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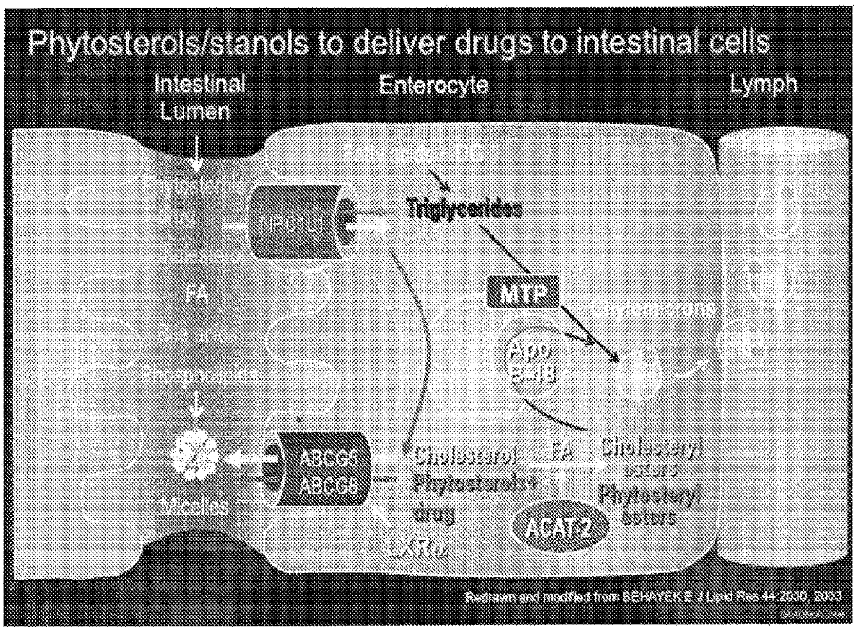
(54) Title: PLANT STEROIDS AND USES THEREOF

FIGURE 1



(57) **Abstract**: The invention relates to a drug conjugate including a drug and a plant steroid. The drug conjugate may target the drug for intestinal cell delivery, and thus may be used to treat diseases, including intestinal diseases, or to affect intestinal metabolism. The invention therefore also relates to treating intestinal diseases and affecting intestinal metabolism with the drug conjugate.

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PLANT STEROIDS AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to plant steroids, including plant sterols, and more particularly, using plant steroids as drug delivery vehicles.

BACKGROUND OF THE INVENTION

[0002] Negative side effects can be associated with systemic exposure to oral drugs that are absorbed into the blood stream through the intestine, and circulate throughout the body. For some drugs, in particular those that are indicated for gastrointestinal symptoms or for those that exert their effects through the gastrointestinal system, it is desirable to keep the drugs within gastrointestinal tissues, such as intestinal cells, rather than circulating through the body. Accordingly, there is a need in the art for methods of specifically targeting drugs to intestinal cells.

SUMMARY OF THE INVENTION

[0003] Provided herein is a compound comprising a plant steroid and a drug. The plant steroid may be attached to the drug, and the attachment may be a conjugated bond or an amine bond. The drug may be an anti-inflammatory drug, a glucocorticoid, a LXR agonist, a TNFα inhibitor, a NF-κB inhibitor or agonist, a selective COX-2 inhibitor, a non-selective non-steroidal anti-inflammatory drug (NSAID), methotrexate, leflunomide, mesalamine, balsalaside, osalazine, sulfasalazine, an aminosalicylate, cyclosporine, mercaptopurine, azathioprine, atropine, a microsomal triglyceride transfer protein (MTP) inhibitor, an acyl CoA:cholesterol acyltransferase-2 (ACAT 2) inhibitor, a farnesoid X receptor (FXR) agonist, a diacylglycerol actyltransferase (DGAT) inhibitor, a glucagon-like peptide-1 (GLP-1) agonist, an ileal bile acid transport (IBAT) inhibitor, an antibiotic, or an antiviral. The drug may also be prednisone.

[0004] The plant steroid may be phytosterol or phytostanol. The sterol may be stigmasterol, brassicasterol, campestenol, or campesterol. Cholesterol may also be attached to the drug. Also provided herein is a method for lowering cholesterol levels, which may comprise administering an oxyphytostanol to a mammal in need thereof.

[0005] Also provided herein is a drug conjugate of formula (I), D-L-P (I),

in which D is a drug constituent, L is a linkage, and P is a plant steroid constituent. Cholesterol may be substituted for the plant steroid constituent. D may be a drug as described above. For example, D may be a glucocorticoid or a LXR agonist constituent. The glucocorticoid may be budesonide, and the LXR agonist may be GW-3965 or TO-901317. P may be a phytosterol or phytostanol constituent. P may be stigmasterol, campesterol, 24(S),25-epoxycholesterol, or 5-6-epoxycampesterol. L may be a bond, and may comprise at least one chemical functional group, such as ether, amide, sulfonamide, or ester. L may comprise at least 3 atoms, and may comprise at least 10 atoms.

[0006] The drug conjugate may be a campesterol/budesonide drug conjugate, a 24(S),25-epoxycholesterol/budesonide drug conjugate, a campesterol/GW-3965 drug conjugate, a campesterol/TO-901317 drug conjugate, a 24(S),25-epoxycholesterol/GW-3965 drug conjugate, or a 24(S),25-epoxycholesterol/TO-901317 drug conjugate. The drug conjugate may be prednisolone stigmasteroltrisethyleneglycolalcohol succinate, prednisolone stigmasterol succinate, or prednisolone stigmasteroltrisethyleneglycol acetate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1 shows how phytosterols and phytostanols are used to deliver drugs to intestinal cells.

[0008] Figure 2 shows an example of a Volcano plot of gene expression changes associated with treatment of Caco-2 cells with a sterol-conjugated prednisone.

[0009] Figures 3A-F show gene expression changes in Caco-2 cells treated with various sterol-conjugated prednisone compounds.

[0010] Figure 4 shows the gene response of key gene families to 2.5μM of Prednisone, SE-22-II, SE-24-II and SE-41-II.

[0011] Figure 5 shows nuclear hormone receptors, which are affected by prednisolone, SE-22, SE-24, and SE-41.

[0012] Figure 6 shows gene responses that affect nuclear hormone receptors in Caco-2 cells.

[0013] Figure 7 shows a list of classical and orphan hormone receptors and their ligands.

[0014] Figure 8 shows a schematic of a nuclear hormone receptor. A typical nuclear receptor is composed of several functional domains. The variable NH2-terminal region (A/B) contains the ligand-independent AF-1 transactivation domain. The conserved DNA-binding domain (DBD), or region C, is responsible for the recognition of specific DNA sequences. A variable linker region D connects the DBD to the conserved E/F region that contains the ligand-binding domain (LBD) as well as the dimerization surface. The ligand-independent transcriptional activation domain is contained within the A/B region, and the ligand-dependent AF-2 core transactivation domain within the COOH-terminal portion of the LBD.

[0015] Figures 9A and B show a reference list of interleukin-related genes affected by prednisolone, SE-22, SE-24 and SE-41.

[0016] Figure 10 shows a visual plot of affected interleukins and interleukin receptors.

[0017] Figures 11A-L show the spectral data confirming the structures of SE-24-II (Figs. 11A-C and J-L), SE-22-II (Figs. 11D-F and M-O), and SE-41-II (Figs. 11G-I and P-R).

DETAILED DESCRIPTION

[0018] The inventors have made the surprising discovery that plant steroid drug conjugates can be used to target intestinal cells for treatment of diseases of the intestine or to affect intestinal metabolism, without the adverse effects of systemic drug circulation. Specifically, attaching drugs to plant steroids by bonds that are temporarily or permanently (somewhat or entirely) resistant to digestion can result in targeted delivery of therapies to the intestinal cells without significant systemic absorption into the bloodstream.

1. Definitions

[0019] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control.

[0020] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. The terms “a,” “an” and “the” include singular and plural referents unless the context clearly dictates otherwise. Thus, for example, references to a composition for delivering “a drug” include reference to one, two or more drugs. The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts, compounds or structures. The present invention also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0021] For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

[0022] The terms "drug," "drug moiety," "drug constituent," "therapeutic," "therapeutic agent," and variants thereof, as used herein, refer to any drug or other agent that is intended for delivery to a targeted cell or tissue.

[0023] The term "linkage," "linker," and variants thereof, as used herein, refers to any moiety that connects the plant steroid constituent and the drug constituent. The linkage can be a covalent bond or a chemical functional group that directly connects the drug and the plant steroid. The linkage can contain a series of covalently bonded atoms and their substituents which are collectively referred to as a linkage. In certain embodiments, linkages can be characterized by a first covalent bond or a chemical functional group that connects the drug to a first end of the linker and a second covalent bond or chemical functional group that connects the second end of the linker to the plant steroid.

[0024] "Treatment" or "treating," when referring to protection of an animal from a disease, means preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering a composition of the present invention to an animal prior to onset of the disease. Suppressing the disease involves administering a composition of the present invention to an animal after induction of the disease but before its clinical appearance. Repressing the disease involves administering a composition of the present invention to an animal after clinical appearance of the disease.

2. Compounds of the Invention

[0025] Provided herein are compounds for targeted drug delivery. In particular, provided herein are agents or drug conjugates comprising a plant steroid and a drug. Drug conjugates of the present invention comprise compounds of Formula (I)

$$\text{D-L-P} \qquad \text{(I)}$$

wherein

D is a drug constituent;

L is a linkage; and

P is a plant steroid constituent.

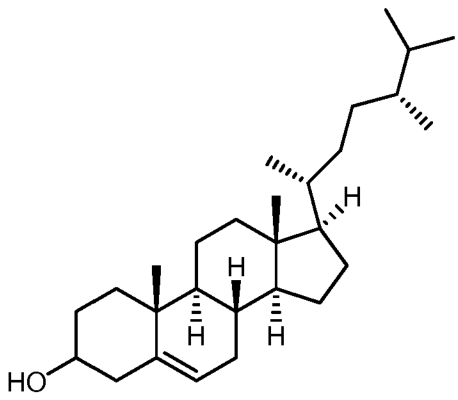
[0026] In certain embodiments, the plant steroid (P) can be a phytosterol or a phytostanol. Phytosterols, and their saturated forms termed phytostanols, are a group of steroid alcohols that occur naturally in plants. These compounds cannot be synthesized by humans and therefore always originate in the diet. Phytosterols are poorly absorbed in the intestines (0.4-3.5 %) while phytostanol absorption is even lower (0.02-0.3%). These plant based compounds are abundant in the diet, and enter the intestinal cells by the sterol transporter NPC 1L1. Once in the intestinal cells, plant sterols and stanols regulate nuclear receptors such as LXR and have other potential metabolic effects. Unlike intestinal cholesterol, however, these plant sterols and stanols are poor substrates for ACAT (an enzyme that is required to convert the sterol into an oleate for absorption into the lymph), and are actively transported out of the intestinal cells and back into the lumen by ABCG5 and ABCG8. For this reason, phytosterols and stanols typically are poorly absorbed into the bloodstream. Accordingly, phytosterols and stanols are suitable for use in targeted delivery of therapeutics to intestinal cells without systematic introduction into circulation.

[0027] In some embodiments, the resulting steroid-linker-drug conjugates are gradually or partially metabolized to one or more of its constituents (either steroid, linker, drug, steroid-linker, or linker-drug constituents or metabolic derivatives thereof). In certain embodiements, conjugates of this kind are useful as a delivery vehicle of the active moiety in the conjugate, beyond the early GI digestive stage. Once delivered down-stream, such conjugates may be metabolized to release the active moiety at the target site. Such conjugates may have pro-drug features. Although cholesterol may be used as a constituent in such partially stable conjugates, due to the risk of elevating cholesterol in the subject, phytosterols may be preferred as a constituent in such conjugates. In other embodiments, the resulting sterol-linker-drug conjugates are quite stable and metabolized to a limited extend in the body. For such relatively stable sterol-linker-drug conjugates there is limited preference of phytosterols over cholesterol.

[0028] Phytosterols suitable for use as the plant steroid constituent include, but are not limited to, β-sitosterol, campesterol, stigmasterol (stigmasta-5,22-dien-3β-ol), and brassicasterol (ergosta-5,22-dien-3β-ol). Phytostanols appropriate for use as the plant steroid constituent include, but are not limited to, sitostanol, campestanol, brassicastanol, and stigmastanol. In certain embodiments, the phytostanol may exist as an esterified form to provide a stanol ester. Alternatively, the plant steroid constituent may be a plant sterol that is oxidized, and thus may be an oxyphytosterol or an oxyphytostanol.

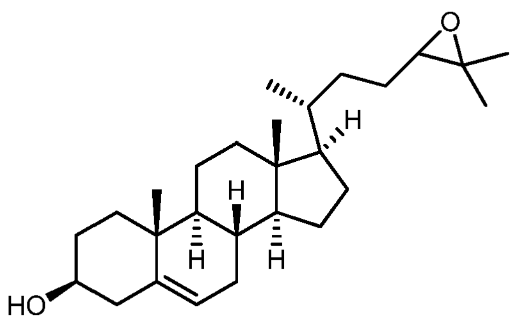
[0029] In another embodiment, the plant steroid (P) may be replaced with cholesterol. Cholesterol may be attached to the drug (D) in the same manner via the same linker (L) as described for plant sterols herein. Cholesterol may also be functionalized as described for plant sterols herein.

[0030] In a preferred embodiment, the plant steroid constituent of the drug conjugate is a compound of Formula (1a), also referred to as campesterol.



(1a)

[0031] In certain embodiments, amino, sulfur, and other derivatives of sterols may be used, including but not limited to, oxidized sterols such as 24(S),25-epoxycholesterol (Formula 1b) or 5-6-epoxycampesterol.

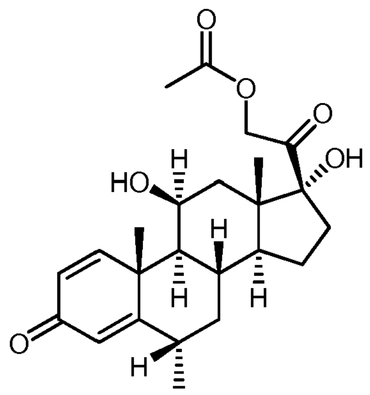


(1b)

[0032] In some embodiments of the present invention, the steroid may link directly to the drug. For instance, the –OH group on the steroid may react with a carboxy group on the drug to form an ester linkage. In other embodiments, a linker molecule may be used to provide a linkage between the steroid and drug constituents, tailored to optimize its drug delivery or metabolic stability characteristics as well as to optimize the synthesis of the desired type of molecule. In other embodiments the –OH group may be substituted with an amine, sulfur or other functionality.

