtained as pale-yellow solid (1.30 g, 96.5%).

[0193] (R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide (0.12 g, 0.40 mmol) in THF (5 mL) was reacted with succinic anhydride (0.048 g, 0.48 mmol), according to the General Procedure C. The crude was taken in DMSO (1.5 mL) and passed through a syringe filter-disc (Whatman, PTFE, 0.45 μ m, 13 mm), then purified from reverse-phase HPLC column. The fraction containing the pure material were combined and lyophilized to obtain 0.10 g of 3-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid as white solid. HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 8.03 min (CH₃CN-H₂O = 45:55). Mp 194-6 °C. ¹H NMR (DMSO-d₆, δ in ppm): 12.18 (br. s, 1 H), 8.36 (br. s, 1 H), 8.10 (d, J = 8.0 Hz, 1 H), 7.25 - 7.15 (m, 5 H), 6.95 (d, J = 7.2 Hz, 2 H), 6.67 (br. s, 1 H), 4.44 - 4.28 (m, 1 H), 4.08 (s, 2 H), 3.00 (d, J = 12.4 Hz, 1 H), 2.69 (dd, J = 12.4, 7.8 Hz, 1 H), 2.91 (s, 6 H), 2.45 - 2.22 (m, 4 H). MS: [EI] m/e 398.5 [M+H]⁺. Anal: (C₂₂H_{28.56}N₃O_{4.78}) C, H, N.

4-((R)-1-(4-(Dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid

[0194] It was prepared according to the General Procedure C. From 0.12 g of (R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide and glutaric anhydride (0.055 g, 0.48 mmol), 0.086 g of the desired acid was obtained as white solid (Scheme 10). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 7.97 min (CH₃CN-H₂O = 47:53). Mp 208-9°C. ¹H NMR (DMSO-d₆, δ in ppm): 12.18 (br. s, 1 H), 8.42 (br. s, 1 H), 8.04 (d, J = 8.4 Hz, 1 H), 7.25 - 7.15 (m, 5 H), 6.95 (d, J = 7.6 Hz, 2 H), 6.65 (br. s, 1 H), 4.44 (apparent q, J = 8.4 Hz, 1 H), 4.19 (d, J = 4.4 Hz, 2 H), 2.90 (dd, J = 13.6, 4.4 Hz, 1 H), 2.91 (s, 6 H), 2.65 (dd, J = 12.6, 10.8 Hz, 1 H), 2.16 (apparent t, J = 7.0, 4 H), 1.54 (apparent t, J = 7.0, 2 H). MS: [EI] m/e 412.5 [M+H]⁺. Anal: (C₂₃H_{29.66}N₃O_{4.33}) C, H, N.

4-((R)-1-(4-(Dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid

[0195] It was prepared according to the General Procedure C. From 0.12 g of (R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide and 3,3-(dimethyl)glutaric anhydride (0.069 g, 0.48 mmol), 0.097 g of the desired acid was obtained as white solid (Scheme 10). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.55 min (CH₃CN-H₂O = 55:45). Mp 76-7°C. ¹H NMR (CDCl₃, δ in ppm): 7.48 (d, J = 7.6 Hz, 1 H), 7.18 - 7.11 (m, 6 H), 6.92 (d, J = 8.4 Hz, 2 H), 6.67 (br. s, 2 H), 6.43 (br. s, 1 H), 4.44 (apparent q, J = 7.6 Hz, 1 H), 4.16 (d, J = 4.8 Hz, 2 H), 3.05 - 2.95 (m, 2 H), 2.85 (s, 6 H), 2.18 (d, J = 2.4 Hz, 2 H), 2.16 (d, J = 13.0, 1 H), 2.04 (d, J = 13.0, 1 H), 0.92 (s, 3 H), 0.87 (s, 3 H). MS: [EI] m/e 440.6 [M+H]⁺. Anal: (C₂₅H₃₃N₃O₄) C, H, N.

4-((R)-1-(4-(Dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(tetramethylene)butanoic acid

