(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 December 2008 (04.12.2008)

PCT

(10) International Publication Number
WO 2008/147717 A1

(51) International Patent Classification:
B01J 20/288 (2006.01) ***B01J 20/32*** (2006.01)
B01J 20/289 (2006.01)

(21) International Application Number:
PCT/US2008/063772

(22) International Filing Date: 15 May 2008 (15.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11/753,934 25 May 2007 (25.05.2007) US

(71) Applicant *(for all designated States except US)*: **DIONEX CORPORATION** [US/US]; 1228 Titan Way, Sunnyvale, CA 94088 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LIU, Xiaodong** [CN/US]; 10167 Camberley Lane, Cupertino, CA 95014 (US). **POHL, Christopher, A.** [US/US]; 32572 Monterey Court, Union City, CA 94587 (US). **CHEN, Jinhua** [CN/US]; 1679 Montemar Way, San Jose, CA 95125 (US).

(74) Agents: **SPLITTGERBER, Ute** et al.; Morgan Lewis & Bockius LLP, One Market, Spear Street Tower, San Francisco, CA 94105 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2008/147717 A1

(54) **Title:** COMPOSITIONS USEFUL AS CHROMATOGRAPHY STATIONARY PHASES

(57) **Abstract:** The current invention provides compositions, which are useful as stationary phases for a variety of chromatographic applications, such as high performance liquid chromatography (HPLC). The compositions include a substrate (e.g., silica gel), covalently bound to a compound, which includes both a hydrophobic moiety and a hydrophilic moiety, which is preferably a 1,2-diol moiety. The hydrophobic moiety is sufficiently hydrophobic for the compositions to exhibit reversed phase characteristics and typically incorporates at least 5 carbon atoms in sequence. Based on having both hydrophilic and hydrophobic functionalities, the new stationary phases exhibit unique chromatographic properties. For example, these media can be used in either hydrophilic (HILIC) mode, in which the mobile phase includes a high percentage of an organic solvent, or in reversed phase mode, in which the mobile phase contains a higher percentage of an aqueous solvent. The current invention also provides methods of making and using the compounds and compositions of the invention.

PATENT APPLICATION

COMPOSITIONS USEFUL AS CHROMATOGRAPHY STATIONARY PHASES

FIELD OF THE INVENTION

[0001] This invention relates to compositions, particularly ones useful as stationary phases for chromatographic applications and methods of making the compositions.

BACKGROUND OF THE INVENTION

[0002] The chromatographic separation of polar compounds is associated with a variety of difficulties. Reversed-phase (RP) columns are widely used for separating molecules via hydrophobic interaction. However, conventional alkyl-bonded reversed-phase media (e.g., C18 and C8) are often not suitable for retaining or separating highly polar molecules (Neue, "HPLC Columns – Theory, Technology, and Practice", WILEY-VCH, New York, 1997, 183-203). Likewise, ion-pairing chromatography is often problematic because of long equilibration times and incompatibility with mass spectroscopy (MS).

[0003] Normal-phase chromatography can also be used to separate polar molecules. A wide range of solvents can be utilized to adjust the selectivity of a particular separation (Neue, "HPLC Columns – Theory, Technology, and Practice," WILEY-VCH, New York, 1997, 164-182). However, due to the sensitivity of normal-phase media to the presence of small concentrations of polar contaminants in the mobile phase, lengthy equilibrating times are common and/or reproducibility problems may occur. Moreover, normal-phase chromatography is often inapplicable due to poor solubility of highly polar compounds in organic solvents.

[0004] *HILIC* (HydrophILic Interaction Chromatography or Hydrophilic Interaction LIquid Chromatography) is a form of normal phase liquid chromatography. HILIC fills the gap between reversed-phase and normal phase chromatography. Appropriate HILIC phases can retain polar compounds while using organic mobile phases, which incorporate only a small percentage of water or an aqueous buffer, typically between about 5 and 30% (Neue, "HPLC Columns – Theory, Technology, and Practice," WILEY-VCH, New York, 1997, 217-223).

[0005] In HILIC, retention increases with the polarity of the analyte and decreases with the polarity of the mobile phase. One possible retention mechanism of HILIC is the partitioning of the analytes between a water-rich stationary phase and a water-poor mobile phase (A.J. Alpert, *J. Chromatogr.* 1990, 499, 117–196). Additional benefits of HILIC include complementary selectivity to reversed-phase columns, enhanced sensitivity in mass spectrometry, and simplified sample preparation procedures. Consequently, HILIC provides a mechanism for the separation and analysis of a wide range of analytes, such as carbohydrates, amino acids, peptides, oligonucleotides and phospholipids, but also small-molecule drugs and their metabolites.

[0006] In general, the packing materials for normal phase chromatography and HILIC can be grouped into the following five categories:

(1) Simple, unbonded silica (see e.g., B.A. Olsen, *J. Chromatogr.* 2001, *A* 913, 113–122);

(2) Neutral silica-based packings bearing diol groups (H. Tanaka, X. Zhou, and O. Masayoshi, *J. Chromatogr. 2003, A* 987, 119–125);

(3) Silica-based packings with amide functionality (A.J. Alpert, *J. Chromatogr.* 1990, 499, 117–196);

(4) Silica-based packings bearing anion-exchange or cation-exchange functionalities (Neue, "HPLC Columns – Theory, Technology, and Practice," WILEY-VCH, New York, 1997, 217-223); and

(5) Zwitterionic silica-based packings, such as those currently offered by Sequant.

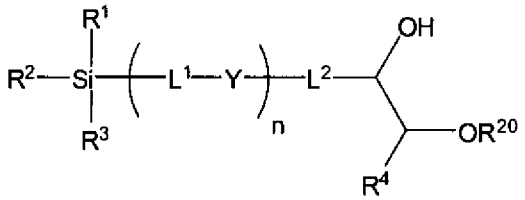
[0007] Unbonded silica was one of the first and most extensively used chromatographic packing material because it was readily available. As discussed above, this material has several drawbacks including poor reproducibility and long equilibration times as well as a limited operating pH range. To overcome these difficulties, silica gels covalently modified with a diol moiety (diol phase) were developed. In these diol phases, silanol groups are functionalized with hydroxyalkyl groups. The resulting stationary phases offer improved reproducibility, shorter equilibration times and a wider operating pH range. In addition, diol-phases are often characterized by reduced secondary interaction compared to its ionizable counterparts.

[0008] However, these and other conventional HILIC materials have inadequate hydrophobicity, making them unsuitable for separating molecules under RP conditions. Therefore, it is highly desirable to develop stationary phases that combine both HILIC and RP characteristics. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

[0009] The current invention provides compositions, which are useful as packing materials/stationary phases for a variety of chromatographic applications, such as high performance liquid chromatography (HPLC). The compositions include a substrate (e.g., silica gel), covalently bound to a molecule, which includes both a hydrophobic moiety as well as a hydrophilic moiety. The hydrophilic moiety is preferably found at a significant distance from the surface of the substrate (e.g., at least 5 carbon atoms away). Based on having both hydrophilic and hydrophobic functionalities, the new packing materials exhibit unique chromatographic properties. For example, these media can be used in either HILIC mode, in which the mobile phase includes a high percentage of an organic solvent, or in RP mode, in which the mobile phase contains a considerable amount of an aqueous solvent. As a result, the new stationary phases are useful for many applications, including the analysis of molecules, which themselves include hydrophilic and hydrophobic moieties, such as ethoxylated surfactants.

[0010] In a first aspect, the present invention provides a composition comprising a compound covalently bound to a substrate. The compound has a structure according to Formula (I), in which the integer n is selected from 0 and 1:



(I)

[0011] In Formula (I), at least one of R^1, R^2 and R^3 is covalently bound to the substrate. R^1, R^2 and R^3 are members independently selected from halogen, OR^{10}, $NR^{10}R^{11}$, $OC(O)R^{12}$, $OS(O)_2R^{12}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and a bond to said substrate. Each R^{10} and each R^{11} is a

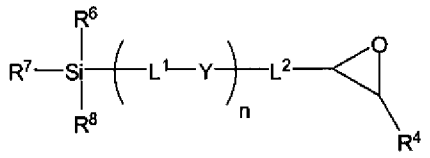
member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and a bond to the substrate. Each R^{12} is a member independently selected from substituted or unsubstituted alkyl (e.g., CH_3, CF_3), substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl.

[0012] In Formula (I), L^1 and L^2 are linker groups independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. In those cases, in which n is 1 and Y is present, at least one of L^1 and L^2 preferably includes a carbon chain having at least 5 carbon atoms, at least 6 carbon atoms, at least 7 carbon atoms or at least 8 carbon atoms in sequence. In those cases, in which n is 0, L^2 preferably includes a carbon chain having at least 5 carbon atoms, at least 6 carbon atoms, at least 7 carbon atoms or at least 8 carbon atoms in sequence. At least two of the carbon atoms in sequence (in L^1, L^2 or both L^1 and L^2) are optionally part of a 5- or 6-membered ring, wherein the ring is a member selected from aryl (e.g., phenyl), heteroaryl (e.g., thiophene) and cycloalkyl (e.g., cyclohexyl or cyclopentyl). The ring can optionally be substituted with a non-polar substituent, such as a C_1-C_4 alkyl group (e.g., methyl).

[0013] The two linker groups L^1 and L^2 are connected through the group Y, which is a member selected from an ether or thioether bond, an amide, sulfonamide, carbonate or carbamate group and a urea or thiourea group. The amide, sulfonamide, carbamate, urea or thiourea groups are optionally substituted. In a particularly preferred embodiment, Y is an amide group, wherein the nitrogen atom is optionally substituted with a lower alkyl group, such as a methyl group.

[0014] R^4 is a member selected from H, acyl, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, wherein R^4 and a substituent of L^2, together with the atoms to which they are attached, are optionally joined to form a 3- to 7-membered ring. In one example, the ring is a substituted or unsubstituted cyclohexyl or cyclopentyl ring. R^{20} is a member selected from H, substituted alkyl, substituted heteroalkyl and substituted heterocycloalkyl, provided that at least one substituent of R^{20} is OH. In one embodiment, R^{20} includes a polyether moiety.

[0015] In a second aspect the invention provides a compound having a structure according to Formula (VI), in which n is an integer selected from 0 and 1. In an exemplary embodiment, the compounds of Formula (VI) are useful as reagents/precursors in the synthesis of the compositions of the invention:

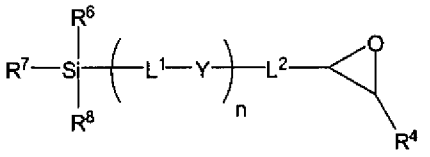


(VI)

[0016] In Formula (VI), R^6, R^7 and R^8 are members independently selected from halogen, OR^{14}, $NR^{14}R^{15}$, $OC(O)R^{16}$, $OS(O)_2R^{16}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. At least one of R^6, R^7 and R^8 is an active silyl group substituent, such as an alkoxy group, a halogen or a primary or secondary amino group. Each R^{14} and each R^{15} is a member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. Each R^{16} is a member independently selected from substituted (e.g, halogen substituted, e.g., CF_3) or unsubstituted alkyl (e.g., CH_3), substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl.

[0017] In Formula (VI), L^1, L^2, R^4, R^{20} and Y are defined as above for Formula (I).

[0018] In a third aspect the invention provides a method of making a composition of the invention. The method includes: (i) contacting a substrate (e.g., silica gel) having a reactive functional group (e.g., a silanol group) with a compound having an epoxide moiety and having a structure according to Formula (VI):



(VI)

wherein n, R^6, R^7, R^8, L^1, L^2, R^4 and Y are defined as above for Formula (VI), under conditions sufficient to form a covalent bond between the compound and the substrate through reaction between the reactive functional group of the substrate and at least one of R^6,

R^7 and R^8. The method may further include (ii) converting the epoxide moiety into an 1,2-diol moiety, for example, by acid-catalyzed hydrolysis or by opening of the epoxide ring using a nucleophilic reagent. In a preferred embodiment, at least one of R^6, R^7 and R^8 is halogen or alkoxy (e.g., methoxy or ethoxy).

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** is a synthetic scheme showing the preparation of diol phase **15**.

[0020] **FIG. 2** is a synthetic scheme showing the preparation of diol phase **20**.

[0021] **FIG. 3** is a synthetic scheme showing the preparation of diol phase **21**.

[0022] **FIG. 4** is a set of two chromatograms comparing the resolution characteristics of diol phase **15** and a commercial diol packing during the analysis of an alkylphenol ethoxylated surfactant (IGPAL CA-630).

[0023] **FIG. 5** is a set of two chromatograms comparing the selectivities of diol phase **15** in reversed-phase and normal phase modes. The two analytes are cytosine (1) and naphthalene (2).