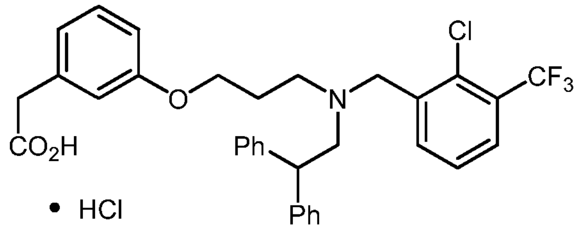
[0033] Drugs suitable for use as the drug constituent (D) of the presently disclosed drug conjugates include any drug or therapeutic agent that is intended for delivery to a targeted cell or tissue. In certain embodiments, the therapeutic agent may be a drug for treating a disease of the intestine or that affects intestinal metabolism. For example, the drug may be used to treat inflammatory bowel disease or may be a chemotherapeutic agent for a cancer such as intestinal or colon cancer. In certain embodiments, the therapeutic agent is an anti-inflammatory, such as for example, a glucocorticoid, a LXR agonist, a TNFα inhibitor, an NF-κB inhibitor or agonist, a selective COX-2 inhibitor, or a non-selective non-steroidal anti-inflammatory drug (NSAID). The therapeutic agent may also be methotrexate, leflunomide, mesalamine, balsalaside, osalazine, sulfasalazine, an aminosalicylate, cyclosporine, mercaptopurine, azathioprine, atropine, a microsomal triglyceride transfer protein (MTP) inhibitor, an acyl CoA:cholesterol acyltransferase-2 (ACAT-2) inhibitor, a farnesoid X receptor (FXR) agonist, a diacylglycerol actyltransferase (DGAT) inhibitor, a glucagon-like peptide-1 (GLP-1) agonist, an ileal bile acid transport (IBAT) inhibitor, an antibiotic, or an antiviral.

[0034] Glucocorticoids include, without limitation, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone (Formula (1c)), prednisone, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, halcinonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate, and fluprednidene acetate.

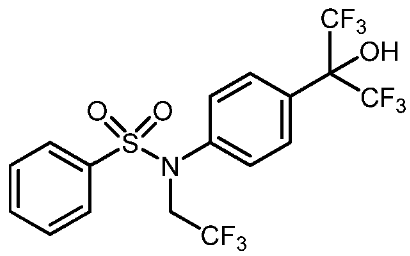


(1c)

[0035] Liver X receptor agonists (LXR agonists) include, without limitation, GW-3965 (GlaxoSmithKline) depicted as Formula (1d), TO-901317 (Tularik) depicted as Formula (1e), MBX-102 (Metabolex), NO-1886 (Otsuka), and Gemcabene (Pfizer).



(1d)



(1e)

[0036] Selective cyclooxygenase-2 inhibitors (COX-2 inhibitors) include, without limitation, rofecoxib, brand name VIOXX™ (Merck & Co., Inc. Whitehouse Station, N.J., USA); celecoxib, brand name CELEBREX™ (Pfizer); valdecoxib, brand name BEXTRA™ (Pharmacia Corp., Peapack, N.J., USA); paracoxib, brand name DYNASTAT™ (Pharmacia Corp.); etoricoxib, brand name ARCOXIA™ (Merck & Co., Inc.); and NS-398 ((N-(2-cyclohexyloxy-4-nitrophenyl) methane sulphonamide).

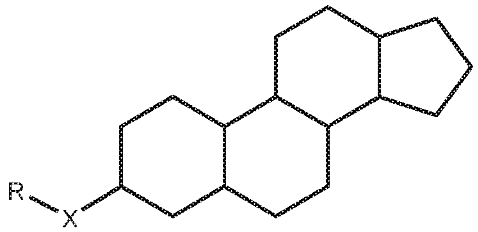
[0037] NSAIDs contemplated for modification in accordance with the present invention include acetaminophen (Tylenol, Datril, etc.), aspirin, ibuprofen (Motrin, Advil, Rufen, others), choline magnesium salicylate (Triasate), choline salicylate (Anthropan), diclofenac (voltaren, cataflam), diflunisal (dolobid), etodolac (lodine), fenoprofen calcium (nalfon), flurbiprofen (ansaid), indomethacin (indocin, indometh, others), ketoprofen (orudis, oruvail), carprofen, indoprofen, ketorolac tromethamine (toradol), magnesium salicylate (Doan's, magan, mobidin, others), meclofenamate sodium (meclomen), mefenamic acid (relafan), oxaprozin (daypro), piroxicam (feldene), sodium salicylate, sulindac (clinoril), tolnetin (tolectin), meloxicam, nabumetone, naproxen, lomoxicam, nimesulide, indoprofen, remifenzone, salsalate, tiaprofenic acid, flosulide, and the like. Presently preferred NSAIDs employed in the practice of the invention include naproxen, aspirin, ibuprofen, flurbiprofen, indomethacin, ketoprofen, carprofen, and the like.

[0038] MTP inhibitors include, without limitation, implitapide, BMS-201038, R-103757, JTT-130, and the like. ACAT-2 inhibitors include, without limitation, fatty acid anilide derivatives, urea-derived compounds, CI976, eflucimibe (F-1251), CP113,818, glibenclamide, avasimibe (CI-1011), pactimibe, rimonabant, AM251, SR144528, pyripyropene A, CP113, PD-138142-15, PD-156759, XD-793-11, AEGR-733, DuP128, and the like. FXR agonists include, without limitation, GW4064, INT-747, MFA-1, fexaramine, WAY-362450, T0901307, 6-ethylchenodeoxycholic acid, AGN29, AGN31, guggulsterone, and the like. DGAT inhibitors include, without limitation, T863, LCQ-908, PF-04620110, A-922500, H128, JTT-553, PF-4415060, xanthohumol, and the like. GLP-1 agonists include, without limitation, exenatide, liraglutide, taspaglutide, AVE-0010, albiglutide, R1583, and the like. IBAT inhibitors include, without limitation, A3309, SC-435, S-8921, 2164U90, BRL 39924A, and the like.

[0039] Preferably, the plant steroid constituent, such as a phytosterol or phytostanol, is linked to the therapeutic constituent through a bond or bonds resistant to digestion. In certain embodiments, the linkage (L) may be a bond, thereby directly linking the drug constituent (D) to the plant steroid constituent (P). Alternatively, the linkage between the plant steroid and the therapeutic includes one or more atoms. Preferably, the linkage includes ten or more atoms, particularly where relative proximity of the drug constituent to the sterol/stanol constituent may interfere with the intestinal cell's ability to recognize/attach to the sterol/stanol constituent for removal from the cell; and/or where relative proximity of the sterol/stanol constituent to the therapeutic may interfere with the therapeutic activity of the drug.

[0040] The linkage (L) between the plant steroid and the therapeutic may include one or more ether, amide, sulfonamide, and/or ester bonds. Ether bonds have a high degree of stability in biological systems, and thus may be preferred in certain embodiments. Amide and sulfonamide bonds resist typical digestion by esterases and proteases, but are often not resistant to liver metabolism. Accordingly, in certain embodiments, amide or sulfonamide linkages provide an extra safety measure compared to ether bonds, as a minor degree of absorption of the drug conjugate can be neutralized by liver metabolism, and thus avoid accumulation of in circulation. In certain embodiments, the linkage may include one or more ester bonds, which can be relatively stable to digestion. The linkage may comprise trisethylene glycol, succinic acid, multiple units thereof, or a combination of the foregoing.

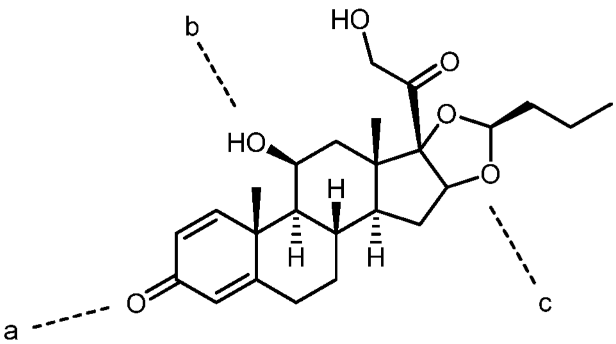
[0041] The plant steroid may be conjugated to the drug using a functional linkage as follows.



where X is O, S, N, NH, SO, SO_2, SONH, or the like; and R is H, or substituted alkyl or aralkyl with at least one functionality suitable for drug conjugation. The steroid nucleus structure above may be replaced by any plant steroid structure described herein.

[0042] In certain embodiments, the phytosterol or phytostanol can be linked to a drug constituent through a typical phytosterol or phytostanol hydroxyl group. Attachment to the plant steroid hydroxyl group can be accomplished without sacrificing the lack-of-absorbance feature of the plant steroids. For example, fatty acid esters of phytosterols and phytostanols have previously been used as agents to reduce LDL-cholesterol levels. Despite the chemical modification of the phytosterol or phytostanol hydroxyl group into a fatty acid ester, the lack-of-absorbance feature of the plant steroids is retained. Accordingly, a typical phytosterol or phytosterol hydroxyl group may be targeted for chemical modification in the attachment of the plant steroid to a drug constituent.

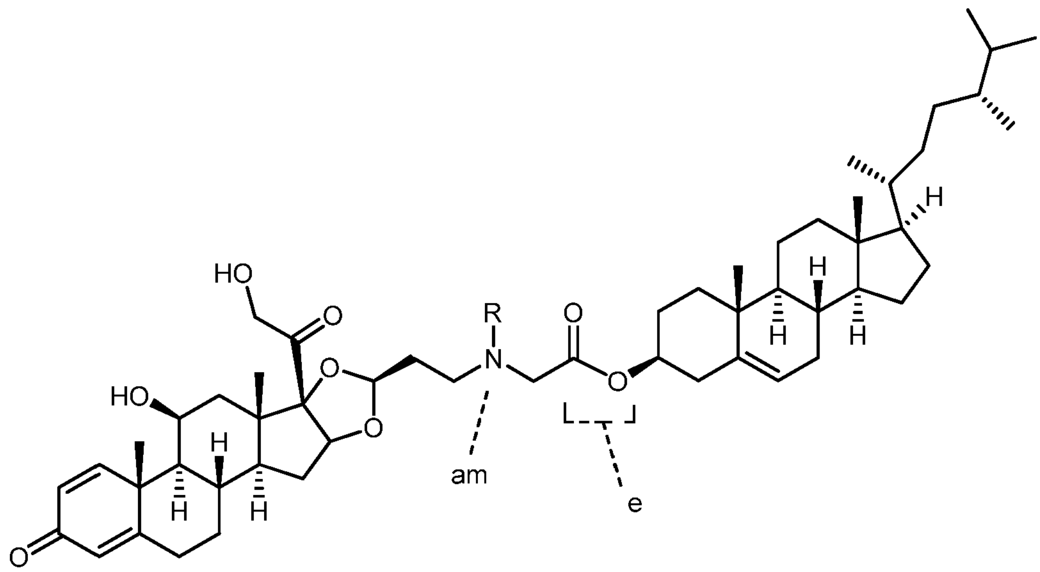
[0043] In one embodiment, a plant steroid constituent may be attached to a glucocorticoid. The plant steroid may be linked to the glucocorticoid, for example, via a ketone or hydroxyl functional group of the glucocorticoid. However, when comparing a broad range of natural and synthetic glucocorticoids, the distal (left side) ketone (=O) and the central hydroxyl (–OH) groups are highly conserved across the range. For example, the glucocorticoid of formula (1f), corresponding to budesonide, comprises the distal (left side) ketone (designated “a”) and a central hydroxyl group (designated “b”). While these groups provide excellent functionality for linking a specific glucocorticoid to another constituent, such a link may reduce or potentially eliminate anti-inflammatory activity. Therefore, in certain embodiments, the distal ketone and central hydroxyl groups are conserved and other functionalities in the glucocorticoid are targeted for attachment to the plant steroid.



(1f)

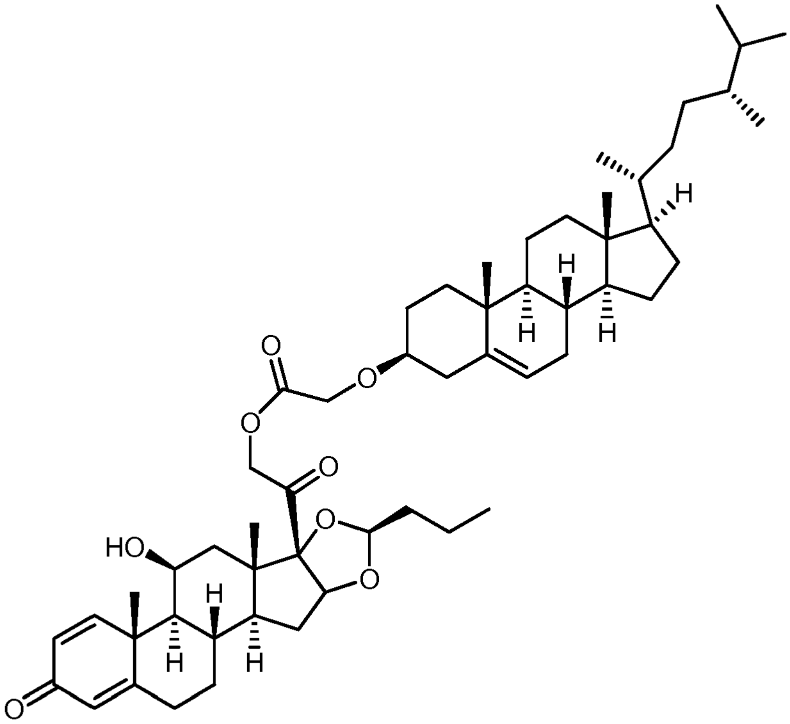
[0044] A subgroup of synthetic glucocorticoids include a 1,3-dioxoalane ring fused to the typical glucocorticoid cyclopentane ring on the opposed distal side (right side) of the glucocorticoid molecule. The compound of formula (1f), budesonide, comprises a distal (right side) 1,3-dioxolane ring (designated “c”). This 1,3-dioxolane ring demonstrates substantial variation in attached substituent groups across the subgroup of synthetic glucocorticoids. Accordingly, in certain embodiments, these variable sites on the acetonide ring can be used for linking a glucocorticoid drug constituent to a plant steroid constituent.