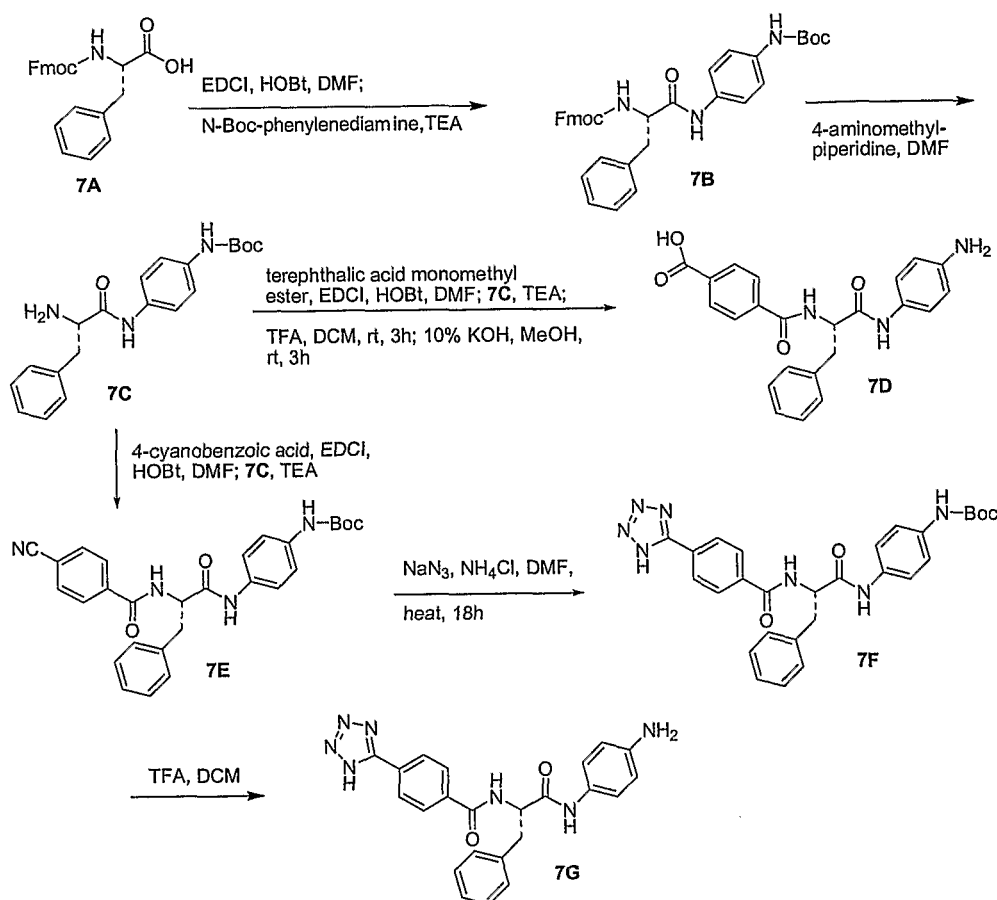
[0196] It was prepared according to the General Procedure C. From 0.12 g of (R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide and 3,3-(tetramethylene)glutaric anhydride (0.081 g, 0.48 mmol), 0.105 g of the desired acid was obtained as white solid (Scheme 10). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient, CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.56 min (CH₃CN-H₂O = 62:38). Mp 88-90°C. ¹H NMR (CDCl₃, δ in ppm): 7.60 (d, J = 8.0 Hz, 1 H), 7.18 - 7.13 (m, 6 H), 6.91 (d, J = 8.4 Hz, 2 H), 6.62 (d, J = 8.0 Hz, 2 H), 4.66 (apparent q, J = 7.8 Hz, 1 H), 4.15 (two sets of dd, J = 14.4, 5.6 Hz, 2 H), 3.05 - 2.94 (m, 2 H), 2.84 (s, 6 H), 2.29 (d, J = 13.6 Hz, 1 H), 2.18 (apparent t, J = 12.2, 2 H), 2.04 (d, J = 13.6, 1 H), 1.53 (br. s, 4 H), 1.40 (br. s, 2 H), 1.31 (br. s, 2 H). MS: [EI] m/e 466.6 [M+H]⁺. Anal: (C₂₇H_{35.5}N₃O_{4.25}) C, H, N.

4-((R)-1-(4-(Dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(pentamethylene)butanoic acid

[0197] It was prepared according to the General Procedure C. From 0.12 g of (R)-N-(4-(dimethylamino)benzyl)-2-amino-3-phenylpropanamide and 3,3-(pentamethylene)glutaric anhydride (0.088 g, 0.48 mmol), 0.12 g of the desired acid was obtained as white solid (Scheme 10). HPLC [Phenomenex Luna 5 μ C₁₈ (2) (gradient,

CH₃CN/H₂O), ϕ = 15 mL/min] t_R = 9.57 min (CH₃CN-H₂O = 66:34). Mp 100-2°C. ¹H NMR (CDCl₃, δ in ppm): 7.37 (br., 1 H), 7.18 - 7.11 (m, 6 H), 6.95 (br. t, J = 6.8 Hz, 2 H), 6.68 (br. s, 2 H), 6.43 (br. s, 1 H), 4.66 (apparent t, J = 7.2 Hz, 1 H), 4.17 (br. s, 2 H), 3.05 - 2.95 (m, 2 H), 2.86 (s, 6 H), 2.25 - 2.12 (m, 4 H), 1.32 (br. s, 6 H), 1.21 (br. s, 2 H), 1.11 (br. s, 2 H). MS: [EI] m/e 480.5 [M+H]⁺. Anal: (C₂₈H_{37.5}N₃O_{4.25}) C, H, N.

SCHEME-11



(S)-{4-[2-(9H-Fluoren-9-yloxy-carbonylamino)-2-phenyl-acetyl-amino]-phenyl}-carbamic acid tert-butyl ester (7B)

[0198] To a solution of Fmoc-L-Phe-OH (7A, 2 g, 5 mmol) in DMF (20 mL) was added HOBt (800 mg, 5 mmol), EDCI (1.1 g, 5.7 mmol) and the mixture stirred at room temperature for 20min. N-Boc-1,4-phenylene diamine (1.1 g, 5 mmol) was added followed by TEA (525 mg, 724 μ L) and the mixture stirred at room temperature for 5 h. The solution was concentrated under reduced pressure and water was added and the mixture sonicated to

precipitate the product which was collected by filtration and dried to give an off white solid (2.9 g, 5 mmol).