[0024] **FIG. 6** is a set of 9 chromatograms comparing the resolution characteristics of diol phase **15** during the analysis of an alkylphenol ethoxylated surfactant (IGPAL CA-630) when using mobile phases with varying acetonitrile/buffer ratios.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0025] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents, which would result from writing the structure from right to left, *e.g.*, $-CH_2O-$ is intended to also recite $-OCH_2-$.

[0026] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups

such as methyl, ethyl, n-propyl (e.g., $-CH_2-CH_2-CH_3$, $-CH_2-CH_2-CH_2-$), isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups that are limited to hydrocarbon groups are termed "homoalkyl". The term "alkyl" can also mean "alkylene" or "alkyldiyl" as well as alkylidene in those cases where the alkyl group is a divalent radical.

[0027] The term "alkylene" or "alkyldiyl" by itself or as part of another substituent means a divalent radical derived from an alkyl group, as exemplified, but not limited, by $-CH_2CH_2CH_2-$ (propylene or propane-1,3-diyl), and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to about 30 carbon atoms, preferably from 1 to about 25 carbon atoms, more preferably from 1 to about 20 carbon atoms, even more preferably from 1 to about 15 carbon atoms and most preferably from 1 to about 10 carbon atoms. A "lower alkyl", "lower alkylene" or "lower alkyldiyl" is a shorter chain alkyl, alkylene or alkyldiyl group, generally having about 10 or fewer carbon atoms, about 8 or fewer carbon atoms, about 6 or fewer carbon atoms or about 4 or fewer carbon atoms.

[0028] The term "alkylidene" by itself or as part of another substituent means a divalent radical derived from an alkyl group, as exemplified, but not limited, by $CH_3CH_2CH_2=$ (propylidene). Typically, an alkylidene group will have from 1 to about 30 carbon atoms, preferably from 1 to about 25 carbon atoms, more preferably from 1 to about 20 carbon atoms, even more preferably from 1 to about 15 carbon atoms and most preferably from 1 to about 10 carbon atoms. A "lower alkyl" or "lower alkylidene" is a shorter chain alkyl or alkylidene group, generally having about 10 or fewer carbon atoms, about 8 or fewer carbon atoms, about 6 or fewer carbon atoms or about 4 or fewer carbon atoms.

[0029] The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

[0030] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N, Si, S and B, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, B, S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2,-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (*e.g.*, alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-CO_2R'-$ represents both –C(O)OR' and –OC(O)R'.

[0031] The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1 –(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1 –piperazinyl, 2-piperazinyl, and the like.

[0032] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0033] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, substituent that can be a single ring or multiple rings (preferably from 1 to 3 rings), which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, S, Si and B, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[0034] For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

[0035] Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0036] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generically referred to as "alkyl group substituents," and they can be one or more of a variety of groups selected from, but not limited to: substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$OS(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and –NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R", R"' and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"' and R"" groups when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF_3 and –CH_2CF_3) and acyl (*e.g.*, -$C(O)CH_3$, -$C(O)CF_3$, -$C(O)CH_2OCH_3$, and the like).

[0037] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are generically referred to as "aryl group substituents." The substituents are selected from, for example: substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and –NO_2, -R', -N_3, -$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on

the aromatic ring system; and where R', R", R"' and R"" are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"' and R"" groups when more than one of these groups is present.

[0038] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –T-C(O)-$(CRR')_q$-U-, wherein T and U are independently –NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –A-$(CH_2)_r$-B-, wherein A and B are independently –CRR'-, -O-, -NR-, -S-, -S(O)-, -$S(O)_2$-, -$S(O)_2$NR'- or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –$(CRR')_s$-X-$(CR"R"')_d$-, where s and d are independently integers of from 0 to 3, and X is –O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or –$S(O)_2$NR'-. The substituents R, R', R" and R"' are preferably independently selected from hydrogen or substituted or unsubstituted (C_1-C_6)alkyl.

[0039] As used herein, the term "silyl group substituent" can be one or more of a variety of groups selected from, but not limited to: substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, acyl, -OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$OS(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and –NO_2. R', R", R"' and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"' and R"" groups when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be

combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, $-CF_3$ and $-CH_2CF_3$) and acyl (*e.g.*, $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

[0040] As used herein, the term "non-reactive silyl group substituent" means a "silyl group substituent" that does not react with a substrate of the invention to form a covalent bond between the silyl group substituent and the substrate. Exemplary "non-reactive silyl group substituents" include alkyl (e.g., methyl, ethyl, propyl, butyl and other lower alkyl groups) or aryl groups (e.g., phenyl and thiophenyl).

[0041] As used herein, the term "reactive silyl group substituent" means a "silyl group substituent" that is capable of reacting with a substrate of the invention to form a covalent bond between the silyl group substituent and the substrate. Exemplary "reactive silyl group substituents" include those groups that are conventionally defined as leaving groups, such as halogens (e.g., Cl and Br). Other exemplary "reactive silyl group substituents" include alkoxy groups (e.g., methoxy or ethoxy) and primary and secondary amino groups.

[0042] As used herein, the term "acyl" describes a substituent containing a carbonyl residue, C(O)R. Exemplary species for R include H, halogen, substituted or unsubstituted alkyl, substituted or unsubstituted hetroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted heterocycloalkyl.

[0043] As used herein, the term "fused ring system" means at least two rings, wherein each ring has at least 2 atoms in common with another ring. "Fused ring systems may include aromatic as well as non aromatic rings. Examples of "fused ring systems" are naphthalenes, indoles, quinolines, chromenes and the like.

[0044] As used herein, the term "heteroatom" includes oxygen (O), nitrogen (N), sulfur (S), silicon (Si) and boron (B).

[0045] The symbol "R" is a general abbreviation that represents a substituent group that is selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl,

substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted heterocycloalkyl groups.

[0046] When compounds of the present invention contain relatively basic or acidic functionalities, salts of such compounds are included in the scope of the invention. Salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid or base, either neat or in a suitable inert solvent. Examples of salts for relative acidic compounds of the invention include sodium, potassium, calcium, ammonium, organic amino, or magnesium salts, or a similar salts. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge *et al., Journal of Pharmaceutical Science* 1977, 66: 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0047] When a residue is defined as "O‾" (COO^-), then the formula is meant to optionally include H or a cationic counterion. Preferably, the salt form of the compound is pharmaceutically acceptable.

[0048] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

[0049] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention. "Compound or a pharmaceutically acceptable salt or solvate of a compound" intends the inclusive meaning of "or", in that a material that is both a salt and a solvate is encompassed.

[0050] Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are encompassed within the scope of the present invention. Optically active (R)- and (S)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included.

[0051] The graphic representations of racemic, ambiscalemic and scalemic or enantiomerically pure compounds used herein are taken from Maehr, *J. Chem. Ed.*, 62: 114-120 (1985): solid and broken wedges are used to denote the absolute configuration of a chiral element; wavy lines indicate disavowal of any stereochemical implication which the bond it represents could generate; solid and broken bold lines are geometric descriptors indicating the relative configuration shown but not implying any absolute stereochemistry; and wedge outlines and dotted or broken lines denote enantiomerically pure compounds of indeterminate absolute configuration.

[0052] The terms "enantiomeric excess" and diastereomeric excess" are used interchangeably herein. Compounds with a single stereocenter are referred to as being present in "enantiomeric excess," those with at least two stereocenters are referred to as being present in "diastereomeric excess."

[0053] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example,

the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

II. Introduction

[0054] The current invention relates to compositions including a compound covalently bound to a substrate, wherein the compound contains at least one hydrophobic linker as well as a polar head group, such as a 1,2-diol moiety. The compositions of the invention are useful as stationary phases or packing materials in the field of chromatography, such as high performance liquid chromatography (HPLC). In a preferred embodiment, the compositions are useful for the chromatographic separation of both polar (hydrophilic) and non-polar (hydrophobic) molecules. The optimal balance between the hydrophilic and the hydrophobic moieties of the substrate-bonded compounds creates unique chromatographic properties for the new packing materials, which are useful for a variety of applications including the analysis of surfactants (e.g., hydrophobe distribution of ethoxylated nonionic surfactants). In addition, this invention relates to the synthesis of new silanes incorporating an epoxy group as well as methods for their immobilization on a substrate, such as silica gel.

[0055] In a preferred embodiment, the new packing materials can be used in either HILIC mode, in which the mobile phase has a high concentration of an organic solvent, or in reverse phase (RP) mode, in which the mobile phase includes an aqueous solvent. As stationary phases/packing materials for chromatography, the compositions of the invention offer many advantages, which include:

1. The new materials are capable of retaining highly polar molecules, which are not retained by conventional reversed-phase chromatography.

2. The selectivity of the new packing material is complementary to reversed-phase columns.

3. Compared to unbonded silica (i.e., normal phase columns), the new phases are characterized by faster equilibration times and better reproducibility.

4. Compared to conventional diol-phases with a three-carbon linkage, the new phases are characterized by improved hydrophobicity and chemical stability.

5. The new materials can be used with mobile phases including a high content (e.g., greater than 80%) of an organic solvent and therefore provide enhanced sensitivity in mass spectrometry (e.g., enhanced ESI-MS response).

6. The new materials can be used in both reversed-phase and normal phase modes.

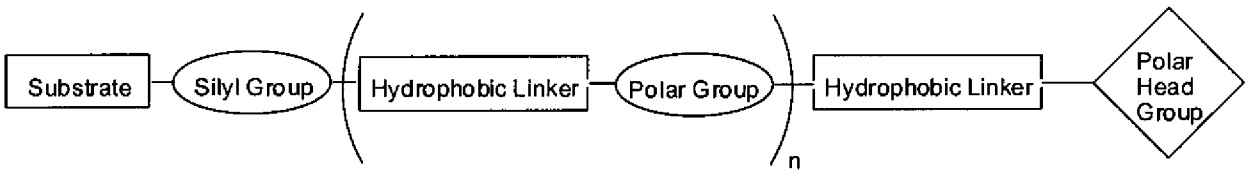
7. The new materials can be used to analyze molecules, which are difficult to analyze using conventional chromatographic media. For example, compounds, which can be analyzed efficiently, include nonionic ethoxylated surfactants and other highly polar molecules (e.g., ureas) as well as peptides and lipids.

III. Compositions

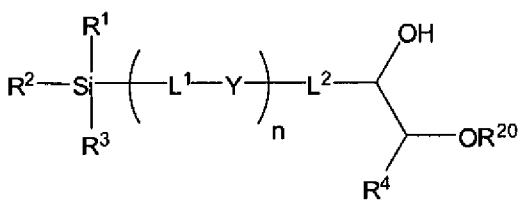
Stationary Phases

[0056] The current invention provides compositions, which are useful as stationary phases or packing materials for a variety of chromatographic applications. Alternatively, the compositions of the invention may be used in other products useful for separation, detection and analysis of compounds, such as membranes, filters and microfluidic devices.

[0057] The compositions include a substrate (e.g., silica gel) and a compound, which is covalently bound to the substrate via a silyl group. The compound includes at least one hydrophobic linker as well as a polar head group. The compositions of the invention can generally be described by the following structure, wherein n is either 0 or 1:



[0058] In one embodiment, the polar head group is a diol moiety. Thus, in a first aspect, the invention provides a composition that includes a compound covalently bound to a substrate, wherein the compound has a structure according to Formula (I), in which n is an integer selected from 0 and 1:



(I)

Silyl Group Substituents

[0059] In Formula (I), R^1, R^2 and R^3 are silyl group substituents. At least one of R^1, R^2 and R^3 is covalently bound to a substrate of the invention. In an exemplary embodiment, one of R^1, R^2 and R^3 is covalently bound to the substrate. In another exemplary embodiment, two of R^1, R^2 and R^3 are covalently bound to the substrate. In yet another exemplary embodiment, each of R^1, R^2 and R^3 is covalently bound to the substrate.

[0060] In one example, R^1, R^2 and R^3 are members independently selected from halogen, OR^{10}, $NR^{10}R^{11}$, acyl, $OC(O)R^{12}$, $OS(O)_2R^{12}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl, wherein each R^{10} and each R^{11} is a member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and a bond to a substrate of the invention (e.g., silica gel). Each R^{12} is a member independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl.

[0061] In another example, at least one of R^1, R^2 and R^3 is a non-reactive silyl group substituent. In this context, a "non-reactive silyl group substituent" does not react with the substrate to form a covalent bond between the silyl group substituent and the substrate. Exemplary non-reactive silyl group substituents include alkyl groups or aryl groups. In a preferred embodiment, at least one of R^1, R^2 and R^3 is a member selected from substituted or unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl and the like). In yet another example, two of R^1, R^2 and R^3 are non-reactive silyl group substituents. For example, two of R^1, R^2 and R^3 are members independently selected from substituted or unsubstituted alkyl, such as substituted or unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl and the like). In a particularly preferred embodiment, one or two of R^1, R^2 and R^3 are methyl.

Linker

[0062] In Formula (I), L^1 and L^2 in Formula (I) are linker groups, which in one embodiment, are independently selected from substituted or unsubstituted alkyl, substituted

or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl.