[0045] For example, one exemplary drug conjugate of the present invention, depicted herein as formula (1g), comprises campesterol linked to the budesonide via the budesonide 1,3-dioxolane ring, wherein R is hydrogen, substituted or unsubstituted alkyl, or substituted or unsubstituted aralkyl. The campesterol/budesonide drug conjugate of formula (1g) includes a linkage (L) between the plant steroid and the glucocorticoid. The linkage includes an ester function (designated “e”) at the campesterol and an amine function (designated “am”) at the glucocorticoid.



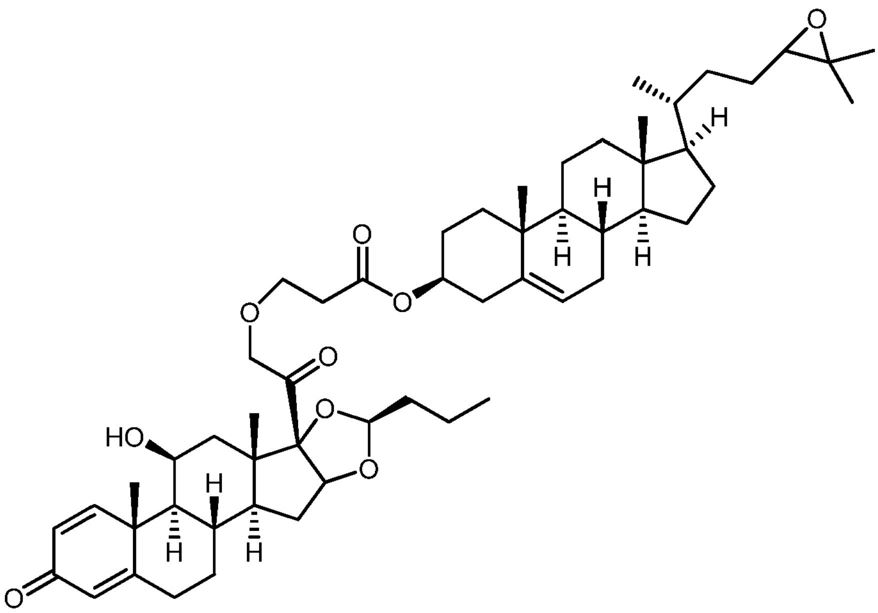
(1g)

[0046] Across the entire range of synthetic glucocorticoids substantial variation is found within sub-molecular groups attached to the north-side of the typical cyclopentane ring on the opposed distal side (right side) of the glucocorticoid molecule. These sub-molecular groups typically contain hydroxyl and ketone functionalities that are suitable for attaching the glucocorticoid to a plant steroid constituent. For example, one exemplary drug conjugate of the present invention, depicted herein as formula (1h), comprises budesonide and campesterol linked through an ether function at the campesterol and an ester function at the glucorticoid. The glucocorticoid is linked to the plant steroid via a sub-molecular group (an α-hydroxy ketone) that is attached to a fused ring carbon of the cyclopentane and 1,3-dioxolane rings of the glucocorticoid.



(1h)

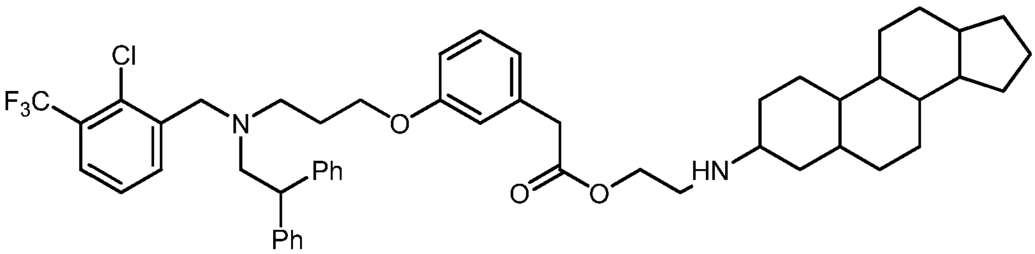
[0047] In another example, an exemplary drug conjugate of the present invention, depicted herein as formula (1i) comprises budesonide linked to 24(S),25-epoxycholesterol through an ether function proximal the budesonide and an ester function at the plant steroid constituent. The glucocorticoid is linked to the plant steroid via a sub-molecular group (an α-hydroxy ketone) attached to a fused ring carbon of the cyclopentane and 1,3-dioxolane rings of the glucocorticoid.



(1i)

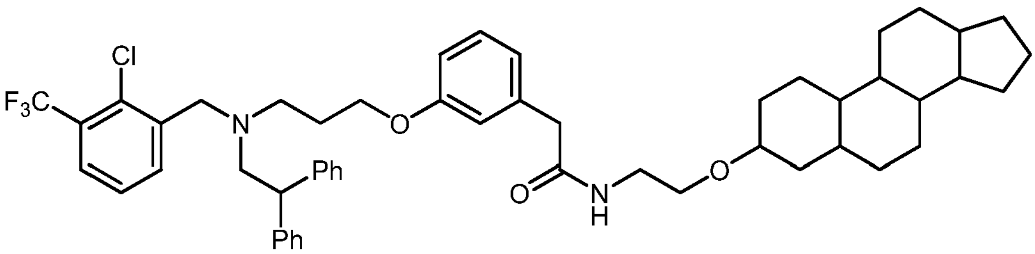
[0048] In certain exemplary embodiments, a plant steroid constituent may be attached to a LXR agonist. LXR agonists, such as TO-901317 or GW-3965, comprise functional groups useful for creating a link to a plant steroid constituent. For example, GW-3965 includes a carboxy group function and TO-901317 includes a hydroxyl group function, both of which can be synthetically modified to link the LXR agonist to a plant steroid constituent.

[0049] One exemplary drug conjugate of the present invention, depicted herein as formula (1j), includes the LXR agonist GW-3965 linked to a phystosterol or phytostanol constituent (depicted generically), via the GW-3965 carboxy group and the left side hydroxyl group of the plant steroid. The linkage between the LXR agonist and plant steroid includes ester function at the LXR agonist and amine function at the plant steroid.



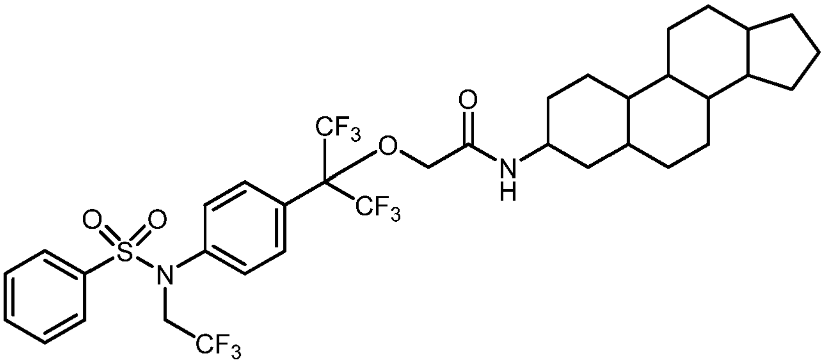
(1j)

[0050] Another exemplary drug conjugate, depicted herein as formula (1k), includes GW-3965 linked to a phytosterol or phytostanol constituent via the GW-3965 carboxy group and the left side hydroxyl group of the plant steroid, wherein the linkage between the LXR agonist and plant steroid includes amide function at the LXR agonist and ether function at the plant steroid.



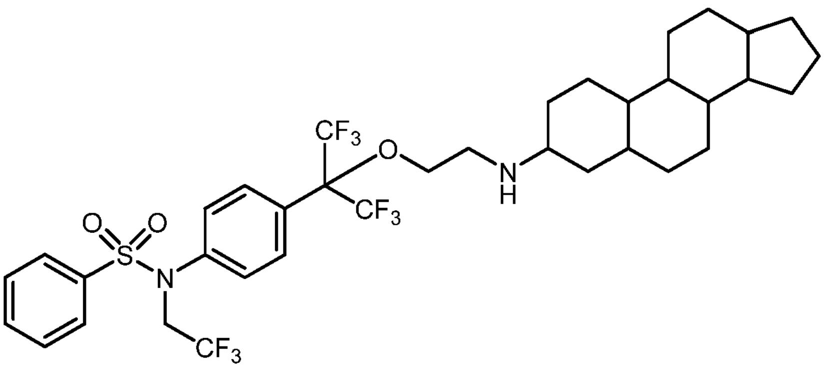
(1k)

[0051] Another exemplary drug conjugate, depicted herein as formula (1m), includes TO-901317 linked to a phytosterol or phytostanol constituent via the TO-901317 hydroxyl group and the left side hydroxyl group of the plant steroid, wherein the linkage between the LXR agonist and plant steroid includes ether function at the LXR agonist and amide function at the plant steroid.



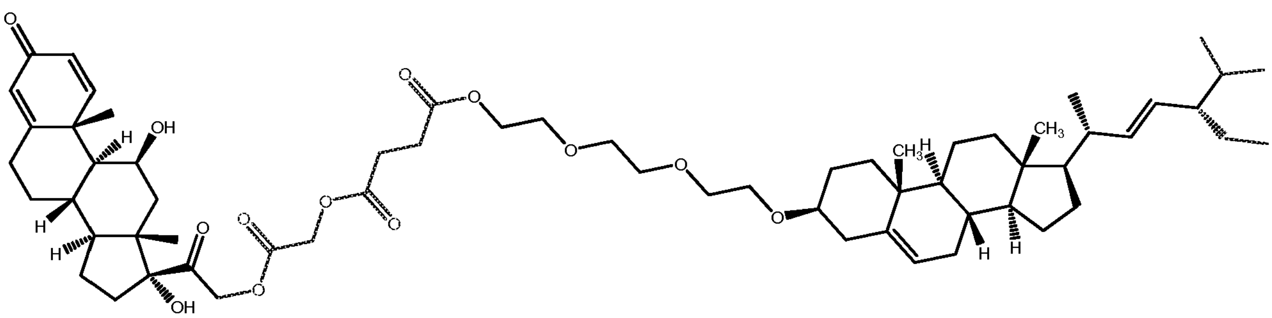
(1m)

[0052] Another exemplary drug conjugate, depicted herein as formula (1n), includes TO-901317 linked to a phytosterol or phytostanol constituent via the TO-901317 hydroxyl group and the left side hydroxyl group of the plant steroid, wherein the linkage between the LXR agonist and plant steroid includes ether function at the LXR agonist and amine function at the plant steroid.



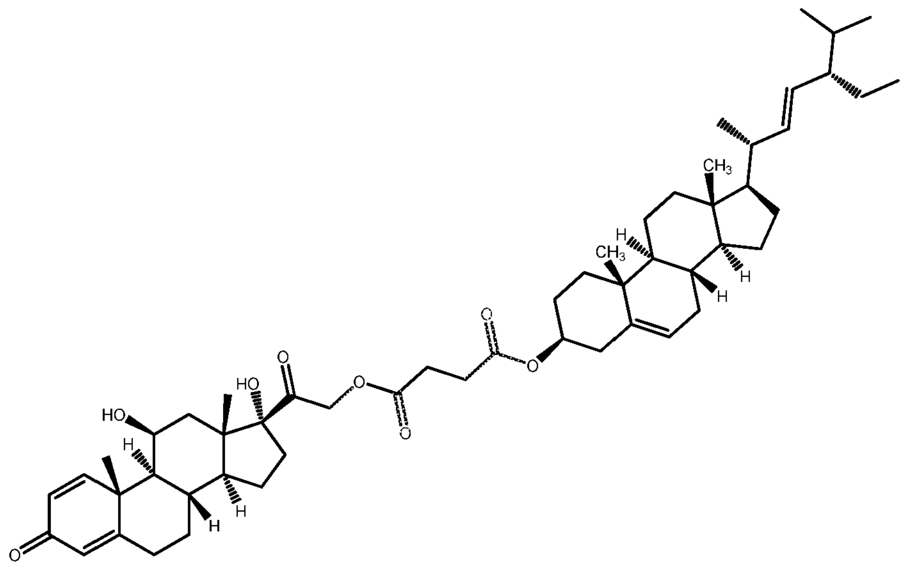
(1n)

[0053] Other exemplary drug conjugates include prednisolone stigmasteroltrisethyleneglycolalcohol succinate (formula (1o)), prednisolone stigmasterol succinate (formula (1p)), and prednisolone stigmasteroltrisethyleneglycol acetate (formula (1q)).



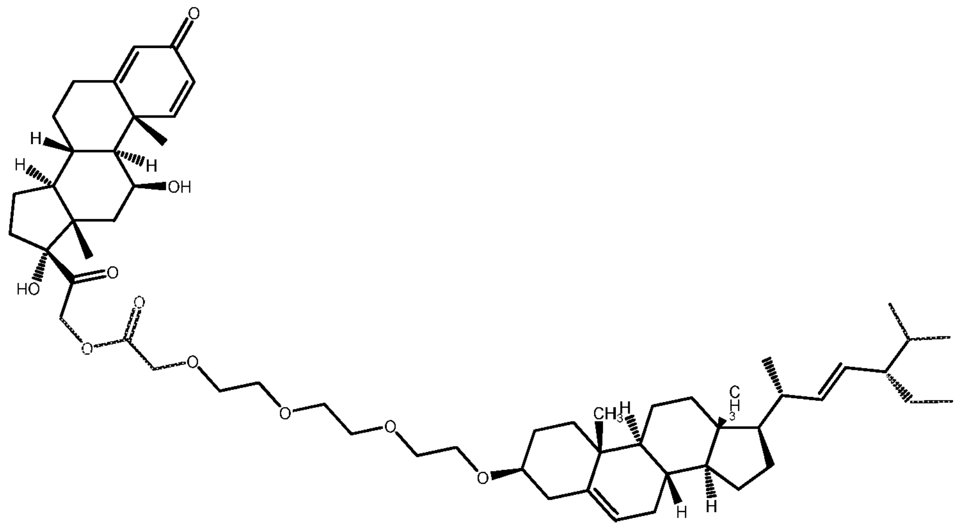
(1o)

SE-22-II



(1p)

SE-24-II



(1q)

SE-41-II

3. Methods of treatment

[0054] In another aspect, provided herein are methods of treating a disease by administering a drug conjugate in accordance with the present invention to a mammal in need thereof. The drug conjugate may be specifically delivered to intestinal cells as shown in Figure 1.