(S)-[4-(2-Amino-2-phenyl-acetyl-amino)-phenyl]-carbamic acid tert-butyl ester (7C)

[0199] To a solution of {4-[2-(9H-Fluoren-9-yloxy-carbonylamino)-2-phenyl-acetyl-amino]-phenyl}-carbamic acid tert-butyl ester (**7B**) (2.8 g, 5 mmol) in DMF (50 mL) was added 4-aminomethyl piperidine (10 x, 5.7 g, 50 mmol) and the solution stirred at room temperature for 18 h. The mixture was filtered to remove solids and the filtrate concentrated under reduced pressure. The residue was taken up in DCM and washed with saturated NaCl, 5.5 phosphate buffer solution (3 x 25 mL), saturated NaCl, dried (Na₂SO₄), filtered, and concentrated to give a solid product (3.48 mmol, 1.24 g) that was used as is.

(S)-{N-[(4-Amino-phenyl-carbamoyl)-phenyl-methyl]-terephthalamic acid} (7D)

[0200] To a solution of terephthalic acid mono methyl ester (133 mg, 0.74 mmol) in dry DCM (50 mL) was added EDCI (142 mg, 0.74 mmol) and HOBt (114 mg, 0.74 mmol) and the reaction mixture stirred for 3 h at room temperature. [4-(2-Amino-2-phenyl-acetyl-amino)-phenyl]-carbamic acid tert-butyl ester (**7C**) (288 mg, 0.67 mmol) was added followed by TEA (75 mg, 103 uL) and the mixture stirred at room temperature for 15 h. The DCM was extracted with water and saturated NaCl solution and dried to give a residue. The residue was dissolved in DCM (3 mL) and TFA (2 mL) added and the mixture stirred at room temperature for 3 h. The solution was concentrated to give a brown oil residue. The oil was dissolved in MeOH (5 mL) and 10% KOH (3 mL) added and the mixture stirred for 3 h at room temperature. The solution was adjusted to pH~5, the methanol was concentrated and the resulting solid collected and dried to give 150 mg of solid. This crude product was purified by reverse phase HPLC using ACN/H₂O (5-95 % ACN) and lyophilized to give the product as a white solid (42 mg). MP 151°C. ¹H NMR (400 MHz) δ 9.83 (s, 1H), 8.87 (d, J=8.4Hz, 1H), 7.98 (d, J=8.4Hz, 2H), 7.90 (d, J=8.8Hz, 2H), 7.39 (d, J=7.2Hz, 2H), 7.22 (m, 5H), 6.5 (m, 2H), 4.80 (m, 1H), 3.10 (m, 4H). M⁺ 404.5. Anal. C₂₃H₂₁N₃O₄ + 1 H₂O

(S)-{4-[2-(4-Cyano-benzoylamino)-3-phenyl-propionyl-amino]-carbamic acid tert-butyl ester (7E)}

[0201] To a solution of 4-cyanobenzoic acid (1.54 mmol, 227 mg) in DMF (15 mL) was added EDCI (296 mg, 1.54 mmol) and HOBt (236 mg, 1.54 mmol) and the solution

stirred at room temperature for 30 min. [4-(2-Amino-2-phenyl-acetyl-amino)-phenyl]-carbamic acid tert-butyl ester (7C) (500 mg, 1.4 mmol) was added followed by TEA (214 μ L) and the mixture stirred for 4h at room temperature. The solution was poured into water (250 mL) and the solid collected by filtration and dried under reduced pressure to give the product as a white solid (638 mg, 1.32 mmol) that was used as is.