[0063] In a preferred embodiment, the compounds of the invention include at least one hydrophobic linker. When n is 1, at least one of L^1 and L^2 in Formula (I) includes a hydrophobic moiety. When n is 0, L^2 includes a hydrophobic moiety. In this context, a "hydrophobic moiety" includes a carbon chain having a preferred number of carbon atoms in sequence, wherein this number is defined by a lower and an upper limit. With respect to the lower limit the hydrophobic moiety has preferably at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, or at least 12 carbon atoms in sequence. In a particularly preferred embodiment, the hydrophobic moiety has at least 7 carbon atoms in sequence. With respect to the higher limit, the hydrophobic moiety includes preferably not more than about 50 carbon atoms in sequence, not more than about 30 carbon atoms, not more than about 25 carbon atoms, not more than about 20 carbon atoms, or not more than about 15 carbon atoms in sequence. Exemplary ranges for the number of carbon atoms in sequence may be formed between the above described higher and lower limits. In yet another embodiment, the hydrophobic moiety includes more than 50 carbon atoms in sequence.

[0064] Within the hydrophobic moiety, at least two of the carbon atoms in sequence are optionally part of a ring (e.g., a 5- or 6-membered ring), wherein the ring is a member selected from aryl, heteroaryl, cycloalkyl and a fused ring system that can include aryl, heteroaryl and cycloalkyl rings. The ring is optionally substituted with a non-polar (hydrophobic) substituent, such as an unsubstituted alkyl group (e.g., methyl, ethyl or propyl group). In a preferred embodiment, the hydrophobic moiety is sufficiently hydrophobic for the compositions to exhibit reversed phase characteristics.

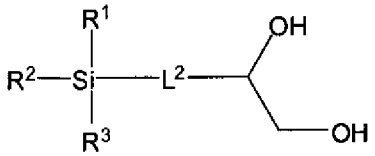
Polar Group Y

[0065] When in Formula (I) n is 1, then the compound of the invention includes a polar group Y. This group can be any suitable group useful to connect the two linker groups L^1 and L^2. In one embodiment, L^1 and L^2 are connected through an ether bond, a thioether bond, an amide or sulfonamide group or, alternatively, through a carbonate, carbamate, urea or thiourea group. In a particularly preferred embodiment, Y is an amide group: $-C(O)NR^{13}-$ or $NR^{13}C(O)-$, wherein R^{13} is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or

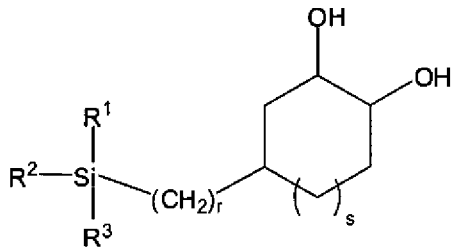
unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. In a preferred embodiment, R^{13} is hydrogen or lower alkyl, such as methyl.

Polar Head Group

[0066] The polar head group can be any moiety that includes at least one, preferably two hydroxyl groups. In one embodiment, the polar head group is a substituted or unsubstituted 1,2-diol moiety as shown in Figure (I), *supra*. R^4 is an alkyl group substituent. In one example, R^4 is a member selected from H, acyl, substituted or unsubstituted alkyl (e.g., C_1-C_4 alkyl) and substituted or unsubstituted heteroalkyl (e.g., C_1-C_4 heteroalkyl). In a preferred embodiment, R^4 is H. An exemplary structure according to this embodiment is shown below:



[0067] In another preferred embodiment, R^4 and a substituent of L^2, together with the atoms to which they are attached, are optionally joined to form a 3- to 7-membered ring selected from substituted or unsubstituted cycloalkyl and substituted or unsubstituted heterocycloalkyl. The ring is preferably cycloalkyl and is optionally substituted with a non-polar (hydrophobic) substituent, such as an unsubstituted alkyl group (e.g., methyl, ethyl or propyl group). An exemplary structure according to this embodiment is shown below:



wherein s is an integer selected from 0 and 1; and r is an integer selected from 2 to 30, preferably from 2 to 20, more preferably from 2 to 10 and most preferably from 2 to 6.

[0068] In Formula (I), R^{20} is member selected from H, substituted alkyl, substituted heteroalkyl and substituted heterocycloalkyl, provided that at least one substituent of R^{20} is OH. In one embodiment, R^{20} is a moiety, which is derived from the nucleophilic opening of an epoxide ring using a nucleophilic reagent having a nucleophilic oxygen. In one example, R^{20} includes a polyether moiety. In another example, R^{20} is H.

Substrate

[0069] The substrate of the invention can be any material (e.g., particles) useful as a stationary phase/packing material for chromatography including porous and non-porous solids.

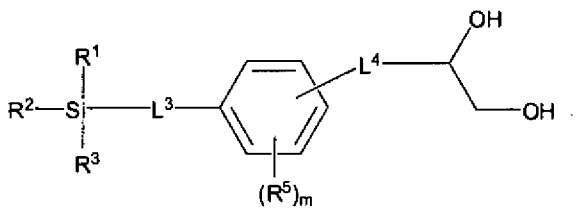
[0070] Exemplary substrates include silica-based (e.g., silicon oxide), titanium-based (e.g., titanium oxide), germanium-based (e.g., germanium oxide), zirkonium-based (e.g., zirkonium oxide) and aluminum-based (e.g., aluminum oxide) materials. Other substrates include cross-linked and non-crosslinked polymers, carbonized materials and metals. Compounds of the invention can also be incorporated into polymeric networks, sol-gel networks or hybrid forms thereof. In one embodiment, the substrate is a silica-based substrate. Exemplary silica-based substrates include silica gel, glass, sol-gels, polymer/sol-gel hybrids and silica monolithic materials.

[0071] In one example, the substrate includes reactive functional groups, which can be reacted with an activated silyl group of a compound of the invention, e.g., those of Formula (VIa) and (VIb), to form a covalent bond between the substrate and the compound. Reactive functional groups of the substrate include silanol groups as well as alkoxy silane, halosilane and aminosilane moieties. Other exemplary reactive groups include metal-hydroxides, such as titanium hydroxide and zirconium hydroxide.

[0072] In a particularly preferred embodiment, the substrate is silica gel. Suitable silica gels include non-porous and/or porous silica particles of different pore sizes, preferably from 20 Å to 3000 Å and more preferably, from 60 Å to 2000 Å; and of different particle sizes, preferably, from 0.2um to1000 um, and more preferably, from 2um to 50um.

Exemplary Compositions of the Invention

[0073] In one embodiment, the integer n in Formula (I) is 0 and the linker L^2 includes an aromatic ring, such as a phenyl ring. In one example according to this embodiment, the compound has a structure according to Formula (II):

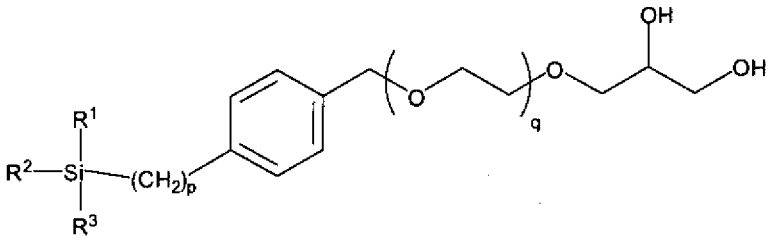


(II)

wherein m is an integer selected from 0 to 4. L^3 is a linker group, which is a member selected from substituted or unsubstituted alkyl. In one example, L^3 is straight or branched alkyl (e.g., C_2-C_{20} alkyl). L^4 is a linker group, which is a member selected from a single bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In one example, L^4 is straight or branched alkyl optionally interrupted by one or more ether or thioether group.

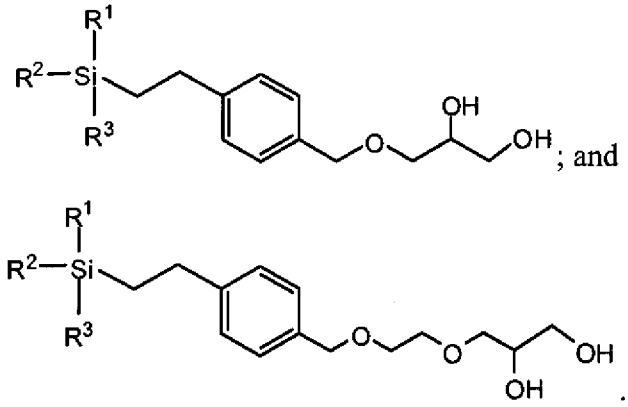
[0074] Each R^5 is an aryl group substituent. In an exemplary embodiment, each R^5 is a member independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl, wherein two adjacent R^5, together with the atoms to which they are attached, are optionally joined to form a 5- to 7-membered ring, wherein the ring can be aromatic or non-aromatic. In one embodiment, the ring is a phenyl ring, thus forming a naphthyl moiety. In a particularly preferred embodiment, each R^5 is a member independently selected from H and a non-polar substituent, such as unsubstituted alkyl. In one example, each R^5 is a member independently selected from H and unsubstituted C_1-C_4 alkyl (e.g., methyl, or ethyl).

[0075] In another exemplary embodiment, the compound of Formula (II) has a structure according to Formula (III):

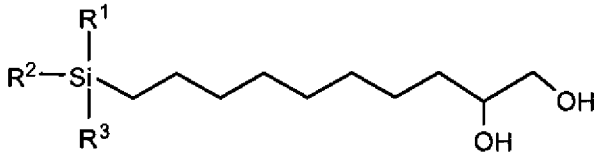


(III)

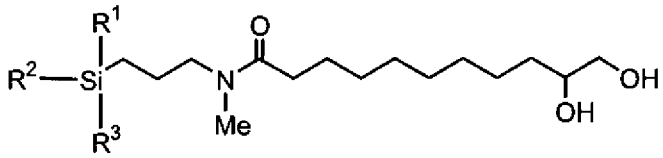
[0076] In Formula (III) p is an integer selected from 2 to 20, preferably from 2-15 and more preferably from 2-10, and q is an integer selected from 0 to 10, preferably from 0 to 5. Exemplary structures according to this embodiment are provided below:



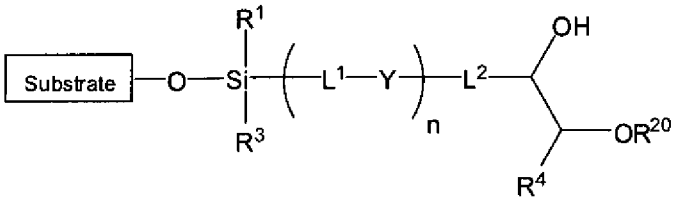
[0077] In yet another exemplary embodiment, in Formula (I), n is 0 and L^2 is straight or branched alkyl. In one example according to this embodiment, L^2 is alkyl-substituted or unsubstituted C_5-C_{30} alkyl, preferably unsubstituted C_6-C_{25} alkyl, and more preferably unsubstituted C_6-C_{20} alkyl. In a particularly preferred embodiment L^2 is C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} or C_{15} unsubstituted alkyl. An exemplary structure according to this embodiment is provided below:



[0078] In a further exemplary embodiment, in Formula (I), n is 1 and L^2 is straight or branched alkyl. In one example according to this embodiment, L^2 is alkyl-substituted or unsubstituted C_5-C_{30} alkyl, preferably unsubstituted C_6-C_{25} alkyl, and more preferably unsubstituted C_6-C_{20} alkyl. In a particularly preferred embodiment L^2 is C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} or C_{15} unsubstituted alkyl. An exemplary structure according to this embodiment is provided below:



[0079] In a particularly preferred embodiment, at least one of R^1, R^2 and R^3 in Formula (I) is OR^{10} wherein R^{10} represents a bond to the substrate (e.g., silica gel). Exemplary compositions of the invention have a structure according to Formula (V):

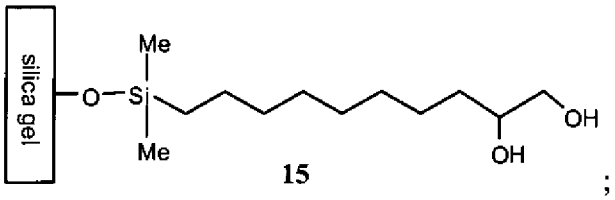


(V)

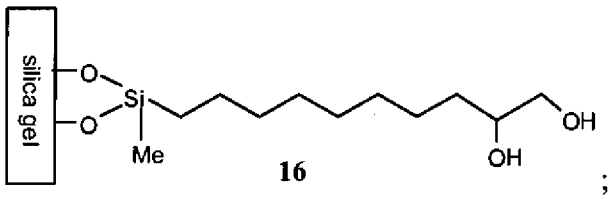
wherein n is an integer selected from 0 and 1 and R^1, R^3, R^4, R^{20}, L^1, L^2 and Y as well as the substrate are defined as above for Formula (I).