[0055] Also provided herein is a method of modulating cholesterol levels such as LDL-C by administering the plant steroid. The modulated cholesterol may be serum or plasma. For example, an oxyphytostanol may be a potent ligand for LXR, which may result in greater LDL-C reduction than non-oxidized plant sterols and stanols, and significantly increase intestinally derived HDL metabolism. The drug conjugate may be used to treat cancer, such as intestinal or colon cancer, inflammatory bowel disease (IBD), celiac disease, irritable bowel syndrome, dyslipidemia, atherosclerosis, obesity, hypertriglyercidemia, diabetes, or intestinal infections.

[0056] The drug conjugate may also be used to treat gastro-intestinal diseases, including, without limitation, Crohn's disease, ulcerative colitis, inflammatory bowel disease, irritable bowel disease, irritable bowel syndrome (either diarrhea or constipation associated), celiac disease, gastro-intestinal inflammation associated with food-allergies or autistic spectrum disorder, gastritis (atrophic, Ménétrier's disease, gastroenteritis), emesis (nausea and vomiting), pyloric stenosis, achlorhydria, gastroparesis, portal hypertensive gastropathy, gastric antral vascular ectasia, gastric dumping syndrome, HMFs (Human Mullular Fibrilation syndrome), enteritis (duodenitis, jejunitis, ileitis), ulcers (peptic, duodenal, or Curling's ulcer), Dieulafoy's lesion, malabsorption (including but not limited to Coeliac, tropical sprue, Whipple's disease, steatorrhea, Milroy disease), colitis (pseudomembranous, ulcerative, ischemic, microscopic, collagenous, lymphocytic), megacolon/toxic megacolon, enterocolitis, necrotizing enterocolitis, functional colonic disease, intestinal pseudoobstruction, and Ogilvie syndrome.

[0057] The drug conjugate may also be used to treat inflammatory conditions, including, without limitation, arthritis, osteo-arthritis, asthma, COPD, allergies, seasonal allergies, food allergies, pruritis, urticaria, atopic allergy, and (atopic) dermatitis.

[0058] The drug conjugate may also be used to treat auto-immune diseases, including, without limitation, acute disseminated encephalomyelitis (ADEM), addison's disease, agammaglobulinemia, alopecia areata, amyotrophic lateral sclerosis (ALS), ankylosing spondylitis, antiphospholipid syndrome, antisynthetase syndrome, autoimmune aplastic anemia, autoimmune cardiomyopathy, autoimmune enteropathy, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune peripheral neuropathy, autoimmune pancreatitis, autoimmune polyendocrine syndrome, autoimmune progesterone dermatitis, autoimmune thrombocytopenic purpura, autoimmune urticaria, autoimmune uveitis, Balo disease (Balo concentric sclerosis), Behçet's disease, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, bullous pemphigoid, Castleman's disease, chronic inflammatory demyelinating polyneuropathy, chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome, cicatricial pemphigoid, Cogan syndrome, cold agglutinin disease, complement component 2 deficiency, contact dermatitis, cranial arteritis, CREST syndrome, Cushing's Syndrome, cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, dermatitis herpetiformis, dermatomyositis, diffuse cutaneous systemic sclerosis, Dressler's syndrome, drug-induced lupus, discoid lupus erythematosus, eczema, endometriosis, enthesitis-related arthritis, eosinophilic fasciitis, eosinophilic gastroenteritis, epidermolysis bullosa acquisita, erythema nodosum, erythroblastosis fetalis, essential mixed cryoglobulinemia, Evan's syndrome, fibrodysplasia ossificans progressive, fibrosing alveolitis (or Idiopathic pulmonary fibrosis), gastritis, gastrointestinal pemphigoid, giant cell arteritis, glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's encephalopathy, Hashimoto's thyroiditis, Henoch-Schonlein purpura, herpes gestationis (gestational pemphigoid), Hidradenitis suppurativa, Hughes-Stovin syndrome, hypogammaglobulinemia, idiopathic inflammatory demyelinating diseases, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura (autoimmune thrombocytopenic purpura), IgA nephropathy, inclusion body myositis, chronic inflammatory demyelinating polyneuropathy, interstitial cystitis, juvenile idiopathic arthritis (juvenile rheumatoid arthritis), Kawasaki's disease, Lambert-Eaton myasthenic syndrome, leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, linear IgA disease (LAD), Lou Gehrig's disease, lupoid hepatitis (autoimmune hepatitis), lupus erythematosus, Majeed syndrome, Ménière's disease, microscopic polyangiitis, Miller-Fisher syndrome (Guillain-Barre Syndrome), mixed connective tissue disease, morphea, Mucha-Habermann disease (pityriasis lichenoides et varioliformis acuta), multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neuromyelitis optica (Devic's disease), neuromyotonia, occular cicatricial pemphigoid, opsoclonus myoclonus syndrome, Ord's thyroiditis, palindromic rheumatism, PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcus), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonage-Turner syndrome, pars planitis, pemphigus vulgaris, pernicious anaemia, perivenous encephalomyelitis, POEMS syndrome, polyarteritis nodosa, polymyalgia rheumatic, polymyositis, primary biliary cirrhosis, primary sclerosing cholangitis, progressive inflammatory neuropathy, psoriasis, psoriatic arthritis, pyoderma gangrenosum, pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, pelapsing polychondritis, Reiter's syndrome, restless leg syndrome, retroperitoneal fibrosis, rheumatoid arthritis, rheumatic fever, sarcoidosis, Schmidt syndrome, Schnitzler syndrome, scleritis, scleroderma, serum Sickness, Sjögren's syndrome, spondyloarthropathy, Still's disease, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, Sweet's syndrome, Sydenham chorea, sympathetic ophthalmia, systemic lupus erythematosis, Takayasu's arteritis, temporal arteritis, thrombocytopenia, Tolosa-Hunt syndrome, transverse myelitis, spondyloarthropathy, urticarial vasculitis, vasculitis, vitiligo, and Wegener's granulomatosis.

[0059] The drug conjugate may also be used to treat dyslipidemia and cardiovascular diseases, including, without limitation, hypercholesterolemia (primary and familial), hypertryglyceridemia (high and very high), mixed dyslipidemia, Fredrickson Type I, II, III, IV, and V dyslipidemia, atherosclerosis, coronary artery disease, coronary heart disease, cerebrovascular disease, peripheral artery disease.

[0060] The drug conjugate may also be used to treat diabetes and associated conditions, including, without limitation, insulin resistance, non insulin dependent diabetes mellitus (NIDDM), Type II diabetes, and Type 1 diabetes.

[0061] The drug conjugate may also be used to treat bacterial, viral, parasite or fungal infections, including, without limitation, gastro-intestinal infections, skin infections, eye-infections, respiratory system infections, ear infections, sexually transmitted diseases, airborne diseases, insect-transmitted diseases, transfusion or transplant transmitted diseases, and mother-to-child transmitted diseases.

[0062] The drug conjugate may also be used to treat cancers, including, without limitation, colon cancer, gastric cancer, pancreatic cancer, skin cancer, liver cancer, myeloma, melanoma, sarcoma, oral cancer, rectal cancer, mesothelioma, lymphoma, and other cancers.

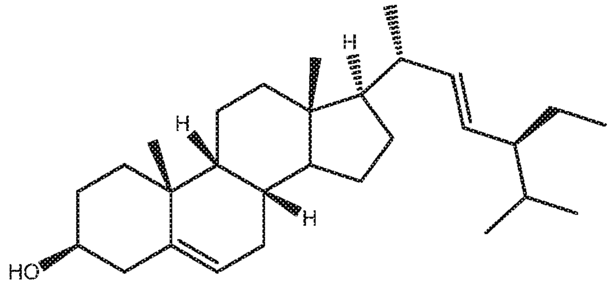
[0063] The present invention has multiple aspects, illustrated by the following non-limiting examples.

Example 1

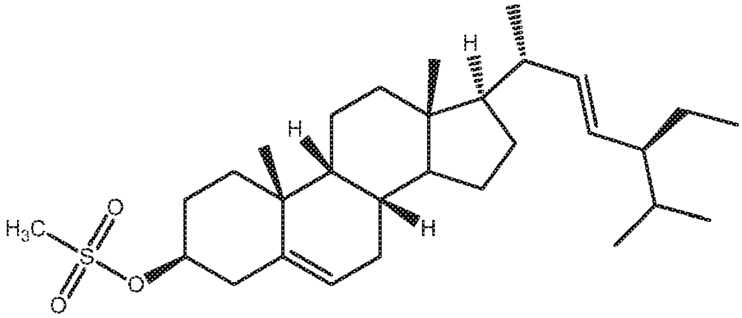
Methods of making plant steroid-conjugated compounds

[0064] Stigmasterol Mesylate (SE-09-II)

[0065] A solution of stigmasterol (formula (1r)) (1.78g) in 25 ml of methylene chloride was cooled to ~ 4°C and 0.9g of triethylamine added followed by the dropwise addition of 0.69 g of methanesulfonyl chloride in 3-4 ml of THF. The reaction mixture was stirred at 4 °C for 3 hr, the cooling bath removed and the reaction mixture stirred overnight at ambient temperature. The reaction was quenched with water and the phases separated. The organic phase was washed with 5% $NaHCO_3$, dried with Na_2SO4 and evaporated under reduced pressure to yield the desired product (stigmasterol mesylate; formula (1s)) used as is in the next step.



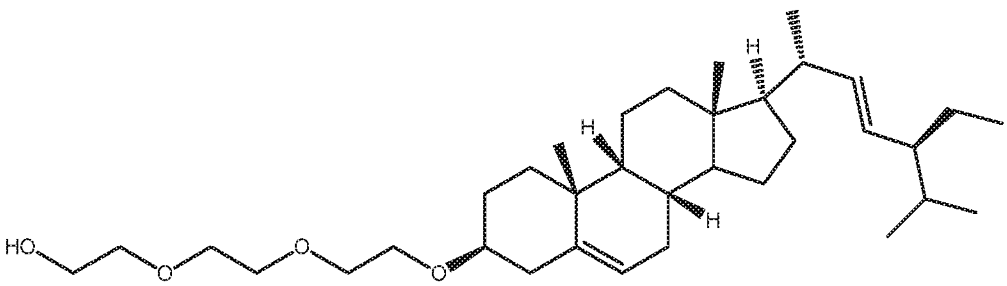
(1r)



(1s)

[0066] Stigmasterol Triethyleneglycol Ether (SE-16-II)

[0067] Stigmasterol mesylate (3.0 g) and triethyleneglycol (21.0g) were dissolved in ~15 ml of 1,4-dioxane and the reaction mixture heated at reflux, under nitrogen, for 3-4 hr. The reaction mixture was cooled to room temperature and the solvent removed under vacuum. The residue was partitioned between methylene chloride and water and the phases separated. The organic phase was washed sequentially with saturated $NaHCO_3$, water and brine, dried over Na_2SO_4 and the solvent removed under vacuum. The residue was purified by column chromatography (silica gel eluted with MTBE). The resulting product (stigmasterol triethyleneglycol ether; formula (1t)) was used in the next step.

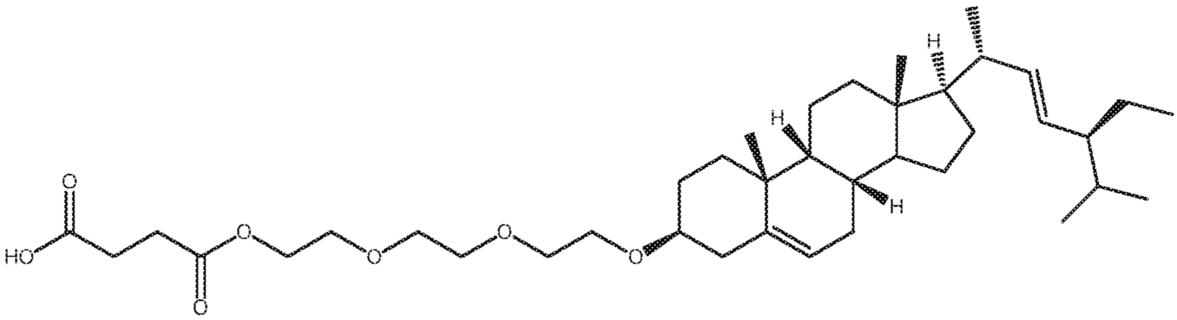


(1t)

SE-16-II

[0068] Stigmasterol triethyleneglycol succinate monoacid (SE-19-II)

[0069] Stigmasterol triethyleneglycol ether (0.55g), succinic anhydride (0.12g), triethylamine (0.20g), and DMAP (20 mg) were dissolved in 15 ml of THF. The reaction mixture was stirred overnight at ambient temperature and ethyl acetate and water added. The phases were separated and the organic layer washed with 5% HCl followed by water. The organic layer was dried and the solvent removed under vacuum. The crude product (stigmaterol triethyleneglycol succinate; formula (1u)) was used as is.



(1u)

SE-19-II

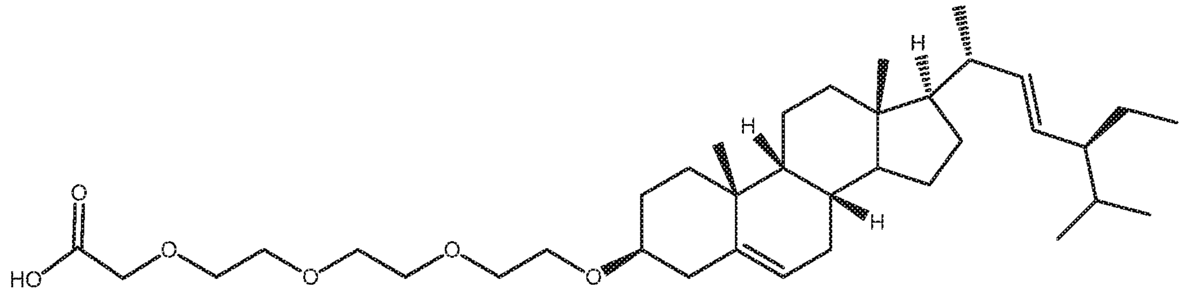
[0070] Stigmasterol triethyleneglycol prednisolone succinate (SE-22-II)

[0071] Stigmasterol triethylene glycol succinate monoacid (0.65 g) was dissolved in toluene (15 ml), cooled to 0°C and oxalyl chloride (0.5 ml) added dropwise. The reaction mixture was stirred at ambient temperature for 1 hr, 60°C for 1 hr, cooled to ambient temperature and evaporated to dryness under reduced pressure.