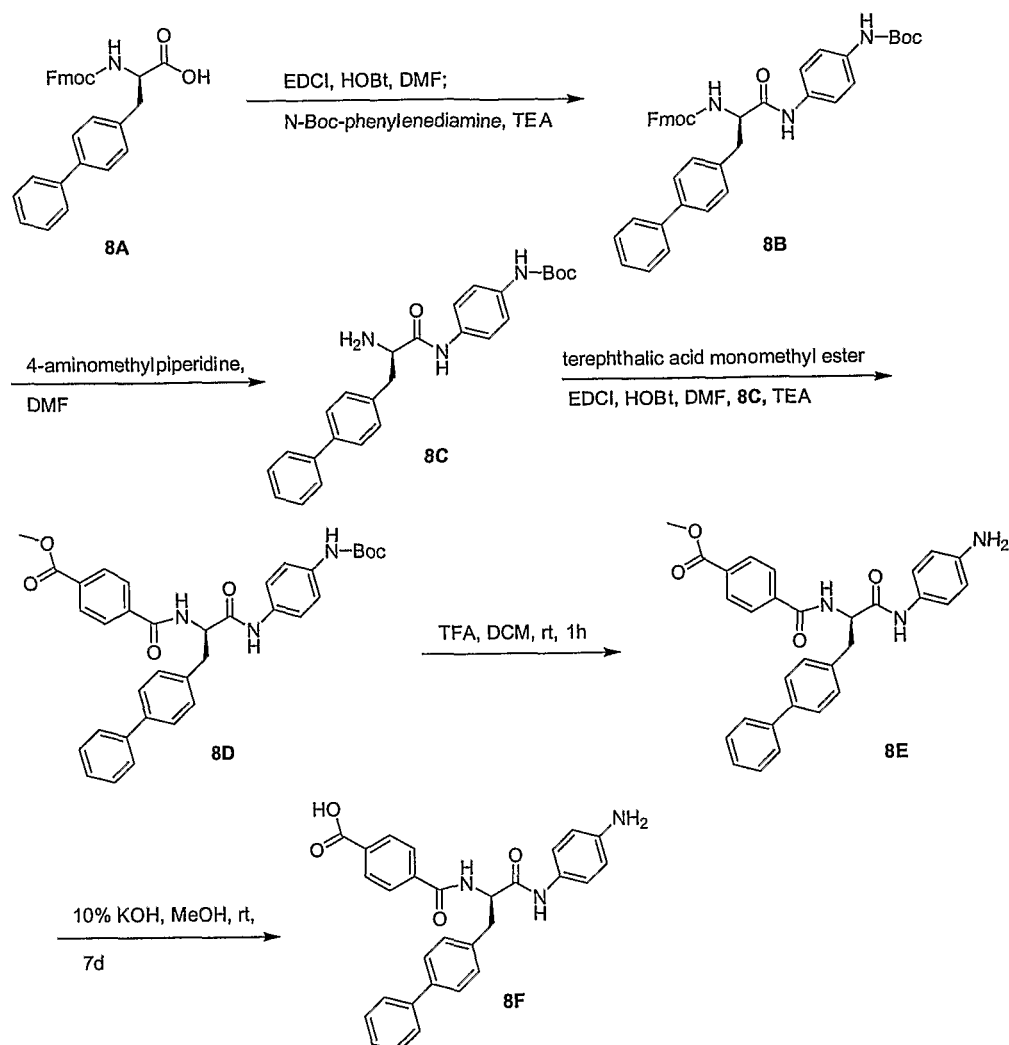
(S)-(4-{3-Phenyl-2-[4-(1H-tetrazol-5-yl)-benzoylamino]-propionylamino}-phenyl)-carbamic acid tert-butyl ester (7F)

[0202] To a solution of {4-[2-(4-Cyano-benzoylamino)-3-phenyl-propionylamino]-carbamic acid tert-butyl ester (7E) (100 mg, 0.21 mmol) in DMF (2 mL) was added NaN_3 (3 x, 40 mg, 0.62 mmol) and NH_4Cl (0.68 mmol, 38 mg) and the mixture heated at 90°C for 18 h. The DMF was removed under reduced pressure and the residue purified by reverse phase HPLC over C18 using ACN/ H_2O (20 %-95 % ACN) to give the product as a white powder solid after lyophilization (31 mg).

(S)-{N-[1-(4-Amino-phenylcarbonyl)-2-phenyl-ethyl]-4-(1H-tetrazol-5-yl)benzamide} (7G)

[0203] To a suspension of (4-{3-Phenyl-2-[4-(1H-tetrazol-5-yl)-benzoylamino]-propionylamino}-phenyl)-carbamic acid tert-butyl ester (7F) (31 mg, 0.06mmol) in DCM (4 mL) was added TFA (1 mL) and the mixture stirred at room temperature for 1.5 h. The solution was concentrated and purified by reverse phase HPLC over C18 using ACN/ H_2O (20 %-95 % ACN) to give the product as a white powder (4.5 mg) after lyophilization. MP 137°C. ^1H NMR (400 MHz) δ 9.88 (s, 1H), 8.86 (d, J=8.0Hz, 1H), 8.06 (d, J=8.0Hz, 2H), 7.97 (d, J=8.4Hz, 2H), 7.36 (d, J=7.6Hz, 2H), 7.24 (m, 4H), 7.14 (m, 1H), 6.56 (d, J=8.4Hz, 2H), 5.70 s, 1H) 4.78 (m, 1H), 3.07 (m, 6H). M^+ 428.5. Anal. $\text{C}_{23}\text{H}_{21}\text{N}_7\text{O}_2 + 2 \text{H}_2\text{O} + 0.4\text{TFA}$

SCHEME-12



(N-[1-(4-Amino-phenylcarbamoyl)-2-D-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester (8E)