[0080] In one embodiment at least one of R^1 and R^3 in Formula (V) is a non-reactive silyl group substituent. In an exemplary embodiment, at least one of R^1 and R^3 is a member selected from substituted or unsubstituted alkyl. In one example, at least one of R^1 and R^3 is unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl and the like). In another example, both R^1 and R^3 are members independently selected from substituted or unsubstituted alkyl, such as unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl and the like). In a preferred embodiment, at least one of R^1 and R^3 is methyl. In another preferred embodiment, both R^1 and R^3 are methyl.

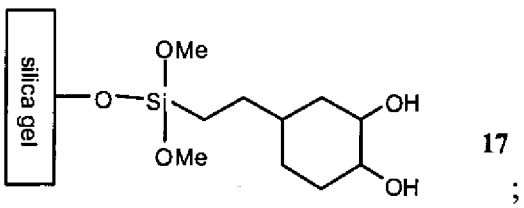
[0081] Exemplary compositions according to Formula (V) include:



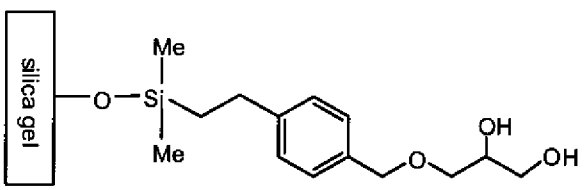
15 ;



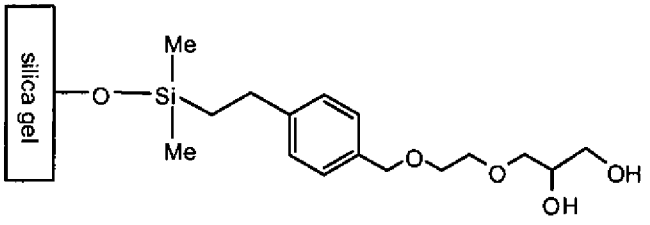
16 ;



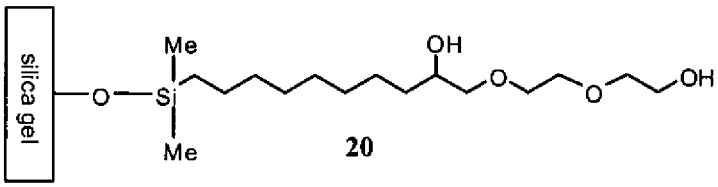
17 ;



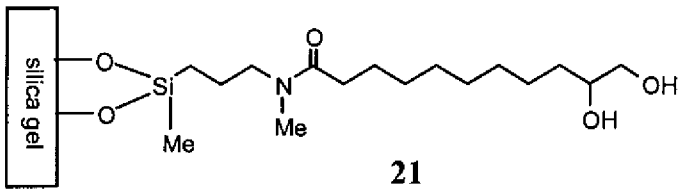
18 ;



19 ;



20 ; and



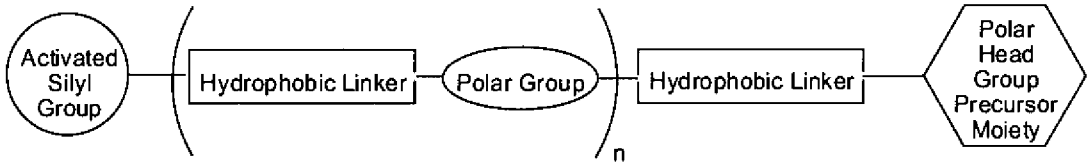
21 .

[0082] Diol phase **15** has been evaluated chromatographically (see Examples 8 to 10). It was found that this material has chromatographic properties different from those of conventional diol phases. The introduction of enhanced hydrophobicity extends the operating range of the new diol phase into reversed-phase conditions (see FIG. 5 and FIG. 6). In addition, the combination of hydrophobic and hydrophilic functionalities provides enhanced resolution for oligomers in ethoxylated molecules, such as alkylphenoethoxylates (IGEPAL CA-630) (see Fig. 4 and FIG. 6).

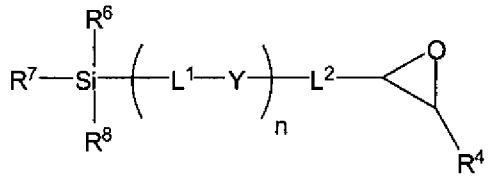
[0083] The current invention provides embodiment, in which the compositions of the invention are contained in a container. The container is preferably a chromatography column. Exemplary chromatography columns include metal columns, glass columns and columns made from a polymeric material, such as plastics. Metal columns may be those commonly used for chromatography procedures employing high pressure (e.g., HPLC). Plastic columns may be those commonly employed for preparative chromatography systems. Such polymeric columns are frequently disposable and are often referred to as cartridges.

Starting Materials

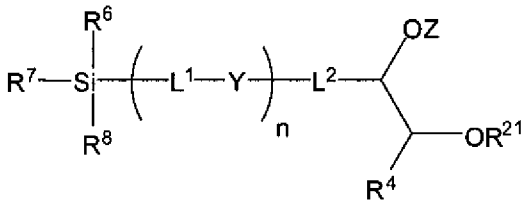
[0084] In a second aspect the invention provides a compound incorporating a reactive silyl group as well as a precursor moiety (which can be converted into a polar head group). Such compounds have the following general formula:



[0085] In one embodiment, the compounds incorporate an epoxide moiety or a protected diol moiety. The compounds have a structure according to Formulae (VIa) and (VIb), wherein n is an integer selected from 0 and 1:



(VIa)



(VIb)

[0086] In Formulae (VIa) and (VIb), L^1, L^2, R^4, R^{20} and Y are defined as above for Formula (I). In Formula (VIb), Z is a protecting group and R^{21} is a member selected from a protecting group, substituted alkyl, substituted heteroalkyl and substituted heterocycloalkyl, provided that at least one substituent of R^{21} is a protected hydroxyl group. In one embodiment, R^{21} is a moiety, which is derived from the nucleophilic opening of an epoxide ring using a nucleophilic reagent having a nucleophilic oxygen. In one example, R^{21} includes a polyether moiety. Z and R^{21} may optionally be joined to form a ring creating a cyclic 1,2-diol protecting group, such as a ketal. Exemplary protecting groups for the protection of hydroxyl groups and 1,2-diol groups of Formula (VIb) include those described in Greene W. and Wuts P. G. M., "Protective Groups in Organic Chemistry", (Wiley, 3rd edition 1999), for example pages 17-245. In one example, Z in Formula (VIb) is a substituted methyl group forming a substituted methyl ether, such as a methoxymethyl (MOM) ether.

[0087] When n is 1, at least one of L^1 and L^2 in Formulae (VIa) and (VIb) includes a hydrophobic moiety. When n is 0, L^2 includes a hydrophobic moiety. In this context, a "hydrophobic moiety" includes a carbon chain having a preferred number of carbon atoms in sequence, wherein this number is defined by a lower and an upper limit. With respect to the lower limit the "hydrophobic moiety" has preferably at least 5, at least 6, at least 7, at least 8,

at least 9, at least 10, at least 11, or at least 12 carbon atoms in sequence. In a particularly preferred embodiment, the hydrophobic moiety has at least 7 carbon atoms in sequence. With respect to the higher limit, the "hydrophobic moiety" includes preferably not more than about 50 carbon atoms in sequence, not more than about 30 carbon atoms, not more than about 25 carbon atoms, not more than about 20 carbon atoms, or not more than about 15 carbon atoms in sequence. Exemplary ranges for the number of carbon atoms in sequence may be formed between the above described higher and lower limits. In yet another embodiment, the hydrophobic moiety includes more than 50 carbon atoms in sequence. Within the hydrophobic moiety, at least two of the carbon atoms in sequence are optionally part of a ring (e.g., a 5- or 6-membered ring), wherein the ring is a member selected from aryl, heteroaryl, cycloalkyl and a fused ring system that can include aryl, heteroaryl and cycloalkyl rings. The ring is optionally substituted with a non-polar (hydrophobic) substituent, such as an unsubstituted alkyl group (e.g., methyl, ethyl or propyl group).

[0088] In an exemplary embodiment, the compounds of Formulae (VIa) and (VIb) are useful as starting materials in the synthesis of the compositions of the invention.

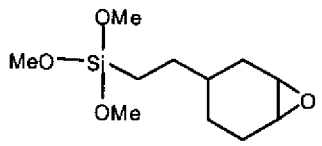
[0089] In Formula (VI), R^6, R^7 and R^8 are silyl group substituents and together with the Si atom form an activated silyl group. An activated silyl group includes at least one reactive silyl group substituent. A reactive silyl group substituent is capable of reacting with a substrate of the invention to form a covalent bond between the compound and the substrate. Thus, at least one of R^6, R^7 and R^8 is a reactive silyl group substituent. Exemplary reactive silyl group substituents include alkoxy groups, halogens and primary or secondary amino groups.

[0090] In one embodiment, R^6, R^7 and R^8 are members independently selected from halogen, OR^{14}, $NR^{14}R^{15}$, $OC(O)R^{16}$, $OS(O)_2R^{16}$, acyl, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. Each R^{14} and each R^{15} is a member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. Each R^{16} is a member independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and

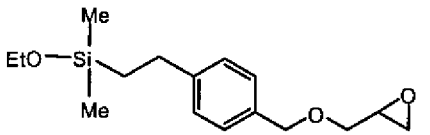
substituted or unsubstituted heterocycloalkyl. In a preferred embodiment, at least one of R^6, R^7 and R^8 is other than OH, unsubstituted alkyl, unsubstituted aryl, unsubstituted heteroaryl and unsubstituted heterocycloalkyl.

[0091] In one example, one of R^6, R^7 and R^8 is a non-reactive silyl group substituent. In another example, two of R^6, R^7 and R^8 are non-reactive silyl group substituents. Exemplary non-reactive silyl group substituents include alkyl groups or aryl groups. In a preferred embodiment, one or two of R^6, R^7 and R^8 are members selected from unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl and the like). In yet another example, two of R^6, R^7 and R^8 are non-reactive silyl group substituents. For example, two of R^6, R^7 and R^8 are members independently selected from substituted or unsubstituted alkyl, such as substituted or unsubstituted C_1-C_6 alkyl (e.g., methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl and the like). In a particularly preferred embodiment, one or two of R^6, R^7 and R^8 are methyl.

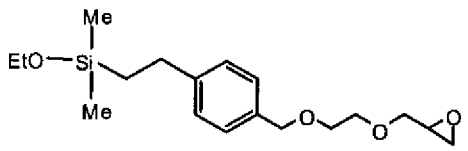
[0092] Exemplary compounds according to Formulae (VIa) and (VIb) include:



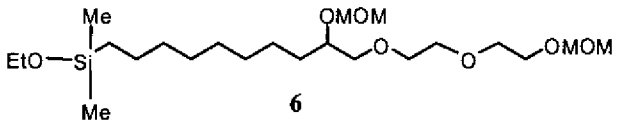
3 ;



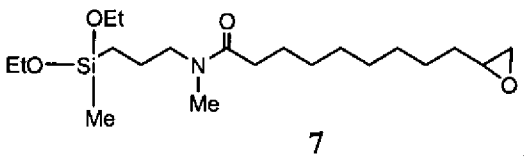
4 ;



5 ;



6 ; and



7 .

IV. Methods

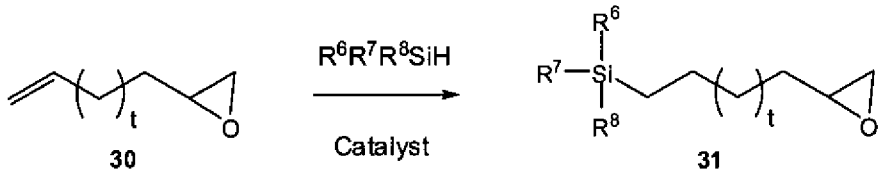
[0093] The compositions and compounds of the invention may be synthesized using methods known in the art and those described herein. Exemplary methods are outlined in Schemes 1-3, below and in Examples 1 to 6. Variation of those methods may be necessary to synthesize compounds of certain embodiments. Those alternative methods will be apparent to a person of skill in the art. Starting materials and reagents useful for preparing the

compositions and compounds of the invention are commercially available or can be prepared using art-recognized methodologies.