[0072] To a cooled (0 – 4°C) solution of 0.36 g prednisolone in a mixture of THF and TEA was added the crude acid chloride prepared above. The reaction mixture was stirred overnight at ambient temperature and then refluxed for 1-3 hrs. The reaction mixture was cooled and ethyl acetate added. The organic phase was separated, washed with water, 1% $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel eluted with methylene chloride and then CH_2Cl_2/EtOAc 9:1. The resulting product was stigmasterol triethyleneglycol prednisone succinate (SE-22-II; formula (1o)).

[0073] Stigmasterol triethyleneglycol oxyacetic acid (SE-39-II)

[0074] To stigmasterol triethyleneglycol (1 g) in THF was added sodium hydride (0.26g, dry basis) as a 60 wt% suspension in mineral oil and 0.51g bromoacetic acid and the reaction mixture stirred overnight at ambient temperature. After cooling to 0°C the excess sodium hydride was destroyed by the slow addition of water. THF was evaporated under reduced pressure and the aqueous solution acidified with 6N HCl and extracted with methylene chloride. The methylene chloride extract was washed with water, dried with Na_2SO4 and evaporated to dryness under reduced pressure to give a waxy solid. This material (stigmasterol triethyleneglycol oxyacetic acid; formula (1v)) was used without further purification.



(1v)

SE-39-II

[0075] Stigmasterol triethyleneglycol oxyacetic acid prednisolone ester (SE-41-II)

[0076] See procedure for synthesis of stigmasterol triethyleneglycol prednisolone diester above. The crude product was purified by column chromatography on silica gel with CH_2Cl_2/EtOAc 9:1, 8:2 as eluent and CH_2Cl_2/MeOH 95:5.

[0077] Stigmasterol succinate (SE-21-II)

[0078] A solution of stigmasterol (0.83g), succinic anhydride (0.32g), pyridine (1.5%) and toluene (15 ml) was refluxed for 24 hr and cooled to room temperature. The toluene solution was washed with water, 0.5N HCl, water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was used as is.

[0079] Stigmasterol prednisolone succinate diester (SE-24-II)

[0080] Stigmasterol prednisolone succinate diester was prepared in the same manner as stigmasterol triethyleneglycol prednisolone diester, substituting stigmasterol succinate monoacid for stigmasterol triethyleneglycol succinate monoacid . The crude product was purified by column chromatography on silica gel eluted with CH_2Cl_2, CH_2Cl_2/EtOAc, 9:1, 8:2.

[0081] Spectral data confirming product structures

[0082] Figures 11A-L show the spectral data confirming the structures of SE-24-II (Figs. 11A-C and J-L), SE-22-II (Figs. 11D-F and M-O), and SE-41-II (Figs. 11G-I and P-R).

Example 2

Plant steroid-conjugated prednisone alter gene expression patterns in a manner similar to the unconjugated drug

[0083] This example shows that a plant steroid-conjugated drug that is minimally absorbed systemically can elicit the same potential therapeutic response in intestinal cells as a non-conjugated drug. Specifically prednisone was conjugated to stigmasterol, which is one of the most poorly absorbed plant sterols, to deliver anti-inflammatory effects to intestinal cells without significant systemic absorption. Caco-2 intestinal cells were used. These types of cells have been used as a cell based assay to evaluate the effects of drugs.

[0084] Gene expression profiles were generated from 2.5 μM and 25 μM treatments of Caco-2 cells (ATCC). The treatments consisted of a reference compound (prednisone) and three experimental compounds: SE-22-II, SE-24-II and SE-41-II. The active forms of these compounds had formulas 1(o), 1(p) and 1(q), respectively.

[0085] All compounds were individually applied to Caco-2 cells (250,000/well). After RNA isolation of each treatment, the isolated RNAs were individually labeled as cDNA probes and applied to Illumina HT-12 array chips. Results from the gene array assays revealed that all compounds (reference and test compounds) affected gene expression compared to non-treated Caco-2 cells. Using the Inforsense Suite of statistical and visualization algorithms (Volcano plots), a broad number of gene classes were observed to be affected by the compound treatments. In particular, for all of the compound treatments tested, >1 fold gene changes were seen in a broad range of genes in a statistically significant manner. A number of genes involved in inflammation, immune system response, cell surface and nuclear hormone signal transduction, fat metabolism and cell cycle/cell differentiation/proliferation were affected. In particular, compounds SE-22 and SE-24 displayed more similar gene profiles to prednisone than did compound SE-41.

[0086] Key Genes Affected per Gene Class: Using the Inforsense Suite of Data Analysis tool (IDBS), a total of 36 Volcano plots were generated against 36 gene array assays (triplicate array assays per nontreatment and treatment of compounds). Volcano plots were generated for each compound data set to statistically identify key genes upregulated and down regulated (see example in Fig. 2). From the volcano plots for each array experiment the most pronounced upregulated and down-regulated genes were identified. Genes were identified on both sides of the volcano plots (shaded in gray in Fig. 2) that were at a correlation coefficient of $p<0.05$. Genes identified in this manner are listed in Figures 3A-F for Caco-2 cells treated with 2.5 μM of each compound. It was also found that the 25 μM treatments demonstrated cytotoxic effects on the gene expression profiles. In addition, 2.5 μM treatments exhibited a far greater linear (dose-dependent) response when looking at several key genes affected in the profiles (e.g., HNF-4, Glypican, and Phosphoenolpyruvate carboxykinase 1).

[0087] Methods

[0088] 1. Cell Culture: Caco-2 Cells treated with Compound Set

[0089] The human colon adenocarcinoma cell line Caco-2 was cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich) with 4.5 g/l glucose, L-glutamine, $NaHCO_3$ and pyridoxine HCl supplemented with 1% (vol/vol) nonessential amino acids, 1% Na-pyruvate, 1% penicillin/streptomycin, and 10% (vol/vol) heat inactivated fetal calf serum, all purchased from Gibco BRL. Cell cultures were transferred weekly by trypsinization and incubated at 37°C in a humidified incubator containing 5% CO_2. After 4 weeks of cell culture, prednisone, SE-22-II, SE-24-II and SE-41-II were each added to separate triplicate wells (250,000 cells/well) at final effective concentrations of 2.5 μM and 25 μM, respectively. Cells were exposed to each compound, respectively, for 24 hours.

[0090] 2. Cell Harvest and RNA Isolation

[0091] Total RNA was isolated according to the TRIzol manufacturer's instructions (Invitrogen). RNA quality was assessed with a 2100 Bioanalyzer (Agilent). RNA from pooled triplicates of the various cell treatments, all with an RNA integrity number >7, was further purified with Qiagen RNeasy columns. From 300 ng of total RNA, the Illumina TotalPrep RNA Amplification kit (Ambion) was used to generate amplified biotinylated cRNA after reverse transcription by the Eberwine procedure. cRNA (900 ng) was hybridized overnight to Illumina HT-12 BeadArrays, which were then washed and stained with streptavidin-Cy3 (Amersham-Pharmacia Biotech) according to the Illumina protocol. Arrays were scanned on a BeadArray Reader (Illumina).

[0092] Specific transcripts within the biotinylated cRNA were measured by fluorescent imaging after direct hybridization to HT-12 bead arrays, which contain 12 arrays per slide, each with an average of 15 beads for each of 48,803 probes measuring 37,846 annotated genes and additional transcripts. Raw measurements of the intensity of each bead were captured directly and processed as "bead-level" for the samples, as described below. For both treated and non-treated samples, measurements were processed as "probe-level" data by GenomeStudio software (Illumina). The software checked that a probe had ≥3 beads present on the array (if not, the probe was considered to be missing), did a local background subtraction for each bead, and then condensed bead-level data to a single probe-level value per probe by removing outliers that were >3 median absolute deviations from the median, recalculating the mean of the remaining values. Raw probe-level values were extracted from the software, without the use of its correction or normalization options, but with the use of its option for imputing missing values.

[0093] 3. Data Analysis

[0094] ANOVA statistical analysis was performed on all Illumina gene expression array files. Volcano plots were generated for all pooled bead array data using Inforsense 5.1 Suite (IDBS).

[0095] 4. Results

[0096] Figure 4 shows the gene response of key gene families to 2.5μM of prednisone, SE-22-II, SE-24-II and SE-41-II.

[0097] 5. Discussion

[0098] Caco-2 cells were treated with various test compounds (prednisone, SE-22-II, SE-24-II, and SE-41-II), and results were compared to the results from cells treated with prednisolone. Non-treated and treated cells were assessed for their gene expression profiles after 2.5μM and 25μM drug treatments (after 24 hours). The Caco-2 cells demonstrated consistent results within replicates of each 2.5μM treatment as compared to the 25μM treatment. All of the compounds tested exhibited bioactivity. One hundred and thirty genes demonstrated a statistically significant >1-fold response ($p<0.005$). The key classes of genes affected included inflammation, immune system, nuclear-transcription-translation, and cell adhesion. Up- and down-regulation of multiple genes associated with prednisolone exposure may depend on the presence of specific transcription factors and/or transcription factor binding motifs (TFBMs) in the promoter regions of the genes affected. In particular, the highest levels of gene responses (>3.0 fold gene expression level change) were seen for nuclear hormone receptor (see schematic in Figure 5), cytokine and chemokine receptor genes and their corresponding receptors. Figure 6 shows gene responses that affect nuclear hormone receptors in Caco-2 cells.

[0099] In the gene profiling assays described herein, specific nuclear hormone receptor genes were affected across all of the compounds tested. Specifically, up- and down-regulation effects were seen for several genes of this class (e.g., Liver receptor homolog-1, NR5A2, Estrogen-Like Receptor NR3C2, Liver X receptor-like NR1H2, Vitamin D receptor NRI1, RAR-related orphan receptor, NR1F2, and Hepatocyte Nuclear Factor-4 Receptor). The protein factors of these genes may have broad affects on a broad array of target genes involved in cell cycle, immune function, cell adhesion and metabolism.

[0100] Nuclear Hormone Receptors

[0101] Nuclear receptors are grouped into a large superfamily and are thought to be evolutionarily derived from a common ancestor. A list of classical and orphan hormone receptors and their ligands is shown in Figure 7. Evolutionary analysis of the receptors has led to a subdivisionin six different subfamilies. One large family is formed by thyroid hormone receptors (TRs), retinoic acid receptors (RARs), vitamin D receptors (VDRs) and peroxisome proliferator-activated receptors (PPARs), as well as different orphan receptors. Ligands for some of these receptors have been recently identified (see Figure 7). The second subfamily contains the retinoid X receptors (RXRs) together with chicken ovalbumin upstream stimulators (COUPs), hepatocyte nuclear factor 4 (HNF4), testis receptors (TR2) and receptors involved in eye development (TLX and PNR). RXRs bind 9-cis-retinoic acid and play an important role in nuclear receptor signaling, as they are partners for different receptors that bind as heterodimers to DNA. Ligands for other receptors have not been identified, whereas long-chain fatty acid acyl-CoA thioesters may be endogenous ligands for HNF4. The third family is formed by the steroid receptors and the highly related orphan receptors estrogen-related receptors (ERRs). The fourth, fifth, and sixth subfamilies contain the orphan receptors NGFI-B, FTZ-1/SF-1, and GCNF, respectively. Most subfamilies appear to be ancient since they have an arthropod homolog, with the exception of steroid receptors that have no known homologs. It has been suggested that the ancestral receptors were constitutive homodimeric transcription factors that evolved to independently acquire the ability to bind a ligand and to heterodimerize. However, the possibility that the ancestral receptor was ligand dependent and that mutations changed the ligand-binding specificity or led to loss of ligand binding during evolution cannot be ruled out.

[0102] Members of this class of receptors are found in number of tissue types (liver, intestine, brain). Nuclear receptors bind to ligands in the cytosol (Class I) or in the nucleus (Class II). Prior to binding to sterols some members of the nuclear hormone receptor family are bound in an inactive state to other protein factors (e.g., heat shock proteins (chaperonins)), once bound to the sterol, nuclear hormone receptors can then bind as homo or hetrodimers. The activated nuclear hormone receptor (homo or heterodimer) then binds to hormone response elements (HREs) in target genes. Once bound, these genes can be transcriptionally or translationally up or down regulated.

[0103] Like other transcriptional regulators, nuclear receptors exhibit a modular structure with different regions corresponding to autonomous functional domains that can be interchanged between related receptors without loss of function. A typical nuclear receptor consists of a variable NH2-terminal region (A/B), a conserved DNA binding domain (DBD) or region C, a linker region D, and a conserved E region that contains the ligand binding domain (LBD). Some receptors contain also a COOH terminal region (F) of unknown function. A schematic of a nuclear receptor is shown in Figure 8. The receptors also contain regions required for transcriptional activation. The hypervariable A/F region of many receptors contains an autonomous transcriptional activation function, referred to as AF-1, that contributes to constitutive ligand independent activation by the receptor. A second transcriptional activation domain, termed AF-2, is located in the COOH terminus of the LBD, but unlike the AF-1 domain, the AF-2 is strictly ligand dependent and conserved among members of the nuclear receptor superfamily.

[0104] Interleukins

[0105] A number of interleukin receptor and interleukins were affected by all of the compounds tested (prednisolone, SE-22, SE-24 and SE-41). Interleukins promote the development and differentiation of T-, B- and hematopoietic cells. In this series of assays the key interleukins affected are shown below. The majority of interleukins are synthesized by helper CD4+ T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. A reference list of this class of affected targets is presented in Figures 9A and B. Also, a visual plot of affected interleukins and interleukin receptors is presented in Figure 10.