[0204] This compound was made in a manner similar to N-[(4-Amino-phenylcarbamoyl)-phenyl-methyl]-terephthalamic acid (7D) by substituting D-biphenylalanine (8A) and forgoing the saponification step as shown in Scheme 8. (32 mg) Mp 289°C. ^1H NMR (400 MHz) δ 9.88 (s, 1H), 8.86 (d, $J=8.0\text{Hz}$, 1H), 8.02 (m, 2H), 7.95 (m, 2H), 7.62 (m, 4H), 7.49 (d, $J=8.0\text{Hz}$, 2H), 7.43 (m, 2H), 7.32 (m, 1H), 7.24 (m, 2H), 6.51 (m, 2H), 4.85 (m, 3H), 3.86 (s, 3H), 3.14 (m, 2H). M^{+1} 494.6. Anal. $\text{C}_{30}\text{H}_{27}\text{N}_3\text{O}_4$

N-[1-(4-Amino-phenylcarbamoyl)-2-D-biphenyl-4-yl-ethyl]-terephthalamic acid (8F)

[0205] To a solution of N-[1-(4-Amino-phenylcarbamoyl)-2-D-biphenyl-4-yl-ethyl]-terephthalamic acid methyl ester (8E) in MeOH was added 10% KOH and the solution stirred at room temperature for 7d. The MeOH was removed under reduced pressure and the aqueous mixture adjusted to pH 5-7 with 20% HCl. The resulting solid was collected and purified by reverse phase HPLC using C18 eluting with ACN/H₂O (5-95 % ACN) and the appropriate fractions lyophilized to give the product as a white solid (13 mg). Mp 282°C. ¹H NMR (400 MHz) δ9.87 (s, 1H), 9.92 (d, J=8.0Hz, 1H), 7.99 (d, J=8.4Hz, 2H), 7.92 (d, J=8.4Hz, 2H), 7.61 (m, 2H), 7.48 (m, 1H), 7.43 (m, 1H), 7.32 (m, 1H), 7.24 (d, J=8.8Hz, 1H) 6.51 (d, J=8.4Hz, 1H), 4.84 (m, 1H), 3.15 (m, 3H). M⁺ 480.3. Anal. C₂₉H₂₅N₃O₂

[0206] Many modifications and variations of the embodiments described herein may be made without parting from the scope, as is apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only.

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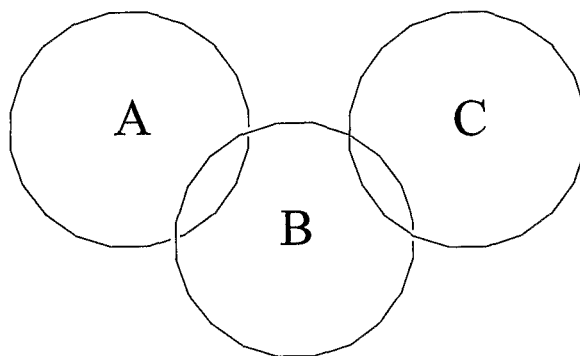
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WHAT IS CLAIMED IS:

1. A mediator of reverse cholesterol transport, comprising the structure:



wherein A, B, and C may be in any order, and wherein:

A comprises an acidic amino acid or a molecular bioisostere thereof;

B comprises an aromatic or lipophilic amino acid or analog thereof; and

C comprises a basic amino acid or a molecular bioisostere thereof,

wherein at least one of A or C comprises the molecular bioisostere thereof.

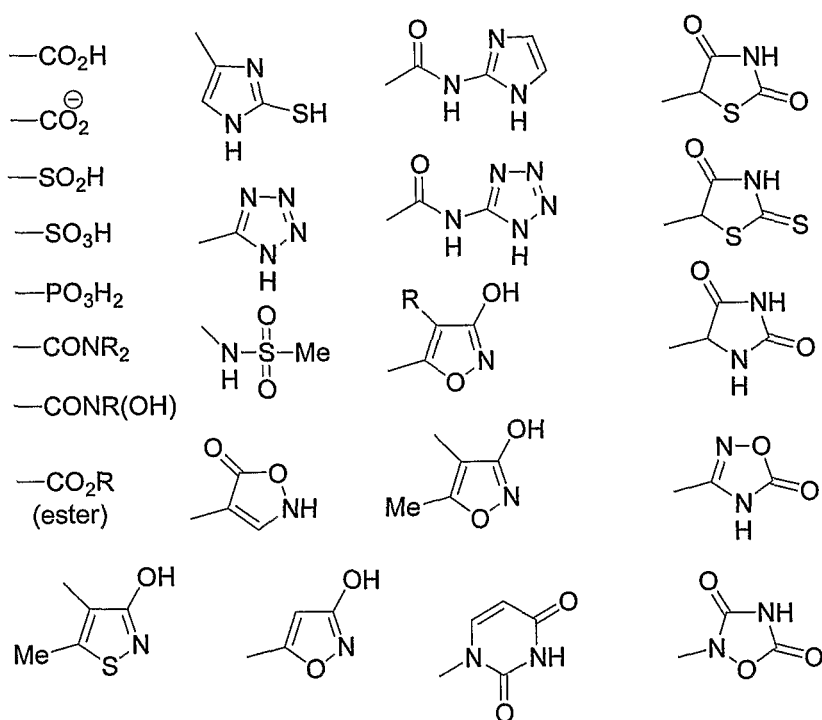
2. The mediator of Claim 1, wherein only one of A or C comprises the bioisostere, and wherein either the alpha amino or alpha carboxy group has been removed from the A or C amino acid that does not comprise the bioisostere.