Synthesis of Compounds According to Formula (VI) (Starting Materials)

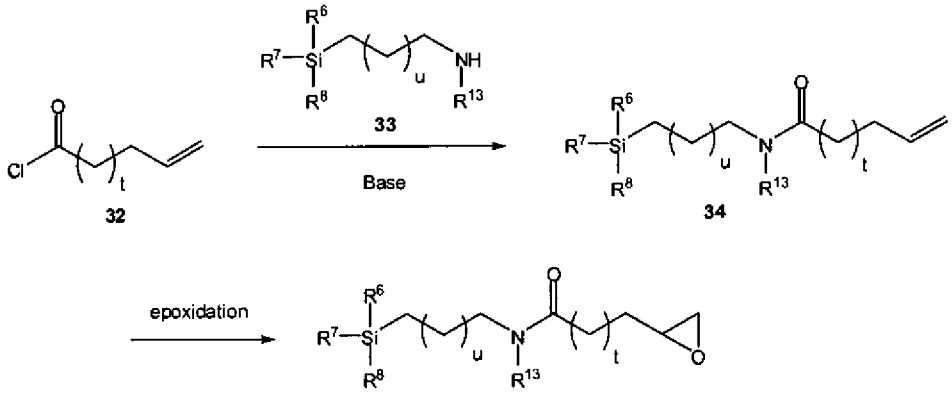
[0094] In one embodiment, compounds of Formula (VIa) are prepared using a procedure outlined in Scheme 1, below, in which the integer t is a member selected from 0 to 30, preferably from 2 to 20 and most preferably from 2 to 15. In Scheme 1, the terminal double bond of compound **30** is hydrosilylated using a silane in the presence of a catalyst, such as a platinum(0) catalyst, to give compound **31**.

Scheme 1



[0095] In another example, compounds according to Formula (VIa), in which n is 1, are synthesized following a procedure outlined in Scheme 2, in which the integer t is selected from 0 to 30, preferably from 4 to about 30, and more preferably from 4 to about 20; and the integer u is selected from 0 to about 30, preferably from 1 to about 20. In Scheme 2, compound **32** having an activate carboxylic acid group (e.g., an acid chloride moiety) is reacted with the amine **33** in the presence of a base (e.g., triethylamine) to form the amide **34**. Subsequently, the terminal double bond of **34** is oxidized to form an epoxide moiety. Epoxidation reagents are known in the art and include perbenzoic acids, such as meta-chloroperbenzoic acid (*m*-CPBA). Epoxidation reactions can be performed stereoselectively resulting in chiral products. Chiral epoxides of the invention can be used to synthesize chiral versions of the compositions of the invention useful for chiral chromatography.

Scheme 2

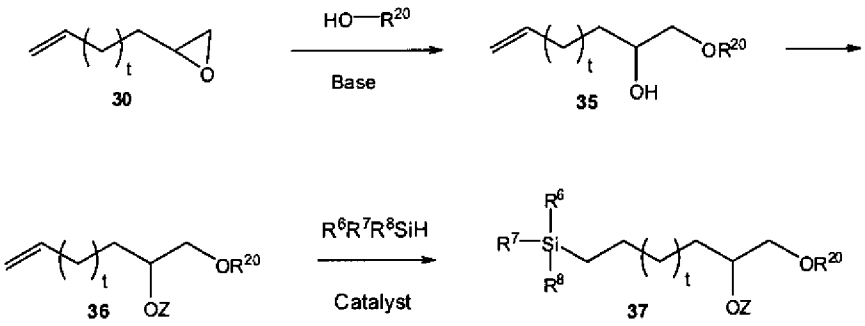


[0096] In Scheme 2, R^{13} is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. In a preferred embodiment, R^{13} is H or lower alkyl, such as methyl.

[0097] Alternatively, compound **34** is first coupled to a substrate of the invention as described herein and the double bond is subsequently oxidized to form an epoxide moiety, which may then be converted to a 1,2-diol moiety. An exemplary synthetic strategy according to this embodiment is presented in FIG. 3 outlining a synthetic route to composition **21**.

[0098] In yet another example, compounds according to Formula (VIb) are synthesized following a procedure outlined in Scheme 3, in which the integer t is a member selected from 0 to 30, preferably from 2 to 20 and most preferably from 2 to 15.

Scheme 3



[0099] In Scheme 3, the epoxide ring of compound **30** is opened using a nucleophilic reagent. The nucleophile may be formed through reaction of a hydroxyl group-containing starting material with a suitable base, such as NaH. The resulting OH-group of compound **35**

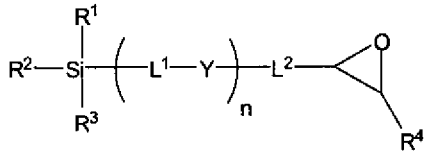
may be protected to form compound **36**, in which Z is a protecting group. Additional hydroxyl groups of the residue R^{20} may also be protected during this step. Suitable protecting groups, such as substituted methyl ethers (e.g., MOM ether) are discussed herein above in the context of describing Formula (VIb). The fully protected analog **36** is then hydrosilylated as described above in Scheme 1 to form compound **37**.

Synthesis of compounds According to Formula (I)

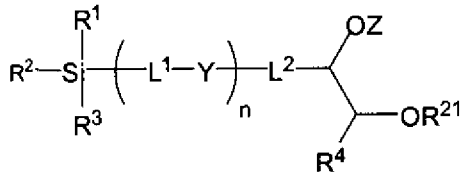
[0100] Compounds of Formula (VIa) and (VIb) can be covalently attached to a substrate (e.g., silica gel) to form a composition of the invention. In one embodiment, a covalent bond between the substrate and the compound is formed through reaction of the substrate with at least one reactive silyl group substituent of the compound. In an exemplary embodiment, the substrate includes reactive functional groups that react with the reactive silyl group substituent to form a covalent bond. Exemplary reactive functional groups of the substrate include silanol and alkoxy silane groups as well as halosilane and aminosilane moieties.

[0101] Typically, reaction between a silica substrate and a compound of the invention is effected by heating a mixture of the compound and a slurry of the silica substrate in an inert solvent (e.g., toluene). For example, the mixture is heated to reflux for from about 2 to about 100 hours, preferably from about 10 to about 80 hours, and more preferably from about 10 to about 60 hours. Optionally, a coupling catalyst is added to control the density of the bonded groups on the surface of the substrate as well as the morphology of the resulting phase. Exemplary coupling catalysts include water as well as organic and inorganic acids (e.g., HCl) and bases (NaOH, amines).

[0102] The above described coupling procedure results in intermediate compositions incorporating a polar head group precursor moiety, such as an epoxide or a protected 1,2-diol moiety. In one embodiment, the intermediate composition has a structure according to Formulae (VIIa) or (VIIb):

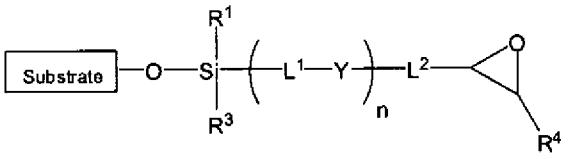


(VIIa)

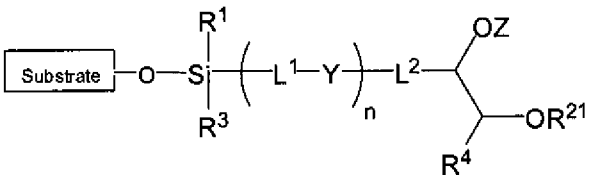


(VIIb)

[0103] In one example the intermediate compositions have a structure according to Formulae (VIIIa) and (VIIIb):



(VIIIa)



(VIIIb)

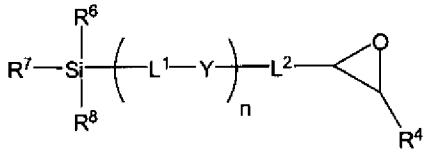
[0104] The above intermediate compositions of Formulae (VIIa), (VIIb), (VIIIa) and (VIIIb) are expected to be useful as stationary phases in chromatography and are within the scope of the current invention.

[0105] The intermediate compositions of the invention may be converted to compositions of the invention, for example those of Formula (I), through conversion of the polar head group precursor to a polar head group, such as a 1,2-diol moiety. In one embodiment, the epoxide moiety of Formula (VIIa) or Formula (VIIIa) is converted to a 1,2-diol moiety. It is well within the abilities of a skilled person to carry out such hydrolysis. For example, acid catalyzed hydrolysis can be carried out by treating the intermediate composition with an aqueous solution containing an organic (e.g., formic acid) or inorganic acid.

[0106] In another embodiment, the protected diol moiety of Formulae (VIIb) and (VIIIb) is deprotected to form a 1,2-diol moiety. The removal of a protecting groups is also well within the abilities of a skilled person. Reaction conditions will depend on the type of protecting group used. Exemplary deprotection procedures include those described in Greene W. and Wuts P. G. M., "Protective Groups in Organic Chemistry", (Wiley, 3rd edition 1999), for example pages 17-245. In one example, Z in Formulae (VIIb) and (VIIIb) is a substituted methyl group forming a substituted methyl ether, such as a methoxymethyl (MOM) ether.

Such ethers may be converted to the corresponding hydroxyl group through acid-catalyzed hydrolysis.

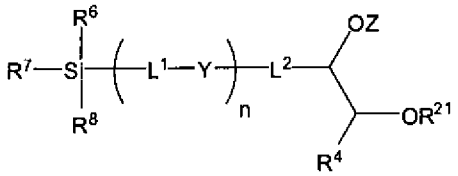
[0107] Accordingly, the invention provides methods of making a composition of the invention. In one embodiment, the method includes: (i) contacting a substrate (e.g., silica gel) having a reactive functional group (e.g., a silanol group) with a compound having an epoxide moiety and having a structure according to Formula (VIa):



(VIa)

wherein n, R^6, R^7, R^8, L^1, L^2, R^4 and Y are defined herein above for Formula (VIa), under conditions sufficient to form a covalent bond between the compound and the substrate through reaction between the reactive functional group of the substrate and at least one of R^6, R^7 and R^8. The method may further include (ii) converting the epoxide moiety into an 1,2-diol moiety, for example, by acid-catalyzed hydrolysis or by opening of the epoxide ring using a nucleophilic reagent as described above. In a preferred embodiment, at least one of R^6, R^7 and R^8 is halogen or alkoxy (e.g., methoxy or ethoxy).

[0108] In another embodiment, the method includes: (i) contacting a substrate (e.g., silica gel) having a reactive functional group (e.g., a silanol group) with a compound having a protected 1,2-diol moiety and having a structure according to Formula (VIb):



(VIb)

wherein n, R^6, R^7, R^8, L^1, L^2, R^4, R^{21}, Z and Y are defined herein above for Formula (VIb), under conditions sufficient to form a covalent bond between the compound and the substrate through reaction between the reactive functional group of the substrate and at least one of R^6, R^7 and R^8. The method may further include (ii) removing the protecting group to form an 1,2-diol moiety, for example, by acid-catalyzed hydrolysis as described above. In a preferred embodiment, at least one of R^6, R^7 and R^8 is halogen or alkoxy (e.g., methoxy or ethoxy).

Chromatographic Methods

[0109] In another embodiment, the invention provides a chromatographic method comprising flowing a mobile phase through a stationary phase comprising a composition of the invention, such as those of Formula (I). In one example, the mobile phase is a liquid. In an exemplary embodiment, the mobile phase includes water. The water content of the mobile phase is preferably between about 0.1% v/v and 60% v/v, more preferably between about 1% and about 20% v/v, even more preferably between about 1% and about 10% v/v and most preferably between about 1% and about 5% v/v.

[0110] In another embodiment, the invention provides a method of separating analytes in a liquid sample comprising flowing the liquid sample through a stationary phase comprising a composition of the invention. In an exemplary embodiment, the liquid sample includes water. The water content of the liquid sample is preferably between about 0.1% v/v and 60% v/v, more preferably between about 1% and about 20% v/v, even more preferably between about 1% and about 10% v/v and most preferably between about 1% and about 5% v/v.

EXAMPLES

Example 1: Synthesis of silyl ligand 1

[0111] To a stirred solution of 50 g 1,2-epoxy-9-decene (e.g., Aldrich), 100 g of $(EtO)Me_2SiH$ (e.g., Gelest) in 30 mL of toluene in a 1-L round bottom flask at ambient temperature were carefully added 0.5 g of Pt(0) catalyst (0.1% wt) (e.g., Gelest). Occasionally, an exothermic reaction is observed upon addition of the catalyst. The flask was equipped with a condenser and the reaction mixture was heated to 50 °C for 8 h. The reaction was monitored using gas chromatography.

[0112] When the conversion was found to be higher than 60% by GC, all volatiles were removed *in vacuo*. Residual volatiles were removed by Kugelrohr Distillation (120 °C/0.05 torr).

Example 2: Synthesis of silyl ligand 2

[0113] To a stirred solution of 50 g 1,2-epoxy-9-decene (e.g., Aldrich), 100 g of $(EtO)_2MeSiH$ (e.g., Gelest) in 30 mL of toluene in a 1-L round bottom flask at ambient temperature were carefully added 0.5 g of Pt(0) catalyst (0.1% wt) (e.g., Gelest).

Occasionally, an exothermic reaction is observed upon addition of the catalyst. The flask was equipped with a condenser and the reaction mixture was heated to 50 °C for 8 h. The reaction was monitored using gas chromatography.