[0106] The protein encoded by interleukin-1 receptor, type 1 gene is a cytokine receptor that belongs to the interleukin-1 receptor family. This protein binds interleukin alpha (IL1A), interleukin beta (IL1B), and interleukin 1 receptor, type I(IL1R1/IL1RA), and acts as a decoy receptor that inhibits the activity of its ligands. Interleukin-4 (IL4) is reported to antagonize the activity of interleukin-1 by inducing the expression and release of this cytokine. This gene and three other genes form a cytokine receptor gene cluster on chromosome 2q12. Two alternatively spliced transcript variants encoding the same protein have been reported.

[0107] Interleukin-7 receptor . Interleukin-7 receptor has been shown to play a critical role in the development of immune cells called lymphocytes - specifically in a process known as V(D)J recombination. This protein is also found to control the accessibility of a region of the genome that contains the T-cell receptor gamma gene, by STAT5 and histone acetylation. Knockout studies in mice suggest that blocking apoptosis is an essential function of this protein during differentiation and activation of T lymphocytes. Functional defects in this protein may be associated with the pathogenesis of severe combined immunodeficiency (SCID)

[0108] Interleukin-1, beta (up-regulated in prednisolone, SE-22, and SE-24; downregulated in SE-41). The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). Interleukin-1 beta is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. This gene and eight other interleukin 1 family genes form a cytokine gene cluster on chromosome 2.

[0109] Interleukin-7 receptor. Interleukin-7 receptor has been shown to play a critical role in the development of immune cells called lymphocytes - specifically in a process known as V(D)J recombination. This protein is also found to control the accessibility of a region of the genome that contains the T-cell receptor gamma gene, by STAT5 and histone acetylation. Knockout studies in mice suggest that blocking apoptosis is an essential function of this protein during differentiation and activation of T lymphocytes. Functional defects in this protein may be associated with the pathogenesis of severe combined immunodeficiency (SCID)

[0110] Other key gene responses via compound treatments (prednisolone, SE-22, SE-24 and SE-41).

[0111] Insulin induced gene 1. Insulin induced gene 1 encodes an endoplasmic reticulum (ER) membrane protein that plays a critical role in regulating cholesterol concentrations in cells. The protein binds to the sterol-sensing domains of SREBP cleavage-activating protein (SCAP) and

HMG CoA reductase, and is essential for the sterol-mediated trafficking of the two proteins. Alternatively spliced transcript variants encoding distinct isoforms have been observed.

[0112] Oysterols. Oxysterols regulate cholesterol homeostasis through liver X receptor (LXR) and sterol regulatory element-binding protein (SREBP) mediated signaling pathway. In the gene profiling experiments, prednisolone, SE-22 and SE-24 upregulated the induced insulin gene 1 by over 10-fold. SE-41 was upregulated only by 1.5-fold.

[0113] Solute Carrier Family 36 Member 1. The solute carrier (SLC) group of membrane transport proteins include over 300 members organized into 51 families. Most members of the SLC group are located in the cell membrane. The SLC gene nomenclature system was originally proposed by the HUGO Gene Nomenclature Committee (HGNC) and is the basis for the official HGNC names of the genes that encode these transporters. Solutes that are transported by the various SL C group members are extraordinarily diverse and include both charged and uncharged organic molecules as well as inorganic ions and the gas ammonia.

[0114] 6. Conclusions

[0115] Overall, the results indicate that the compounds are bioactive. The gene responses have implications for various cell processes (cell differentiation, immune cell and/or immune response, cell adhesion, lipid metabolism, nuclear hormone receptor activation, transcription and translation modification).

CLAIMS

1. A drug conjugate of formula (I),

D-L-P (I)

wherein

D is a drug constituent;

L is a linkage; and

P is a plant steroid constituent.

2. The compound of claim 1, wherein D is prednisone.

3. A drug conjugated according to claim 1, selected from the group consisting of:
prednisolone stigmasteroltrisethyleneglycolalcohol succinate;
prednisolone stigmasterol succinate; and
prednisolone stigmasteroltrisethyleneglycol acetate.

4. The drug conjugate of claim 1, wherein P is a phytosterol or phytostanol constituent.

5. The drug conjugate of claim 4, wherein P is stigmasterol, campesterol, 24(S),25-epoxycholesterol, or 5-6-epoxycampesterol.

6. The drug conjugate of claim 1, wherein D is selected from the group consisting of an anti-inflammatory drug, a glucocorticoid, a LXR agonist, a TNFα inhibitor, a NF-κB inhibitor or agonist, a selective COX-2 inhibitor, a non-selective non-steroidal anti-inflammatory drug (NSAID), methotrexate, leflunomide, mesalamine, balsalaside, osalazine, sulfasalazine, an aminosalicylate, cyclosporine, mercaptopurine, azathioprine, atropine, a microsomal triglyceride transfer protein (MTP) inhibitor, an acyl CoA:cholesterol acyltransferase-2 (ACAT 2) inhibitor, a farnesoid X receptor (FXR) agonist, a diacylglycerol actyltransferase (DGAT) inhibitor, a glucagon-like peptide-1 (GLP-1) agonist, an ileal bile acid transport (IBAT) inhibitor, an antibiotic, and an antiviral.

7. The drug conjugate of claim 6, wherein D is a glucocorticoid or a LXR agonist constituent.

8. The drug conjugate of claim 7, where the glucocorticoid is budesonide and the LXR agonist is GW-3965 or TO-901317.

9. The drug conjugate of claim 1, wherein L is a bond.

10. The drug conjugate of claim 1, wherein L comprises at least one chemical functional group selected from the group consisting of an ether, amide, sulfonamide, and ester.

11. The drug conjugate of claim 1, wherein L comprises at least 3 atoms.

12. The drug conjugate of claim 11, wherein L comprises at least 10 atoms.

13. A drug conjugate according to claim 1, selected from the group consisting of:
a campesterol/budesonide drug conjugate;
a 24(S),25-epoxycholesterol/budesonide drug conjugate;
a campesterol/GW-3965 drug conjugate;
a campesterol/TO-901317 drug conjugate;
a 24(S),25-epoxycholesterol/GW-3965 drug conjugate; and
a 24(S),25-epoxycholesterol/TO-901317 drug conjugate.

14. A compound comprising a plant steroid and a drug, wherein the plant steroid is attached to the drug.

15. The compound of claim 14, wherein the attachment is a conjugated bond or an amine bond.

16. The compound of claim 14, wherein the drug is selected from the group consisting of an anti-inflammatory drug, a glucocorticoid, a LXR agonist, a TNFα inhibitor, a

NF-κB inhibitor or agonist, a selective COX-2 inhibitor, a non-selective non-steroidal anti-inflammatory drug (NSAID), methotrexate, leflunomide, mesalamine, balsalaside, osalazine, sulfasalazine, an aminosalicylate, cyclosporine, mercaptopurine, azathioprine, atropine, a microsomal triglyceride transfer protein (MTP) inhibitor, an acyl CoA:cholesterol acyltransferase-2 (ACAT 2) inhibitor, a farnesoid X receptor (FXR) agonist, a diacylglycerol actyltransferase (DGAT) inhibitor, a glucagon-like peptide-1 (GLP-1) agonist, an ileal bile acid transport (IBAT) inhibitor, an antibiotic, and an antiviral.

17. The compound of claim 16, wherein the drug is prednisone.

18. The compound of claim 14, wherein the plant steroid is a sterol selected from the group consisting of a phytosterol and a phytostanol.

19. The compound of claim 18, wherein the sterol is selected from the group consisting of stigmasterol, brassicasterol, campestenol, and campesterol.

20. A compound comprising cholesterol and a drug, wherein the cholesterol is attached to the drug.

21. A method for lowering cholesterol levels, comprising administering an oxyphytostanol to a mammal in need thereof.

FIGURE 1

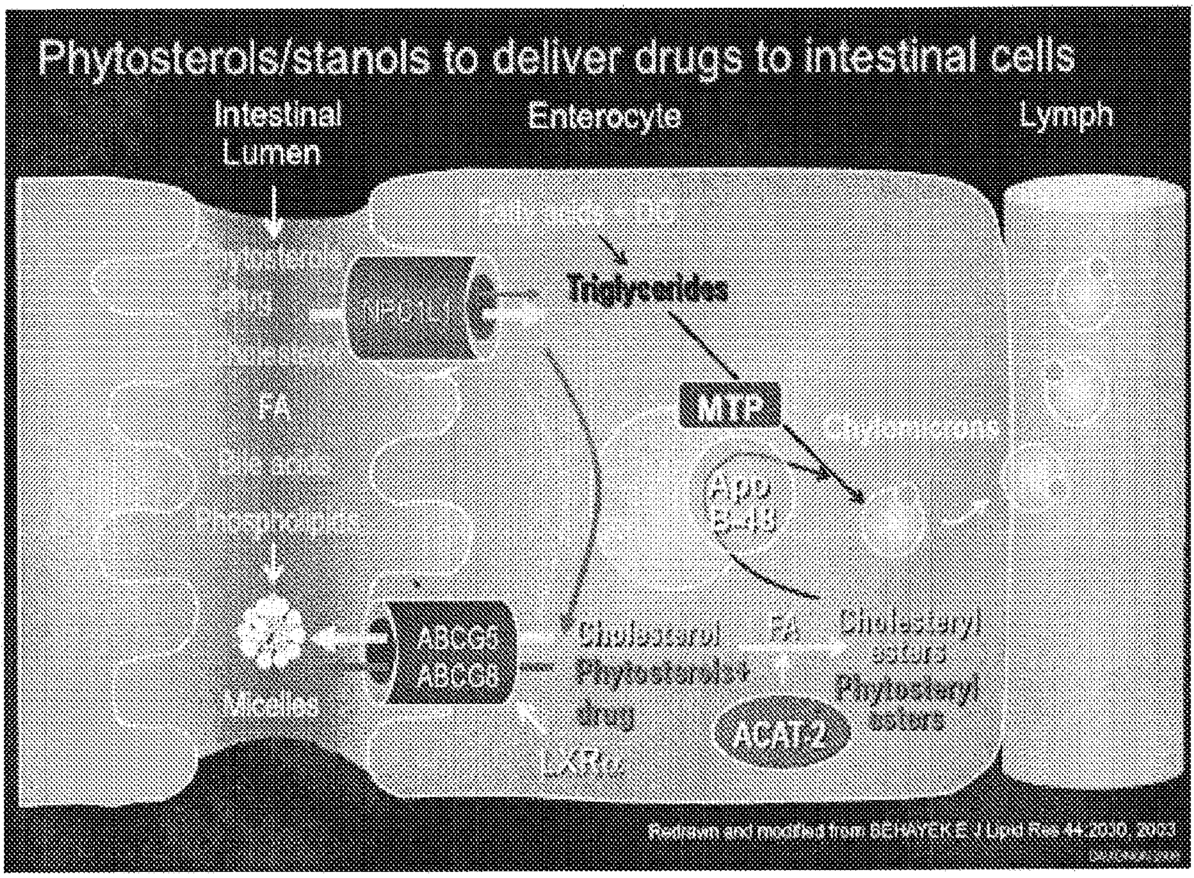


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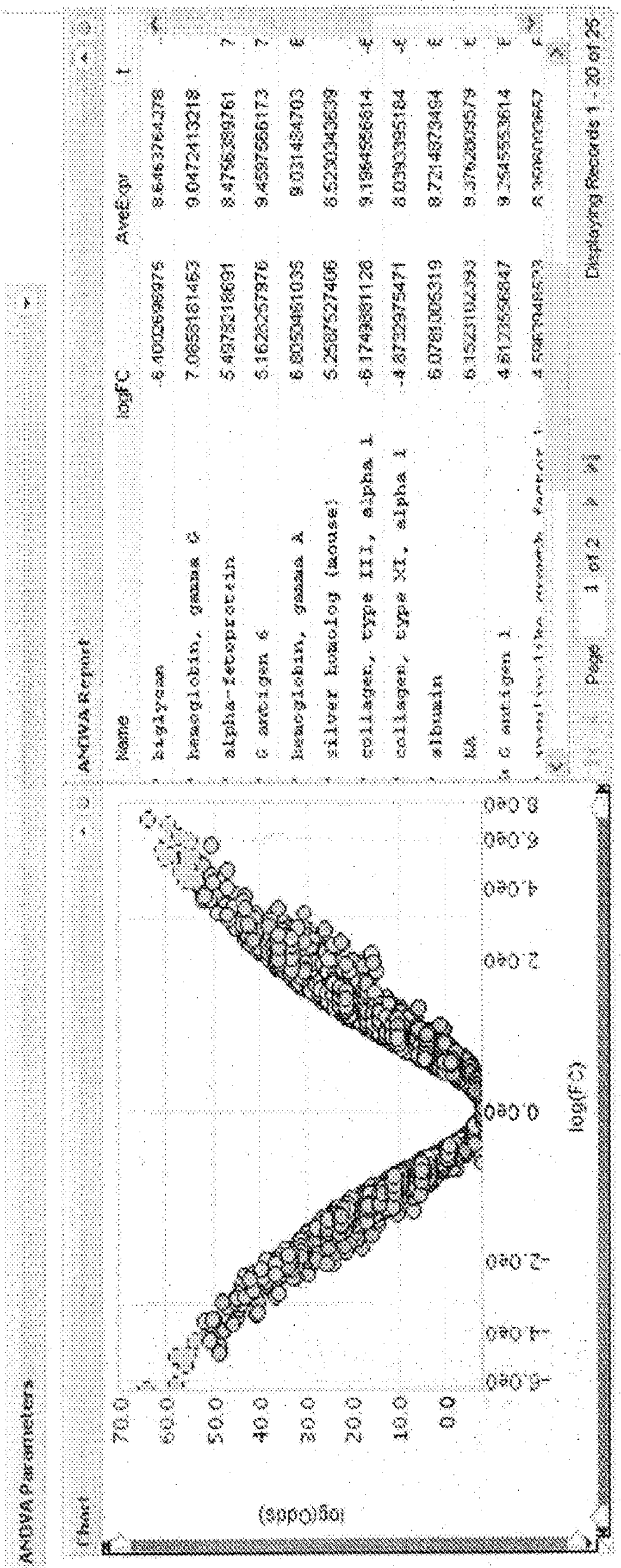
FIGURE 2



163397.3

FIGURE 3A

Genes affected from compound treatments (upregulation indicated in green, down regulation indicated in red)