3. The mediator of Claim 1, wherein if present, an alpha amino group from the amino terminal is capped with a protecting group selected from the group consisting of formyl, acetyl, phenylacetyl, benzoyl, pivoyl, 9-fluorenylmethyloxycarbonyl, 2-naphthyl acid, nicotinic acid, a $\text{CH}_3-(\text{CH}_2)_n-\text{CO}-$ where n ranges from 1 to 20, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, and substituted saturated heteroaryl.

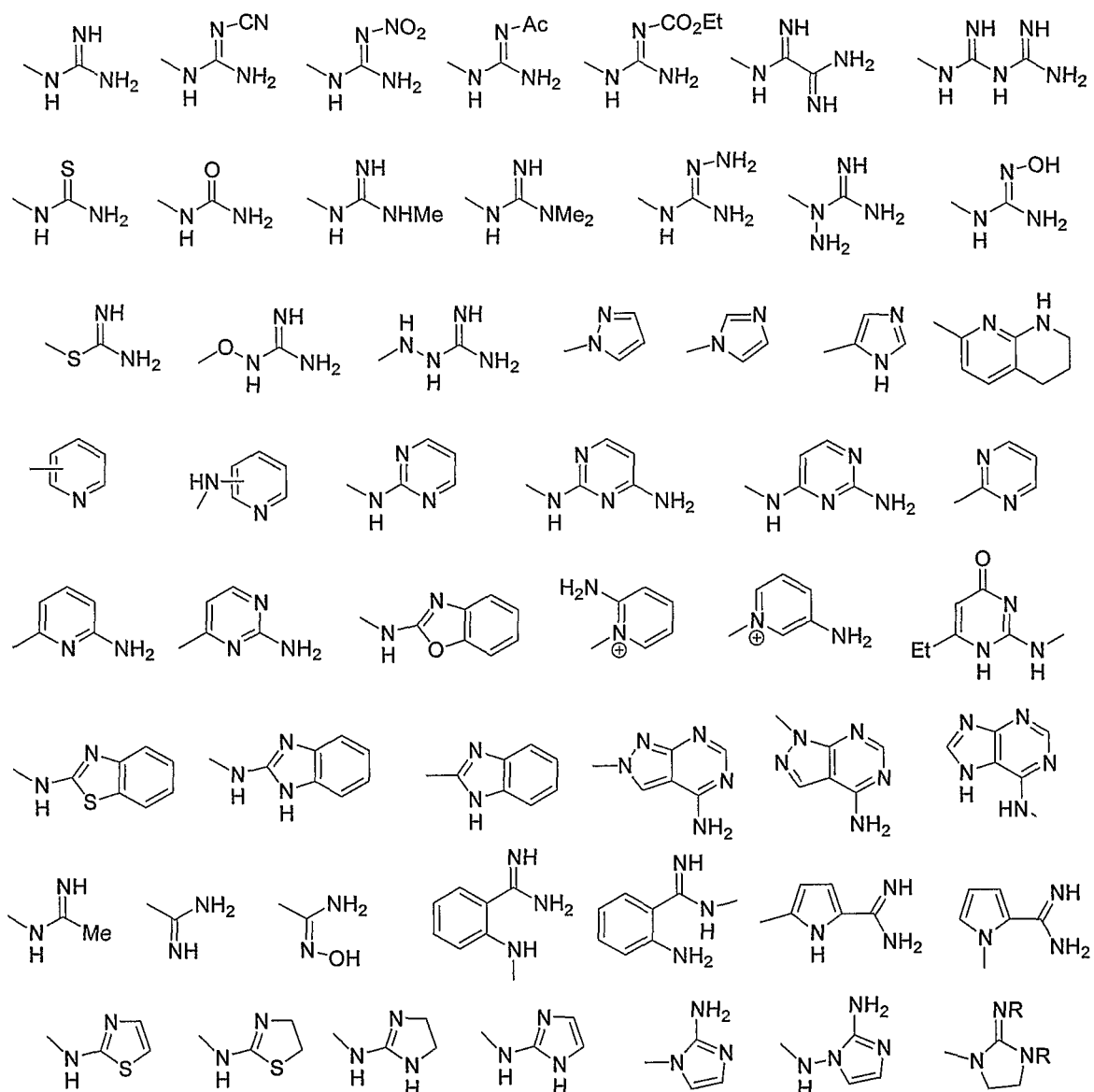
4. The mediator of Claim 1, wherein if present, an alpha carboxy group from the carboxy terminal is capped with a protecting group selected from the group consisting of an

amine, such as RNH₂ where R = H, di-tert-butyl-4-hydroxy-phenyl, naphthyl, substituted naphthyl, Fmoc, biphenyl, substituted phenyl, substituted heterocycles, alkyl, aryl, substituted aryl, cycloalkyl, fused cycloalkyl, saturated heteroaryl, and substituted saturated heteroaryl.