[0114] When the conversion was found to be higher than 60% by GC, all volatiles were removed *in vacuo*. Residual volatiles were removed by Kugelrohr Distillation (140 °C/0.11 torr).

Example 3: Synthesis of silyl ligand 6

[0115] To 5.89 g (0.24 mol, 1.2 eq) of sodium hydride in an oven-dried four necked 1 L flask equipped with a mechanical stirrer, reflux condenser, additional funnel, internal thermometer and nitrogen inlet, were added 600 mL of anhydrous N, N-dimethylformamide. 127.82 g (1.2 mol, 6 eq) of diethylene glycol were added dropwise in one hour. The mixture was stirred at 75 °C for four hours before 30.3 g of 1, 2-epoxy-9-decene (0.2 mol, 1 eq) were added dropwise over 30 minutes. The reaction mixture was stirred at 75 °C overnight and was then cooled to room temperature. The volatiles were removed *in vacuo*. 300 mL of water were added to the residue. Concentrated HCl was then added until pH 2-3. The product was extracted with methylene chloride (3x250 mL). The organic phase was washed with water (3x250 mL) and brine (2x250 mL) and was dried over $MgSO_4$. The volatiles were removed *in vacuo*. The residue was subjected to Kugelrohr distillation (145 °C /0.03 mmHg) to give 42.90 g of intermediate **B** in 83% yield (100 % purity by GC).

[0116] To a solution of 5.20 g of intermediate **B** (20 mmol, 1 eq.) in 60 mL of 1, 2-dimethoxyethane in a 250 mL three necked flask equipped with a funnel and a nitrogen inlet, were added 8.9 mL of diisopropylethylamine. The mixture was cooled to 0 °C and 3.65 g of methoxylmethyl(MOM) chloride in 10 mL 1, 2-dimethoxyethane were added over 30 minutes. The mixture was warmed to room temperature and stirred for 2 days. The mixture was then diluted with 200 mL ether and washed with water (3x75 mL) and brine (3x75 mL). It was dried over magnesium sulfate and the volatiles were removed *in vacuo*. The crude residue was purified by Kugelrohr distillation (150 °C /0.03 mmHg) to give 6.79 g of intermediate **C** in 98% yield (96.45 % purity by GC).

[0117] To a stirred mixture of 6.79 g of intermediate **C** (19.5 mmol, 1 eq.) and 8.0 mL of dimethylethoxyl silane (58.1 mmol, 3 eq.) in a 100 mL flask with condenser were carefully

added a solution of 0.08 g of a Pt(0) catalyst (0.1 % wt) in 2 mL of toluene (an exothermic reaction is sometimes observed). The mixture was refluxed at 52 °C over night. The volatiles were removed *in vacuo* and the residue was purified by Kugelrohr distillation (185 °C /0.03 mmHg) to give 7.56 g of silyl ligand **6** in 85% yield and 85.6 % purity by GC.

Example 4: Synthesis of silyl ligand 7

[0118] To a stirred solution of N-methylaminopropylmethyldimethoxysilane (180 g, 95%) and triethylamine (140 mL) in anhydrous CH_2Cl_2 (1000 mL) in a 3-neck 5-L round bottom flask purged with nitrogen at below 5°C (ice-water bath) was slowly added a solution of 10-undecenoyl chloride (220 g, 95%) in anhydrous CH_2Cl_2 (500 mL). The reaction mixture was stirred at ambient temperature for 16 to 24 hours. GC was used to monitor the reaction. Upon completion of the reaction, the mixture was filtered and the organic phase was washed with D. I. water and dried over Na_2SO_4. The volatiles were removed in *vacuo* to give intermediate **A** as an orange oily liquid (290 g).

[0119] The above intermediate **A** was oxidized with 3-chloroperbenzoic acid using a literature procedure [Tetrahedron Letter, 849 (1965)] to form silyl ligand **7**.

Example 5: General procedure for preparing exemplary intermediate phases 8 – 14

[0120] The coupling reaction between the silica substrate and a compound of the invention having a reactive silyl group were typically carried out in the slurry of the silica gel in an inert solvent such as toluene at elevated temperature. Optionally, water and/or an acid or base catalyst were added to control the surface coverage and morphology of the resulting phase, depending on different application. The reaction mixture was refluxed for 12 to 72 hours. The functionalized silica was then filtered off (e.g., using a glass frit filter) and thoroughly washed with an organic solvent. The solid was dried (e.g., air-dried on the filter) to give bonded silica functionalized with an epoxide moiety or a protected diol moiety (intermediate phases of Formula (VIIa and VIIb)). The preparation of exemplary intermediate phases **8**, **13** and **14** are outlined in Figures 1, 2 and 3, respectively.

Example 6: General procedure for preparing diol phases 15 – 21

[0121] 25 g of the above bonded silica of Formula (VII) was treated with 0.2% formic acid in an accelerated solvent extraction (ASE) cell (100 mL cell) at 50 °C for 4 hours. The silica was then rinsed thoroughly with acetonitrile and dried in a vacuum oven for 3 hours to give

diol phases of Formula (I). The preparation of diol phases **15**, **20** and **21** are outlined in Figures 1, 2 and 3, respectively.

Example 7: Alternative procedure for preparing diol phase 21

[0122] To a slurry of 25 g of raw silica (5 micron, 120 Å, dried *in vacuo* at 200 °C for 20 h) in 75 mL of anhydrous m-xylene in a dry 250-mL flask was added a solution of 25 g of silyl intermediate **A** and the resulting mixture was mixed well (e.g., by shaking and/or sonication). The reaction mixture was refluxed for 120 hours, and the silica was then filtered off and washed with dichloromethane.

[0123] The above silica was treated with a solution of 3-chloroperbenzoic acid in dichloromethane at 5 °C and the reaction mixture was stirred for 4 hours. The silica was then filtered off and washed thoroughly with dichloromethane, and then with acetone to give diol phase **21**.

Example 8: Comparison between diol phase 15 and a commercial diol packing

[0124] Diol phase **15** and a commercial diol packing packing material were compared with respect to their performance during the analysis of an alkylphenol ethoxy surfactant (IGEPAL CA-630). Resulting chromatograms are shown in Figure 4. Under the same chromatographic conditions, the column packed with **15** exhibits better resolution for oligomers compared to its commercial counterpart. The test conditions were as follows:

Column dimensions:	4.6x150 mm with 5 μm particle size
Mobile phase:	99/1 v/v acetonitrile/100 mM ammonium acetate, pH 5.2
Temperature:	30 °C
Flow rate:	1 mL/min
Injection volume:	10 μL
Detection wavelength:	225 nm
Sample:	IGEPAL CA-630 (0.1%).

Example 9: Selectivity comparison of diol phase 15 in reversed-phase and normal phase modes

[0125] The selectivity of diol phase **15** in both reversed-phase mode and normal phase mode was determined using a polar (cytosine) and non-polar (naphthalene) test analytes. Results are shown in Figure 5. In reversed-phase mode (mobile phase containing 52%

acetonitrile), the polar molecule (cytosine) elutes before the hydrophobic molecule (naphthalene). When the mobile phase contains 92% acetonitrile, diol phase **15** exhibits normal phase behavior, in which retention time increases with polarity of the compound. These results indicate that diol phase **15** can be used for both reversed-phase and normal phase applications. The test conditions were as follows:

Column dimensions:	4.6x150 mm with 5 μm particle size
Mobile phase:	52/48 (reversed-phase) or 92/8 (normal phase) v/v acetonitrile/100 mM ammonium acetate, pH 5.2
Temperature:	30 °C
Flow rate:	1 mL/min
Injection volume:	10 μL
Detection wavelength:	254 nm
Sample:	Cytosine (100 ppm) and naphthalene (100 ppm).

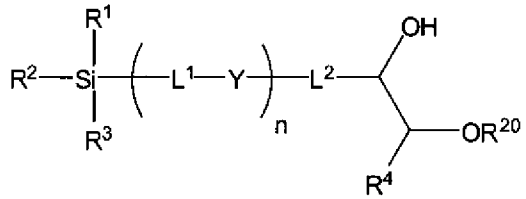
Example 10: Analysis of an alkylphenyl ethoxylated surfactant (IGPAL CA-630) using packing material 15

[0126] The surfactant IGEPAL CA-630 was analyzed using a column packed with diol phase **15** and mobile phases containing various amounts of acetonitrile. Results are summarized in Figure 6. In an aqueous-rich mobile phase, the new packing material behaves like a reversed-phase material, in which retention of an analyte increases when the aqueous content of the mobile phase is high. In organic-rich mobile phases, diol phase **15** acts like a normal phase, in which the retention increases when the organic content of the mobile phase is high. In addition, oligomer resolution is enhanced under normal phase conditions, and diminished under reversed-phase conditions. The test conditions were as follows:

Column dimensions:	4.6x150 mm with 5 μm particle size
Mobile phase:	40/60 to 99/1 v/v acetonitrile/100 mM ammonium acetate, pH 5.2
Temperature:	30 °C
Flow rate:	1 mL/min
Injection volume:	10 μL
Detection wavelength:	225 nm
Sample:	IGEPAL CA-630 (0.1%).

WHAT IS CLAIMED IS:

1 **1.** A composition comprising a compound covalently bound to a substrate, said compound
2 having a structure according to Formula (I):



3 **(I)**

4 wherein

5 n is a member selected from 0 and 1;

6 R^1, R^2 and R^3 are members independently selected from halogen, OR^{10}, $NR^{10}R^{11}$,
7 $OC(O)R^{12}$, $OS(O)_2R^{12}$, substituted or unsubstituted alkyl, substituted or
8 unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted
9 or unsubstituted heteroaryl, substituted or unsubstituted
10 heterocycloalkyl and a bond to said substrate,
11 wherein
12 each R^{10} and each R^{11} is a member independently selected from H,
13 substituted or unsubstituted alkyl, substituted or unsubstituted
14 heteroalkyl, substituted or unsubstituted aryl, substituted or
15 unsubstituted heteroaryl, substituted or unsubstituted
16 heterocycloalkyl and a bond to said substrate; and
17 each R^{12} is a member independently selected from substituted or
18 unsubstituted alkyl, substituted or unsubstituted heteroalkyl,
19 substituted or unsubstituted aryl, substituted or unsubstituted
20 heteroaryl, substituted or unsubstituted heterocycloalkyl,
21 with the proviso that at least one of R^1, R^2 and R^3 is covalently bound to said
22 substrate;

23 L^1 and L^2 are linker groups independently selected from substituted or unsubstituted
24 alkyl, substituted or unsubstituted heteroalkyl, substituted or
25 unsubstituted aryl, substituted or unsubstituted heteroaryl and
26 substituted or unsubstituted heterocycloalkyl,

with the proviso that if n is 1, then at least one of L^1 and L^2 comprises a carbon chain having at least 5 carbon atoms in sequence, and if n is 0, then L^2 comprises a carbon chain having at least 5 carbon atoms in sequence, wherein at least two of said carbon atoms in sequence are optionally part of a 5- or 6-membered ring, wherein said ring is a member selected from aryl, heteroaryl and cycloalkyl, and wherein said ring is optionally substituted with a non-polar substituent;

R^4 is a member selected from H, acyl, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, wherein R^4 and a substituent of L^2, together with the atoms to which they are attached, are optionally joined to form a 3- to 7-membered ring;

R^{20} is a member selected from H, substituted alkyl, substituted heteroalkyl and substituted heterocycloalkyl, provided that at least one substituent of R^{20} is OH; and

Y is a member selected from ether, thioether, amide, sulfonamide, carbonate, carbamate, urea and thiourea.

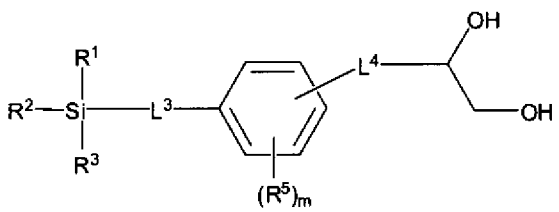
2. The composition of claim **1**, wherein at least one of R^1, R^2 and R^3 is substituted or unsubstituted C_1-C_6 alkyl.

3. The composition of claim **1**, wherein two of R^1, R^2 and R^3 are substituted or unsubstituted C_1-C_6 alkyl.

4. The composition of claim **3**, wherein two of R^1, R^2 and R^3 are methyl.

5. The composition of claim **1**, wherein R^4 is H.

6. The composition of claim **1**, wherein n is 0 and said compound has a structure according to Formula (II):



(II)

wherein

m is an integer selected from 0 to 4;

L^3 is a member selected from substituted or unsubstituted alkyl;

L^4 is a member selected from a single bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

each R^5 is a member independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl, wherein two adjacent R^5, together with the atoms to which they are attached, are optionally joined to form a 5- to 7-membered ring.