	Treatments(Gene fold change for triplicate assays at 2.5 uM), n=3			
Regulated Genes	Prednisone	SE-22	SE-24	SE-41
Tubulin beta	4.2	3.1	2.75	1.2
Keratin 18	4.4	7.1	7.7	1.4
Low-density lipoprotein receptor	6.7	3.3	4.23	1.3
Insulin induced gene 1	10.3	14.4	13.3	1.5
Anterior gradient 2 homolog (Xenopus laevis)	3	6.2	1.2	1.97
Keratin 8	5.5	4.3	2.5	1.8
NAD(P) dependent steroid dehydrogenase-like	3.1	3.3	5.45	1.1
Tubulin, alpha, ubiquitous	4.5	4.7	3.38	2
Fatty acid synthase	6.9	5.51	2.9	1.3
Inhibitor of DNA binding 1	7.3	6.8	3.8	2.4
Alpha-2-HS-glycoprotein	4.2	4.4	6.4	1.1
Apolipoprotein D	8.9	7.4	6.8	2.2
Wnt interacting factor	7.6	4.5	4.5	1.3
hepatocyte nuclear factor-4 NR2A1	6.4	10.7	6.6	1.2
Zymogen granule protein 16	14	6.4	2.3	1.5
Gamma-aminobutyric acid (GABA) A receptor, alpha 2	11.7	4.5	5.5	3.39
Solute carrier family 36 member 1	15.3	3.5	4.3	3.5
Calbindin D9K	4.6	7.7	4.7	7.7
Estrogen -like receptor,glucocorticoid receptor	3.3	6	4.1	2.1
RAR related orphan receptor NR1F2	4.6	3.2	3.4	1.3
Soluble carrier family 1 member 1	3.1	3.2	3.71	1.6

FIGURE 3B

Alpha-fetoprotein	7.7	9.7	1.3	4.1
Apolipoprotein H	5.5	2.3	3.4	1.34
Fibrinogen, gamma polypeptide	4.1	4.3	3.5	4.3
Serine (or cysteine) proteinase inhibitor, clade A, member 6	6.9	5.2	3.6	3.1
Transthyretin	6.2	4.7	4.9	2.2
Vitronectin	4.4	6.6	4.2	2.3
Inter-alpha (globulin) inhibitor, H2 polypeptide	4.3	3.4	5.5	1.4
Retinol binding protein 4	4.2	5.2	8.8	5.2
Metallothionein 1F	4.1	3.8	4.5	3.8
Glypican 3	3.2	4.6	2.77	0
Ornithine decarboxylase 1	3.1	3.5	2.76	0
Heterogeneous nuclear ribonucleoprotein A3	3.5	3.4	2.4	2.17
Human hepatic dihydrodiol dehydrogenase	3.7	3.8	4.3	3.8
Lipopolysaccharide-induced TNF factor	3.1	2.4	5.97	4.4
Hypothetical protein MAC	3.1	2.1	1.4	3.2
Liver receptor homolog 1, NR5A2	3.4	2.5	3.8	3.1
Decay accelerating factor for complement (CD55, Cromer blood group system)	3.3	1.4	7.7	5.5
Vitamin D receptor NRI1	3.5	3.6	1.4	5.5
Dual specificity phosphatase 1	3.2	3.7	4.3	0
Phosphoenolpyruvate carboxykinase 1 (soluble)	2.9	4.7	2.4	0
Ras homolog gene family, member B	3.4	4.88	4.1	3.1
Apolipoprotein A-IV APOA4	3.3	3.4	3.9	0.97

FIGURE 3C

Dual specificity phosphatase 5	3.3	2.2	3.2	1.1
Carcinoembryonic antigen-related molecule 1 (biliary glycoprotein)	3.1	6.4	1.1	3.1
Claudin 4	5.3	0	1.5	1.5
Core promoter element binding protein	3.1	2.4	1.2	1.4
3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2 (mitochondrial)	8.7	4.9	2.2	4.9
Integrin, alpha 6	5.3	4.3	1.7	0
Transmembrane 4 superfamily member 1	5.6	4.5	3.4	0
Coagulation factor II (thrombin) receptor-like 1	7.3	4.5	1.3	0
Uroplakin 1B				
3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1 (soluble)	4.4	3.7	3.9	1.4
Insulin induced gene 1	4.7	3.2	1.2	1.32
Syndecan binding protein (syntenin)	4.4	3.7	2.6	1.7
Annexin A 13	3.7	4	4.7	2.3
Jun D proto-oncogene	3.5	7	1.7	2.4
Plasminogen activator, urokinase receptor	3.1	5	3.4	3.23
Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	3.1	6.3	4.1	2.25
Retinoic acid induced 3	4.1	3.2	4.9	1.2
Prostate differentiation factor	3.2	3.4	1.66	1.1
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	3.7	1.1	3.1	1.6

FIGURE 3D

Microsomal triglyceride transfer protein (large polypeptide, 88 kda)	7.2	5.6	5.9	1.1
Keratin 20	5.2	3.5	1.4	2.7
Thymosin, beta 10 31481_s_at TMSB10	3.7	3.9	2.4	4.4
Low-density lipoprotein receptor	1.4	5.7	3.1	3.3
Adipose differentiation-related protein	4.9	6.6	1.3	2.6
Apolipoprotein C-III	4.6	3.3	2.2	2.3
Ectodermal-neural cortex (with BTB-like domain)	3.8	1.4	4.3	1.1
Hepatic dihydrodiol dehydrogenase	3	2.6	4.6	2.5
Profilin 2	4.2	4.4	2.6	4.2
Tumor rejection antigen (gp96)	3	2.29	0.29	1.33
X-ray repair complementing defective repairin	3	3.3	4.3	0
Estrogen Like Receptor NR3C2	4.2	4.5	2.5	1.33
Casein kinase 2, beta polypeptide	3	1.34	1.44	2.75
Interleukin 13 receptor, alpha 2 (IL 13ra2)	5.1	4.3	1.88	4.3
Interleukin 7 receptor (IL 7r)	14.8	5.7	5.7	5.7
Chemokine (C-X-C motif) ligand 12	11.4	6.4	8	9.2
Transforming growth factor, beta receptor 1	9.3	5.6	8.5	4.56
(TGFbR1)	6.2	2.1	3.5	3.6
Chemokine (C-C motif) receptor 5 (CCR5)	6.4	5.1	2.1	1.6
Interleukin 2 receptor, alpha chain (IL2ra)	5	3.4	4.6	2.7
Chemokine (C-X-C motif) ligand 15 (CXCL	4.5	3.3	5.6	1.3

FIGURE 3E

15)				
Colony-stimulating factor 2 receptor, alpha, lowaffinity (granulocyte-macrophage) (CSF2ra)	4	2.05	1.1	2.3
Chemokine (C motif) XC receptor 1 (XCR1)				
Interleukin 11 receptor, alpha chain 1 (IL 11ra)	4.6	2.4	6.3	5.6
Transforming growth factor, beta 2 (TGFb2)	3.9	5.5	7.3	3.5
Chemokine (C-C motif) receptor 7 (CCR7)	3.4	2.3	3.3	1.11
Interleukin 12 receptor, beta 2 (IL 12rb2)	3.1	2.9	3.2	4.8
Chemokine (C-X-C motif) ligand 1 (CXCL 1)	3.4	8	7	1.2
Chemokine (C-C motif) receptor 1-like 1 (CCR1l1)	3.5	3.8	6.7	2.88
Chemokine (C-C motif) ligand 11 (CCL 11/eotaxin)	3.2	0	1.2	2.2
Chemokine (C-X-C motif) receptor 4 (CXCR4)	4.7	2.1	2.9	1.64
Low-density lipoprotein receptor	3.53	3.44	1.75	1.73
Adipose differentiation-related protein	4.5	5.7	7.9	11.5
Apolipoprotein C-III	4.9	4.3	2.12	1.1
Claudin 3	4.6	8.55	7.73	3.2
Cellular retinoic acid binding protein 2	3.6	0	3.3	1.66
Sequestosome 1	4.1	1.3	2.2	3.1
Liver X receptor like NR1H2	3.8	4.7	1.5	7.3
Aldehyde dehydrogenase 1	4.2	3.3	6.42	8.9

FIGURE 3F

family, member A1				
Heat shock 10 kda protein 1 (chaperonin 10)	3.7	6.5	4.22	1.18
Cyclin D1 (PRAD1: parathyroid adenomatosis 1)	3.2	1.43	3.3	3.7
Chromosome 7 open reading frame 24	4.1	1.2	2.66	4.4
Aldehyde dehydrogenase 3 family, member B1	3	5.6	9.86	5.6
Phosphatidylserine synthase 1	3	4	2.5	6.3
Activin A receptor, type IB	4.4	6	8.55	3.91
Chemokine (C-C motif) ligand 25 (CCL 25)	3.8	3.5	0	3.11
Tumor necrosis factor (TNF)	5.7	2.3	5.5	2.3
Interleukin 21 receptor (IL 21r)	3.5	8.9	5.5	6
Chemokine (C-C motif) receptor-like 2 (CCRl2)	6	6	2.22	1.28
Interleukin 10 receptor, beta (IL 10rb)	7	2.64	3.55	4.4
Interleukin 15 receptor, alpha chain (IL 15ra)	3.7	4.5	5.5	5.5
Interleukin 13 (IL 13)	4.5	8.3	4.6	9.4
Interleukin 1 receptor, type II (IL 1r2)	6	2.7	1.7	4.9
Interleukin 2 receptor, gamma chain (IL 2rg)	4	2.1	0	2.1
Interleukin 1 receptor antagonist (IL 1rn)	3.1	4.5	0	1.82
Interleukin 6 signal transducer (IL 6st)	3.1	9.5	0	9.5
Interleukin 9 receptor (IL 9r)	3.4	7.4	2.3	0.55
Transforming growth factor, beta receptor	4.1	2.7	4.9	3.44

FIGURE 3G

III (TGFbr 3)				
Interleukin 5 receptor, alpha (IL 5ra)	3.3	4.1	5.9	7
Colony-stimulating factor 3 receptor (granulocyte) (CSF3r)	0	0	1.33	0
Lymphotoxin A (LTA)	3.9	0	1.5	0
Colony-stimulating factor 2 receptor, beta 2,	3.2	0	5.5	5.5
low-affinity (granulocyte-macrophage) (CSF2rb2)	3.2	4.4	4.8	4.4
Chemokine (C-C motif) ligand 7 (CCL7)	1.9	3.4	0	3.05
Colony stimulating factor 2 (granulocytemacrophage) (CSF2)				
Chemokine (C-X-C motif) ligand 5 (CXCL 5)	4.4	2.3	1.2	5.4
Interleukin 1 receptor, type 1 (IL 1r1)	13	6.1	5.7	3.3
Chemokine (C-C motif) ligand 17 (CCL 17)	3.11	4.09	1.13	4.3
IL-13 receptor alpha chain (IL 13ra1)	3.8	4.34	2.5	1.07
Chemokine (C-C motif) ligand 1 (CCL 1)	3.1	4.25	4.5	1
Interleukin 18 receptor accessory protein (IL18rap)	3.1	8.22	3.8	1.88
Chemokine (C-C motif) ligand 9 (CCL 9)	2.1	6.59	5.14	3.48
Chemokine (C-C motif) ligand 12 (CCL 12)	3	4.5	4.5	13.44
Chemokine (C-C motif) ligand 8 (CCL 8)	3	7.32	1.74	4.79