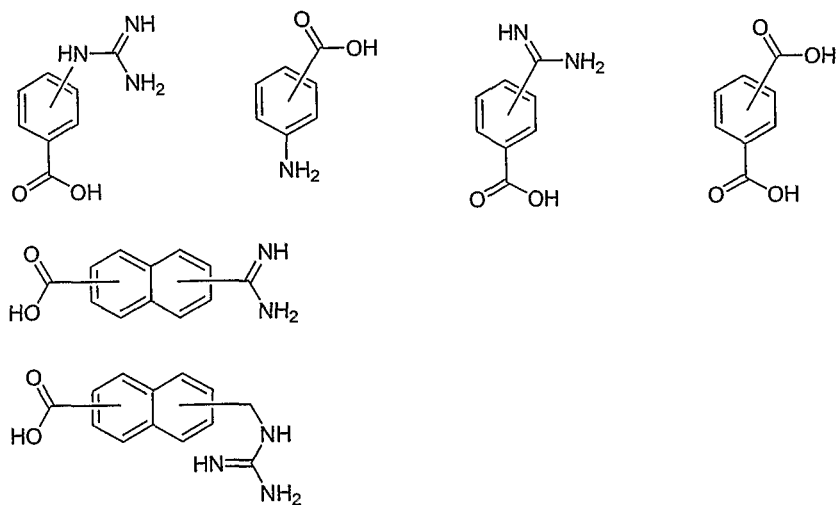
5. The mediator of Claim 1, wherein the acidic group of A comprises a bioisostere selected from the group consisting of:



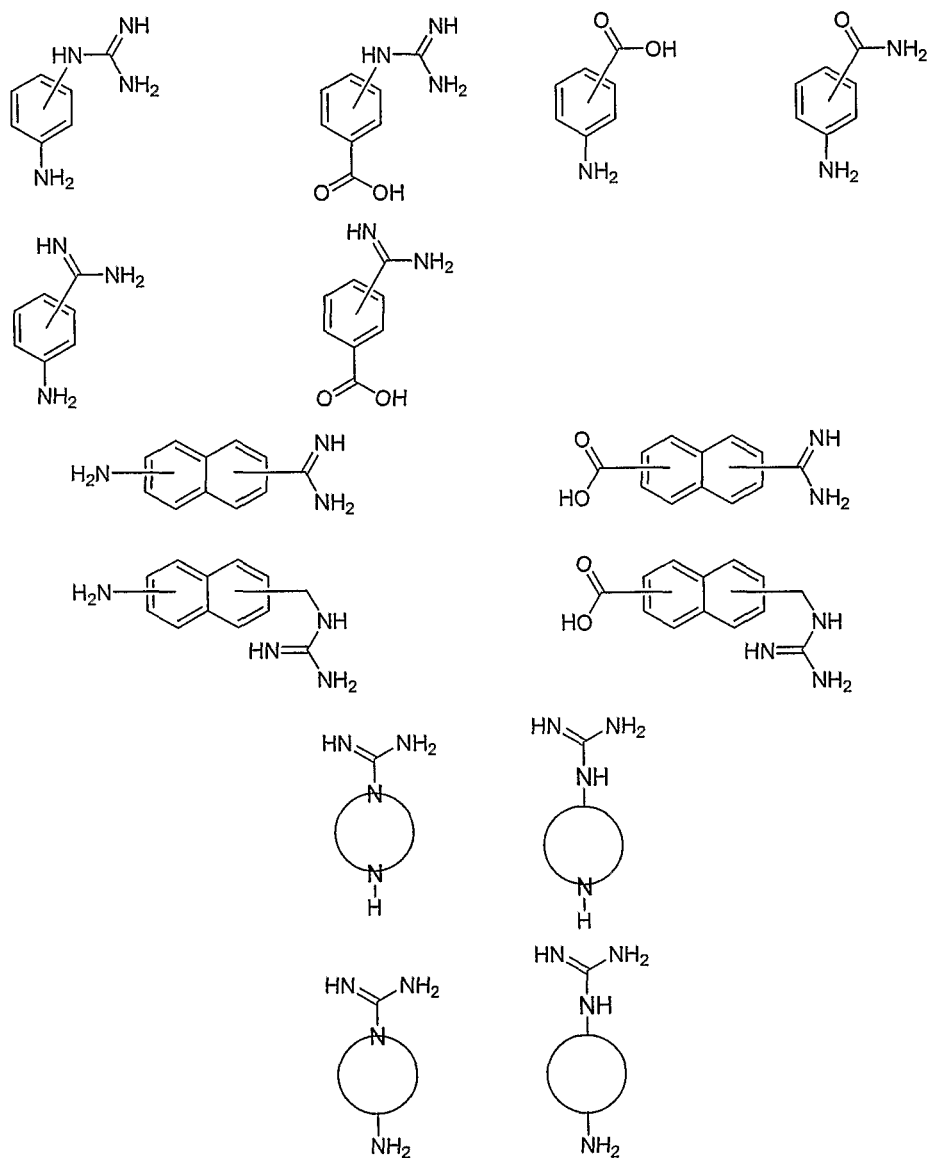
6. The mediator of Claim 1, wherein the basic group of C comprises a bioisostere selected from the group consisting of:



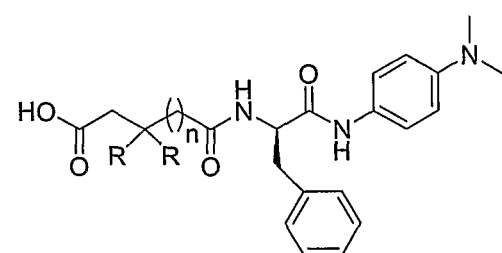
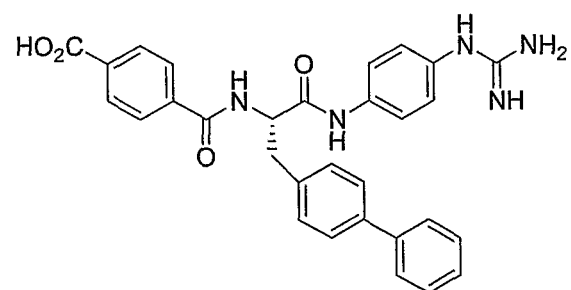
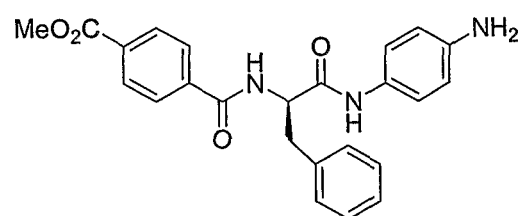
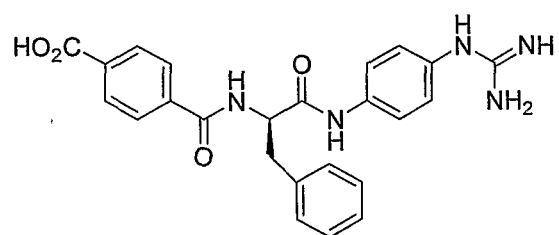
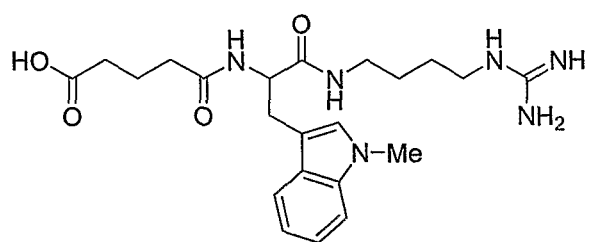
7. The mediator of Claim 1, wherein the bioisostere of A is selected from the group consisting of:



8. The mediator of Claim 1, wherein the bioisostere of C is selected from the group consisting of:

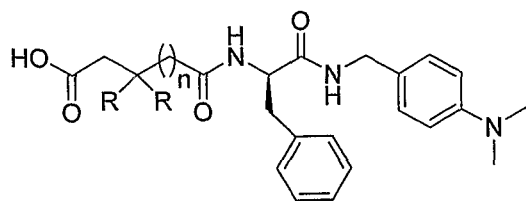


9. The mediator of Claim 1, wherein the mediator is selected from the group consisting of:



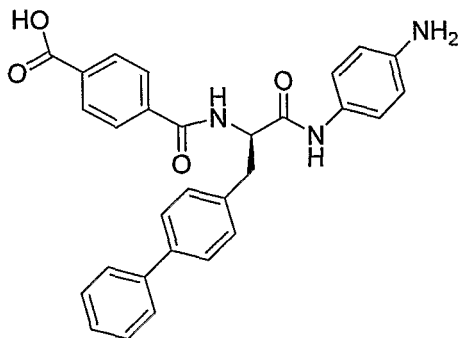
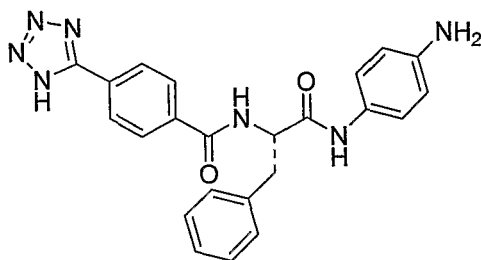
wherein R is H, methyl, cycloalkyl (C3-C7), and n =

1-10



= 1-10

wherein R is H, methyl, cycloalkyl (C3-C7), and n



10. The compound BenOMe - bip - Aniline.

11. The compound 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid.

12. The compound 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid.

13. The compound 4-((R)-1-(4-(dimethylamino)phenylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-(pentamethylene)butanoic acid.

14. The compound 4-((S)-1-(4-guanidinophenylcarbamoyl)-2-(biphenyl)ethylcarbamoyl)benzoic acid.

15. The compound 3-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)propanoic acid.

16. The compound 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)butanoic acid.

17. The compound 4-((R)-1-(4-(dimethylamino)benzylcarbamoyl)-2-phenylethylcarbamoyl)-3,3-dimethylbutanoic acid.