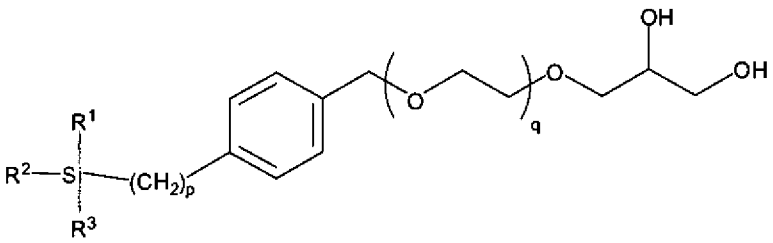
7. The composition of claim **6**, wherein

R^5 is a member selected from H and substituted or unsubstituted alkyl;

L^3 is unsubstituted alkyl; and

L^4 is straight or branched alkyl optionally interrupted by one or more ether or thioether group.

8. The composition of claim **7**, wherein said compound has a structure according to Formula (III):



(III)

wherein

p is an integer selected from 2 to 10; and

q is an integer selected from 0 to 10.

9. The composition of claim **1**, wherein n is 0, R^4 is H and L^2 is substituted or unsubstituted C_5-C_{20} alkyl.

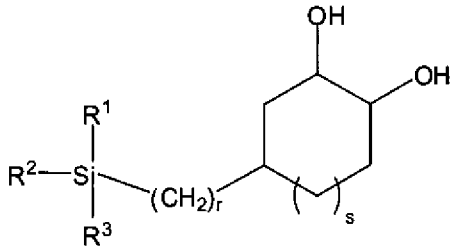
10. The composition of claim **9**, wherein L^2 is unsubstituted C_6-C_{20} alkyl.

11. The composition of claim **10**, wherein L^2 is unsubstituted C_8 alkyl.

12. The composition of claim **1**, wherein n is 1, Y is an amide group, R^4 is H and L^2 is substituted or unsubstituted C_5-C_{20} alkyl.

13. The composition of claim **12**, wherein L^2 is unsubstituted C_6-C_{20} alkyl.

14. The composition of claim **1**, wherein said compound has a structure according to Formula (IV):



(IV)

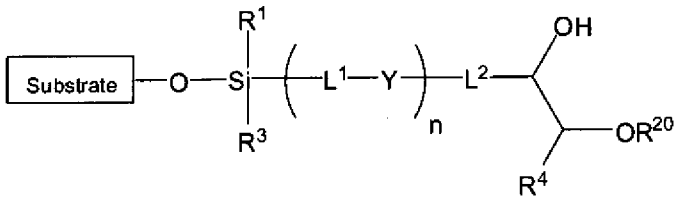
wherein r is an integer selected from 2 to 6 and s is an integer selected from 0 and 1, with the proviso that when r is 2, then s is 1.

15. The composition of claim **1** contained in a chromatography column.

16. The composition of claim **15**, wherein said chromatography column is a member selected from a metal column and a plastic column.

17. The composition of claim **1**, wherein R^{20} is H.

18. The composition of claim **1**, having a structure according to Formula (V):



(V).

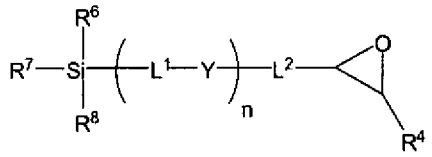
19. The composition of claim **18**, wherein at least one of R^1 and R^3 is substituted or unsubstituted C_1-C_6 alkyl.

20. The composition of claim **19**, wherein at least one of R^1 and R^3 is methyl.

21. The composition of any one of claims **1** to **18**, wherein said substrate is a silica-based substrate.

22. The composition of any one of claims **1** to **18**, wherein said substrate is a member selected from silica gel, sol-gel, a polymer, a polymer/sol-gel hybrid, glass and a silica monolithic material.

23. A compound having a structure according to Formula (VIa):



(VIa)

wherein

n is a member selected from 0 and 1;

R^6, R^7 and R^8 are members independently selected from halogen, OR^{14}, $NR^{14}R^{15}$, $OC(O)R^{16}$, $OS(O)_2R^{16}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

wherein

each R^{14} and each R^{15} is a member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl; and

each R^{16} is a member independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

with the proviso that at least one of R^6, R^7 and R^8 is other than OH, unsubstituted alkyl, unsubstituted aryl, unsubstituted heteroaryl and unsubstituted heterocycloalkyl;

L^1 and L^2 are linker groups independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

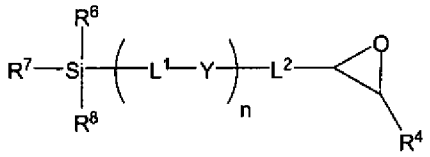
with the proviso that if n is 1, then at least one of L^1 and L^2 comprises a carbon chain having at least 5 carbon atoms in sequence, and if n is 0, then L^2 comprises a carbon chain having at least 5 carbon atoms in sequence, wherein at least two of said carbon atoms in sequence are optionally part of a 5- or 6-membered ring, wherein said ring is a member selected from aryl, heteroaryl and cycloalkyl; and wherein said ring is optionally substituted with a non-polar substituent;

R^4 is a member selected from H, acyl, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, wherein R^4 and a substituent of L^2, together with the atoms to which they are attached, are optionally joined to form a 3- to 7-membered ring; and

Y is a member selected from ether, thioether, amide, sulfonamide, carbonate, carbamate, urea and thiourea.

24. A method of making a compound according to claim **1**, said method comprising:

(i) contacting a substrate having a reactive functional group with a compound having an epoxide moiety and having a structure according to Formula (VIa):



(VIa)

wherein

n is a member selected from 0 and 1;

R^6, R^7 and R^8 are members independently selected from halogen, OR^{14}, $NR^{14}R^{15}$, $OC(O)R^{16}$, $OS(O)_2R^{16}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

wherein

each R^{14} and each R^{15} is a member independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or

unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl; and

each R^{16} is a member independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

with the proviso that at least one of R^6, R^7 and R^8 is other than OH, unsubstituted alkyl, unsubstituted aryl, unsubstituted heteroaryl and unsubstituted heterocycloalkyl;

L^1 and L^2 are linker groups independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl,

with the proviso that if n is 1, then at least one of L^1 and L^2 comprises a carbon chain having at least 5 carbon atoms in sequence, and if n is 0, then L^2 comprises a carbon chain having at least 5 carbon atoms in sequence,

wherein at least two of said carbon atoms in sequence are optionally part of a 5- or 6-membered ring, wherein said ring is a member selected from aryl, heteroaryl and cycloalkyl; and wherein said ring is optionally substituted with a non-polar substituent;

R^4 is a member selected from H, acyl, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, wherein R^4 and a substituent of L^2, together with the atoms to which they are attached, are optionally joined to form a 3- to 7-membered ring; and

Y is a member selected from ether, thioether, amide, sulfonamide, carbonate, carbamate, urea and thiourea,

under conditions sufficient to form a covalent bond between said compound and said substrate through reaction between said reactive functional group and at least one of R^6, R^7 and R^8.

25. The method of claim **24** further comprising:

(ii) converting said epoxide moiety into an 1,2-diol moiety.

26. The method of claim **25**, wherein said converting of step (ii) is accomplished by acid-catalyzed hydrolysis.

27. The method of claim **25**, wherein R^4 is H and said converting of step (ii) is accomplished by contacting the product of step (i) and a reagent comprising a nucleophilic oxygen atom.

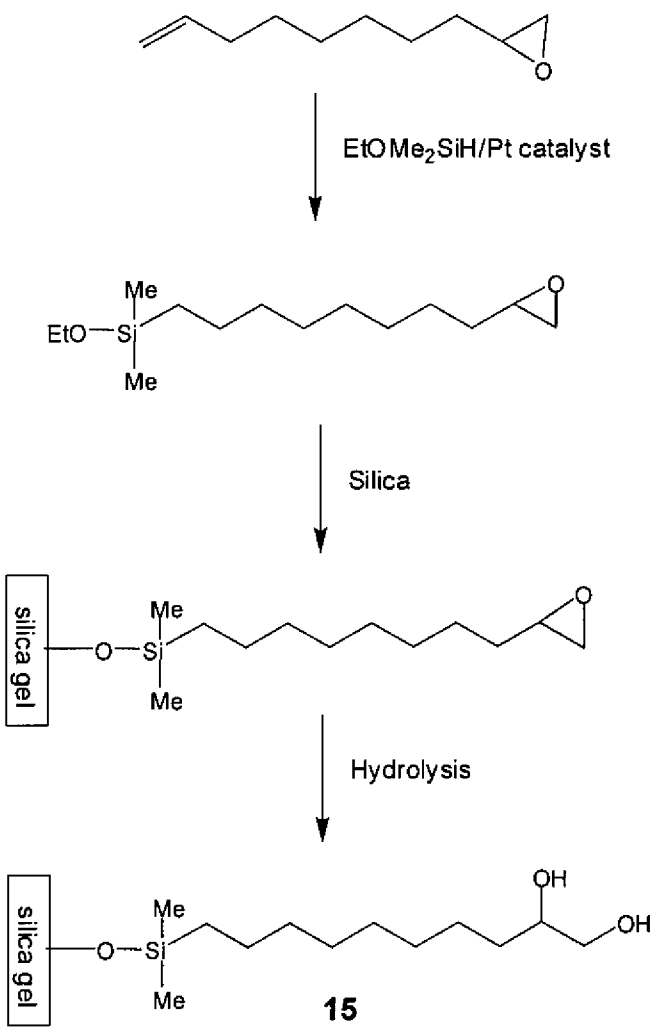
28. The method of claim **24**, wherein said substrate is a silica-based substrate.

29. The method of claim **28**, wherein said substrate is a member selected from silica gel and a silica monolithic material.

30. The method of claim **24**, wherein said reactive functional group of said substrate is a member selected from silanol, alkoxysilane, halosilane and aminosilane.

31. The method of claim **24**, wherein at least one of R^1, R^2 and R^3 is alkoxy.

Figure 1


EtO Me2SiH/Pt catalyst
Me
EtO
Si
Me
O
Silica
silica gel
Me
O
Si
Me
O
Hydrolysis
silica gel
Me
O
Si
Me
OH
OH

15

Figure 2

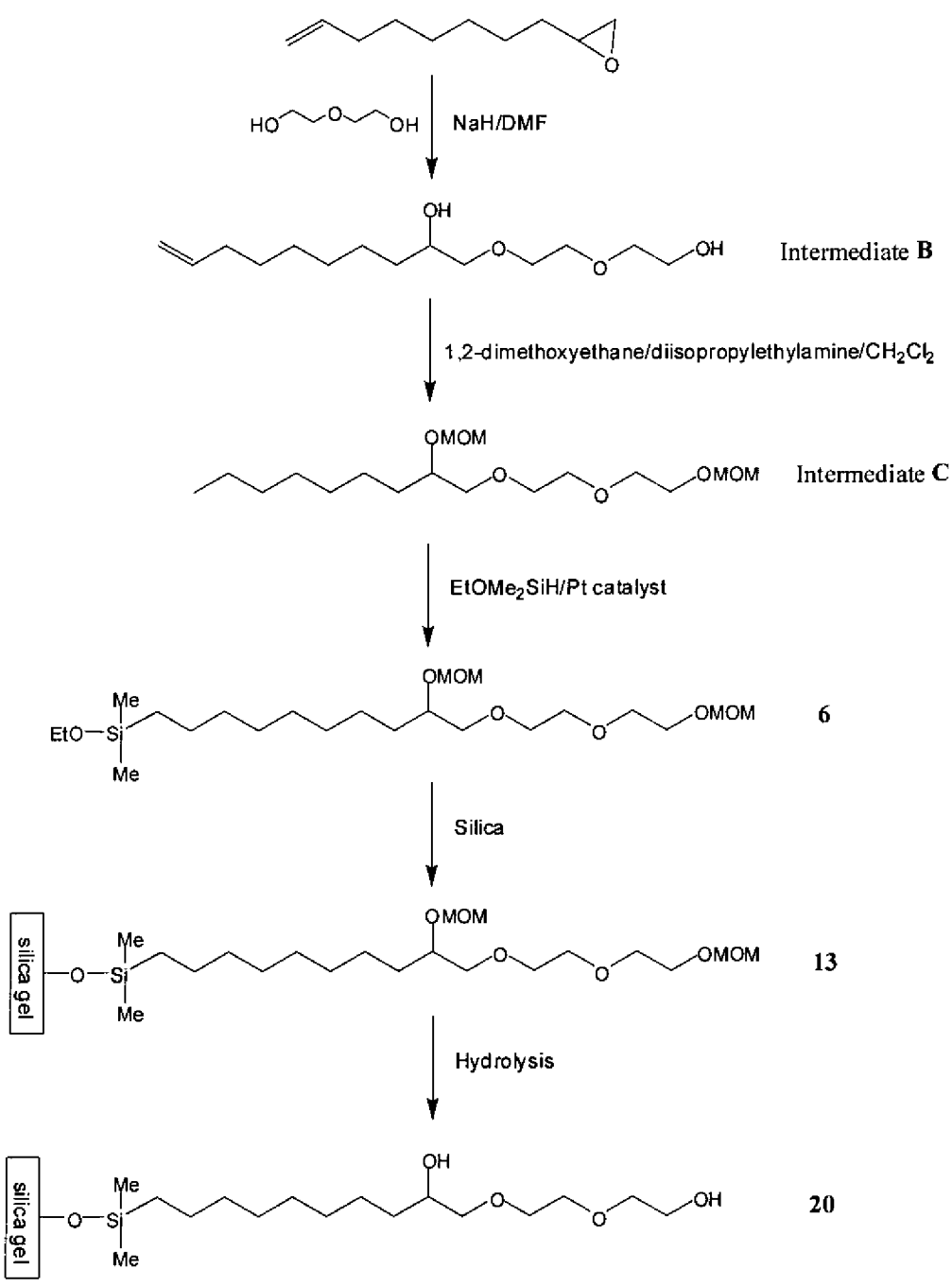

NaH/DMF
Intermediate B
1,2-dimethoxyethane/diisopropylethylamine/CH_2Cl_2
Intermediate C
$EtOMe_2SiH$/Pt catalyst
6
Silica
13
Hydrolysis
20
silica gel

Figure 3

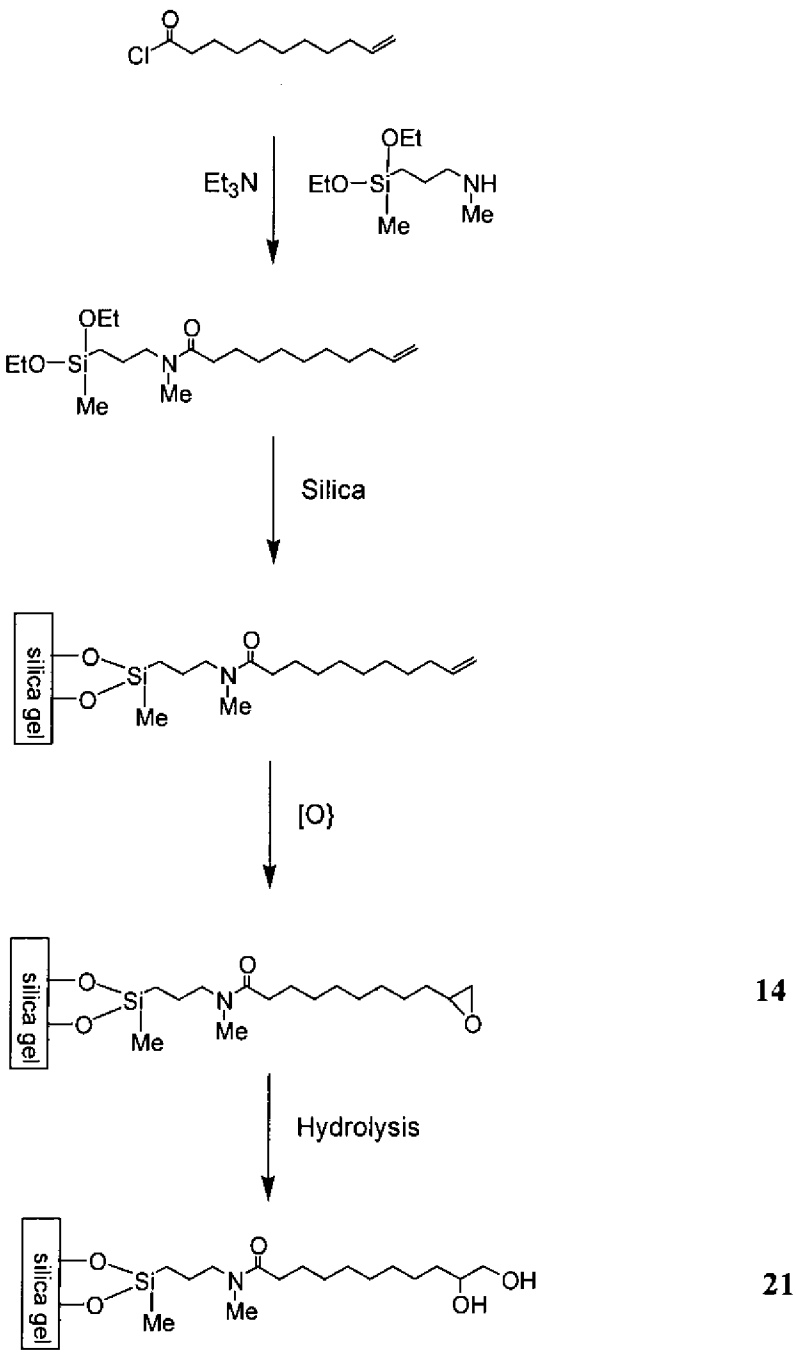
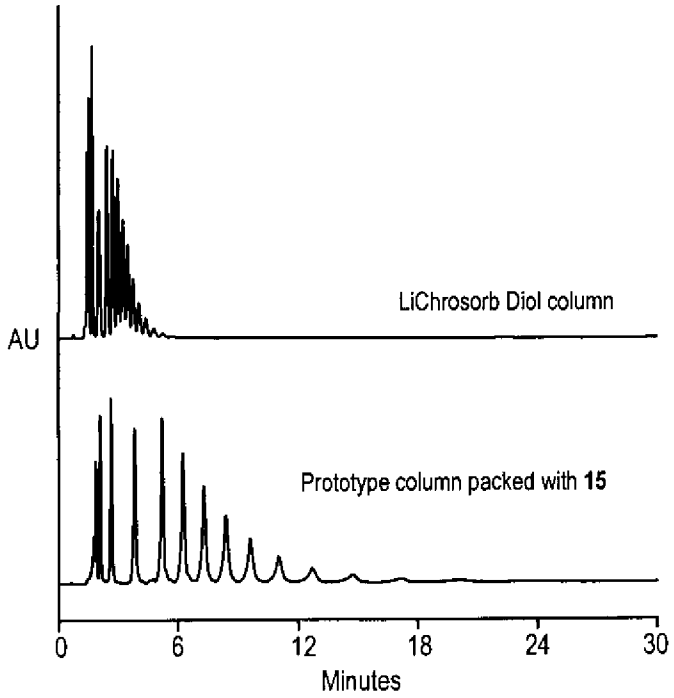
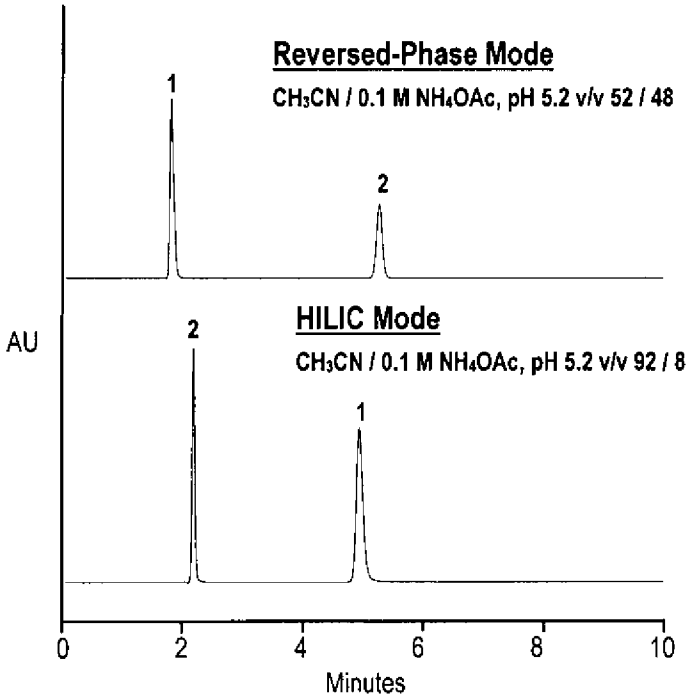

Cl
O
Et3N
OEt
EtO–Si
Me
NH
Me
EtO–Si
OEt
Me
N
Me
O
Silica
silica gel
O
Si
Me
N
Me
O
[O}
O
14
Hydrolysis
OH
OH
21

Figure 4



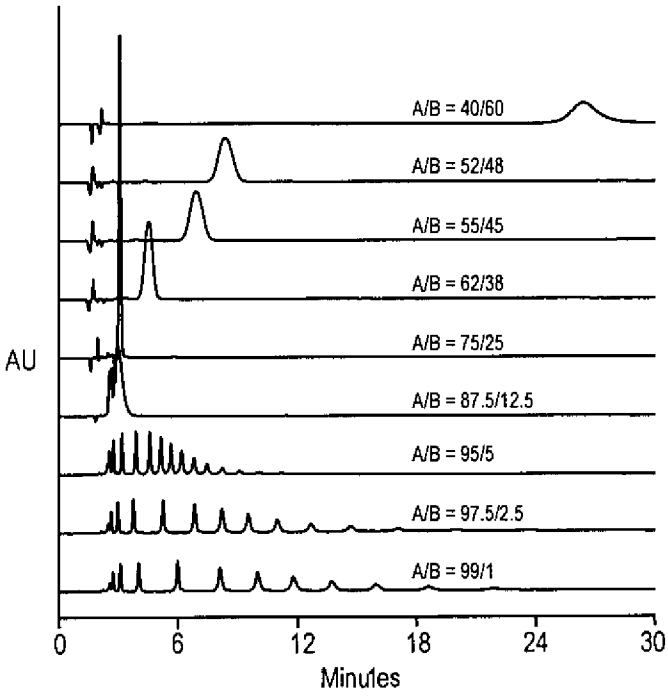
Column: 5 µm
Dimensions: 4.6 x 150 mm
Mobile Phase: 99/1 v/v CH_3CN/0.1 M NH_4OAc, pH5.2
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: UV @ 225 nm
Sample: IGEPAL CA-630 (0.1%)

Figure 5



Column:	Diol Phaes **15**, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	CH_3CN/0.1 NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	UV @ 254 nm
Peaks:	(100 ppm each) 1. Cytosine 2. Naphthalene

Figure 6



Column:	Diol Phase **15**, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	A – CH_3CN
	B – 0.1 M NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	UV @ 225 nm
Sample:	GEPAL CA-630 (0.1%)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/063772

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01J20/288 B01J20/289 B01J20/32

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01J G01N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.J. PESEK: JOURNAL OF CHROMATOGRAPHY A, vol. 687, 1994, pages 33-44, XP002494504 Synthesis and spectrometric characterization of a true diol bonded phase page 42; figure 8 -----	1,5-10, 12-18, 21,22
X	GB 2 074 892 A (VARIAN ASSOCIATES) 11 November 1981 (1981-11-11) page 4, line 12 - line 19 page 4, line 19 - line 27 claims 11-13 page 3, line 75 - line 82 page 5, line 39 - line 46 ----- -/--	1,5-9, 12-18, 21-31

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2008	06/10/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hilgenga, Klaas

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/063772

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/178730 A1 (LI GUANG Q [US]) 18 August 2005 (2005-08-18) page 12, paragraph 162 - paragraph 163 page 8, paragraph 114 page 9, paragraph 118 - paragraph 119 page 5, lines 1-3 page 6, paragraph 83	1-4,6-23
A	D.S. SEIBERT: "Retention properties of a spacer-bonded propanediol sorbent for reversed-phase liquid chromatography and solid-phase extraction" ANALYST, vol. 121, April 1996 (1996-04), pages 511-520, XP008096034 the whole document	
A	US 2006/054559 A1 (LIU XIADONG [US] ET AL) 16 March 2006 (2006-03-16) page 5, paragraph 56 page 6, paragraph 60 - paragraph 73	
A	US 6 375 846 B1 (JARRETT HARRY WELLINGTON [US] ET AL) 23 April 2002 (2002-04-23) column 4, line 15 - line 20; figure 1	
A	NORIYUKI NIMURA: "Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs" JOURNAL OF CHROMATOGRAPHY A, vol. 689, 1995, pages 203-210, XP002494435 page 204, paragraph 2	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/063772

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2074892 A	11-11-1981	CA 1144138 A1	05-04-1983
		DE 3117732 A1	04-03-1982
		JP 57003043 A	08-01-1982
		US 4298500 A	03-11-1981
US 2005178730 A1	18-08-2005	AU 2005215377 A1	01-09-2005
		CA 2554650 A1	01-09-2005
		CN 1917953 A	21-02-2007
		EP 1711258 A1	18-10-2006
		JP 2007522476 T	09-08-2007
		KR 20060129382 A	15-12-2006
		US 2006207923 A1	21-09-2006
		WO 2005079975 A1	01-09-2005
US 2006054559 A1	16-03-2006	AU 2005285037 A1	23-03-2006
		CA 2578266 A1	23-03-2006
		EP 1791849 A1	06-06-2007
		JP 2008512493 T	24-04-2008
		US 2008203027 A1	28-08-2008
		WO 2006031717 A1	23-03-2006
US 6375846 B1	23-04-2002	NONE	