FIGURE 3H

Interleukin 1 beta (IL 1b)	3	2.5	7.47	6.16

FIGURE 4

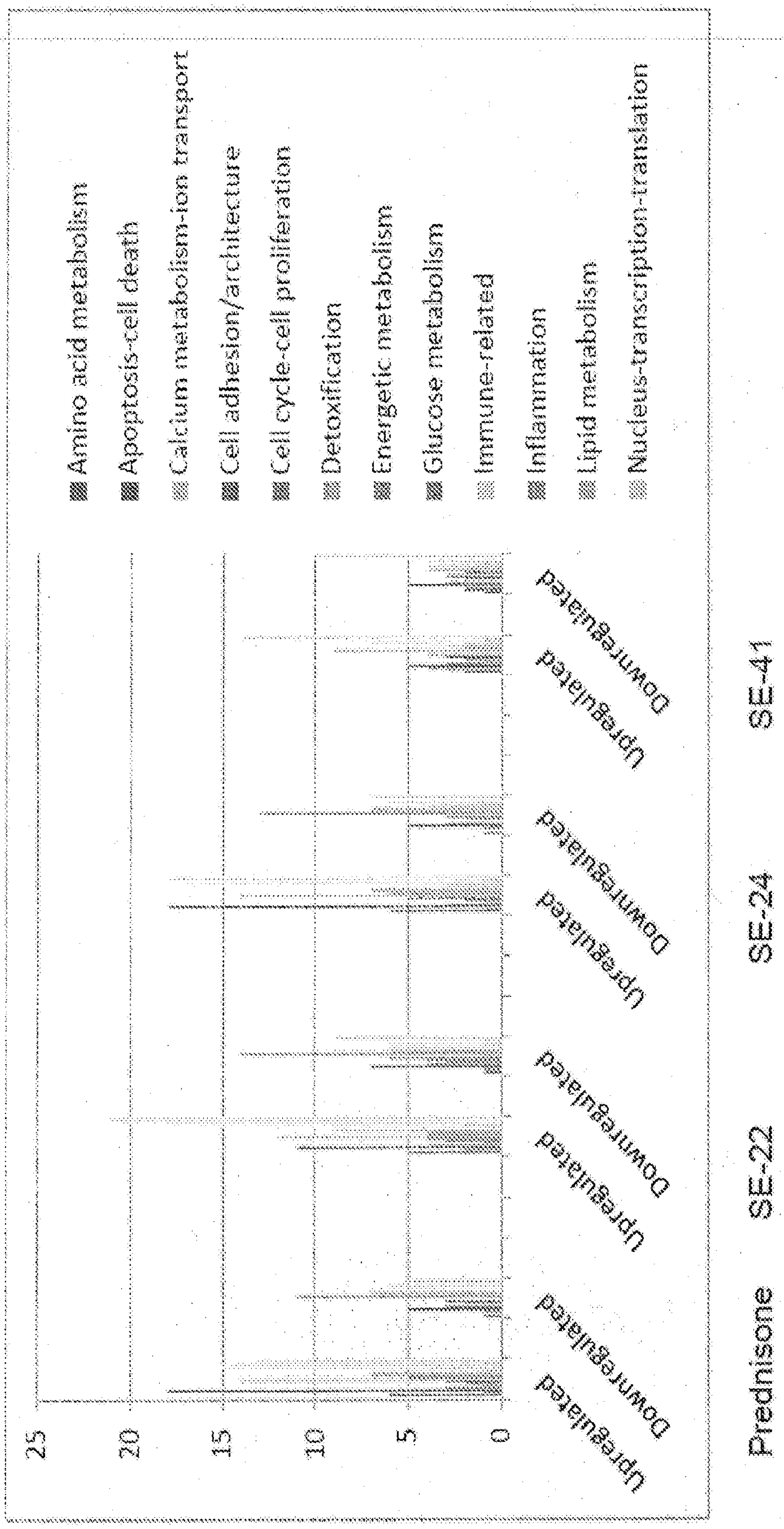


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FIGURE 5

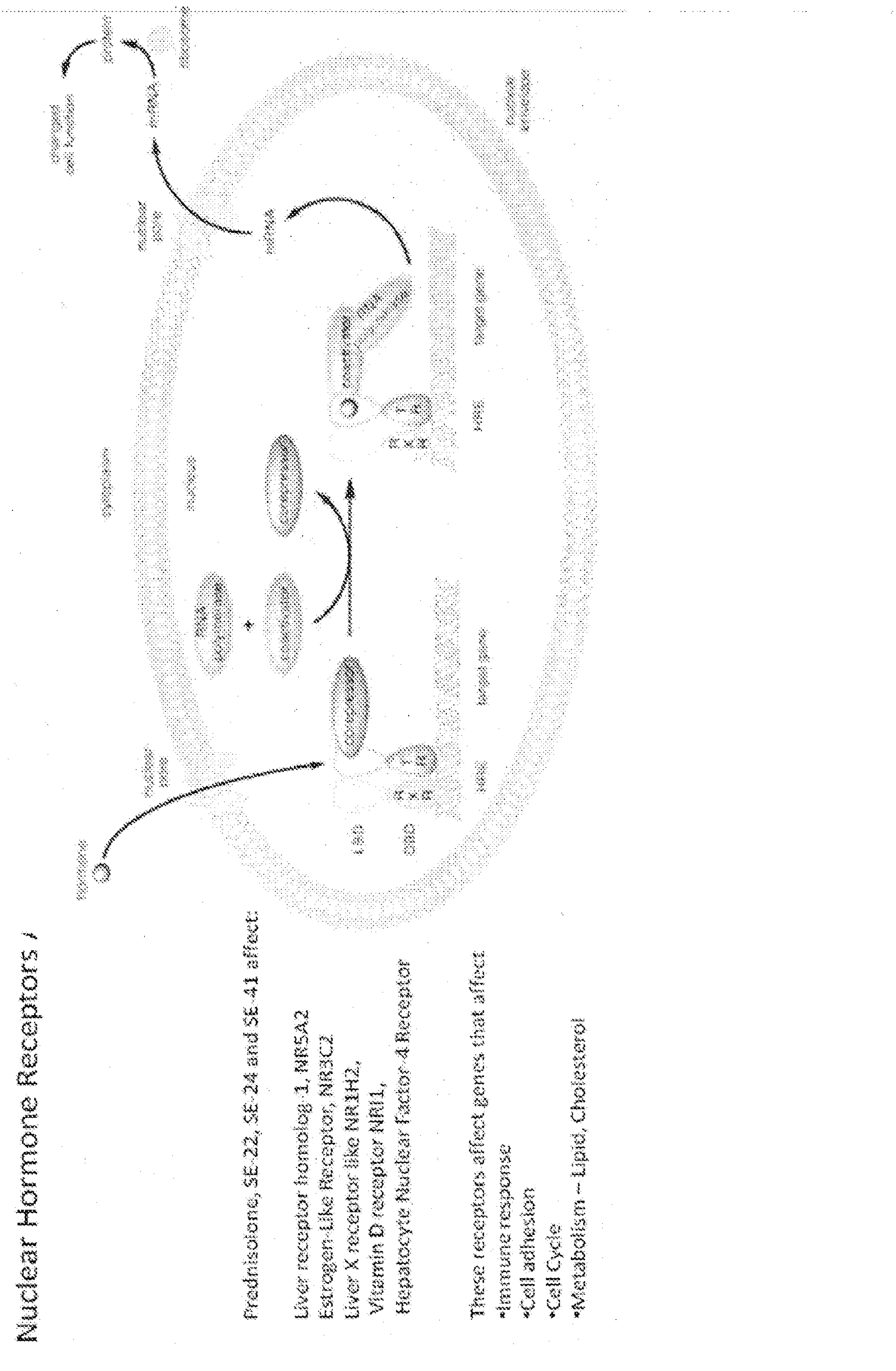
Nuclear Hormone Receptors /
Prednisolone, SE-22, SE-24 and SE-41 affect:
Liver receptor homolog-1, NR5A2
Estrogen-Like Receptor, NR3C2
Liver X receptor like NR1H2,
Vitamin D receptor NR1I1,
Hepatocyte Nuclear Factor-4 Receptor
These receptors affect genes that affect:
•Immune response
•Cell adhesion
•Cell Cycle
•Metabolism – Lipid, Cholesterol

FIGURE 6

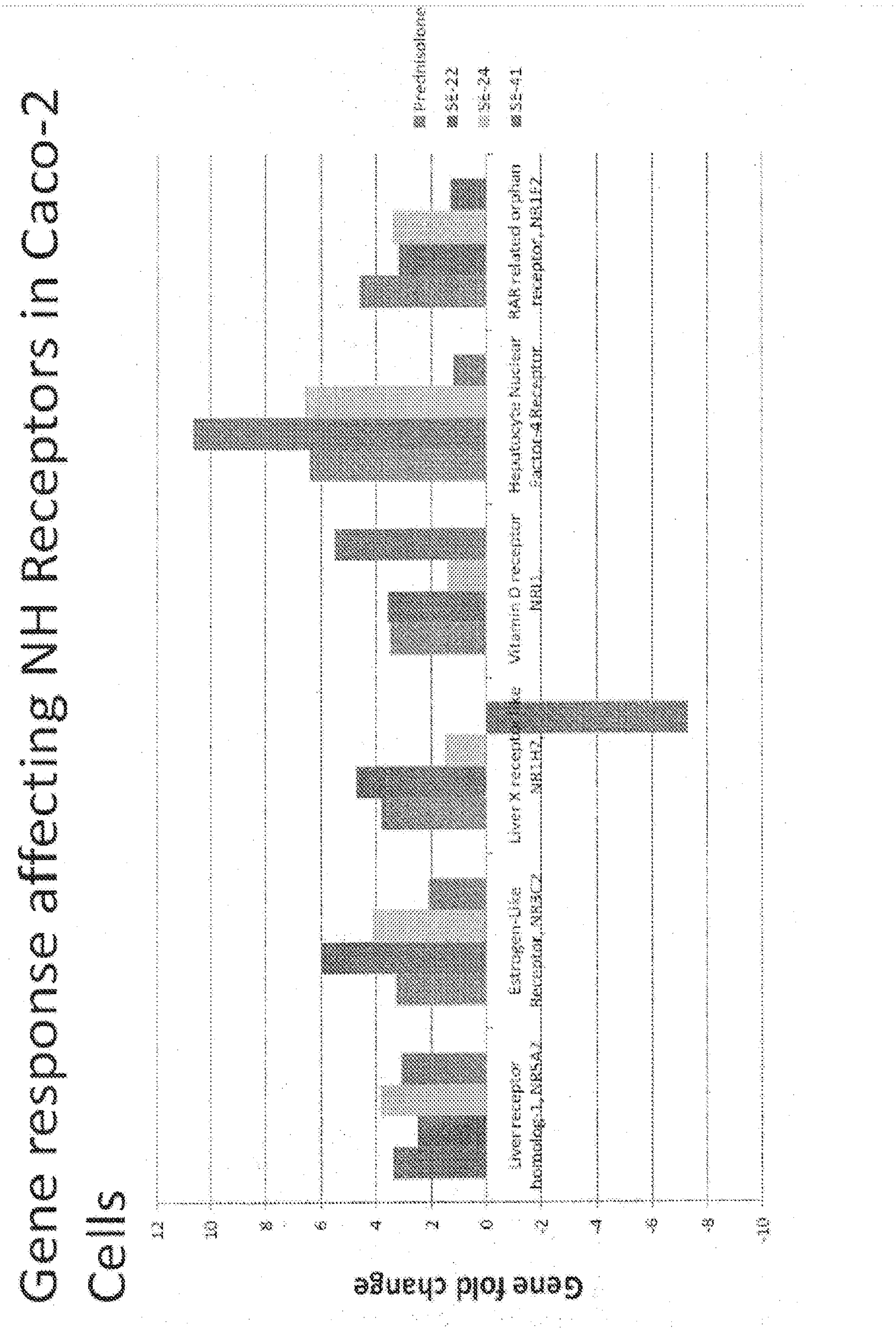
Gene response affecting NH Receptors in Caco-2 Cells
Gene fold change
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
Prednisolone
SE-22
SE-24
SE-41
Liver receptor homolog 1, NR5A2
Estrogen-Like Receptor, NR3C2
Vitamin D receptor
Hepatocyte Nuclear Factor 4 Receptor
RAR related orphan receptor

FIGURE 7

	Receptor	Subtype	Denomination	Ligand	Response Element	Monomer, Homodimer, or Heterodimer
Class I	TR	α, β	Thyroid hormone receptor	Thyroid hormone (T_3)	Pal, DR-4, IP	H
	RAR	α, β, γ	Retinoic acid receptor	Retinoic acid	DR-2, DR-5 Pal, IP	H
	VDR		Vitamin D receptor	$1,25(OH)_2$ vitamin D_3	DR-3, IP-9	H
	PPAR	α, β, γ	Peroxisome proliferator activated receptor	Benzotriene B4; Wy 14,643 Eicosanoids; thiazolidinediones (TZD_s); 15-deoxy-12,14-prostaglandin J_2; polyunsaturated fatty acids	DR-1	H
	PXR		Pregnane X receptor	Pregnanes: C21 steroids	DR-3	H
	CAR/MB67	α, β	Constitutive androstane receptor	Androstanes; 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene	DR-5	H
	LXR	α, β	Liver X receptor	Oxysterols	DR-4	H
	FXR		Farnesoid X receptor	Bile acids	DR-4, IR-1	H
	RevErb	α, β	Reverse ErbA	Unknown	DR-2, Hemisite	M, D
	RZR/ROR	α, β, γ	Retinoid Z receptor/retinoic acid-related orphan receptor	Unknown	Hemisite	M
	UR		Ubiquitous receptor	Unknown	DR-4	H
Class II	RXR	α, β, γ	Retinoid X receptor	9-Cis-retinoic acid	Pal, DR-1	D
	COUP-TF	α, β, γ	Chicken ovalbumin upstream promoter transcription factor	Unknown	Pal, DR-5	D, H
	HNF-4	α, β, γ	Hepatocyte nuclear factor 4	Fatty acyl-CoA thioesters	DR-1, DR-2	D
	TLX		Tailles-related receptor	Unknown	DR-1, Hemisite	M, D
	PNR		Photoreceptor-specific nuclear receptor	Unknown	DR-1, Hemisite	M, D
	TR2	α, β	Testis receptor	Unknown	DR-1 to DR5	D, H
Class III	GR		Glucocorticoid receptor	Glucocorticoids	Pal	D
	AR		Androgen receptor	Androgens	Pal	D
	PR		Progesterone receptor	Progestins	Pal	D
	ER	α, β	Estrogen receptor	Estradiol	Pal	D
	ERR	α, β, γ	Estrogen-related receptor	Unknown	Pal, Hemisite	M, D
Class IV	NGFI-B	α, β, γ	NGF-induced clone B	Unknown	Pal, DR-5	M, D, H
Class V	SF-1/FTZ-F1	α, β	Steroidogenic factor 1 Fushi Tarazu factor 1	Oxysterols	Hemisite	M
Class VI	GCNF		Germ cell nuclear factor	Unknown	DR-0	D
Class 0	SHP		Small heterodimeric partner	Unknown		H
	DAX-1		Dosage-sensitive sex reversal	Unknown		

M, monomer; D, homodimer; H, heterodimer; NGF, nerve growth factor; DR, direct repeat; Pal, palindrome; IP, inverted palindrome.

FIGURE 8

Nuclear Hormone Receptor

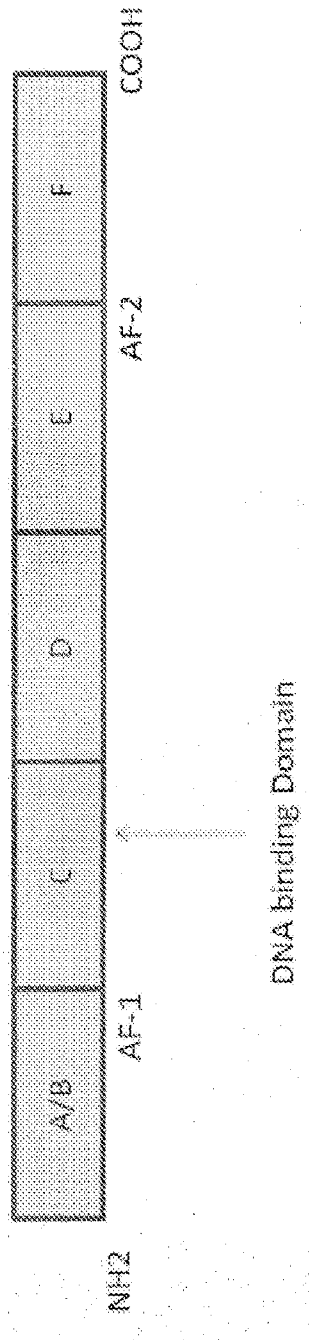

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FIGURE 9

A.

Name	Source	Target receptors	Target cells	Function
IL-1	macrophages, B cells, monocytes, dendritic cells	CD121a/IL1R1, CD121b/IL1R2	T helper cells	co-stimulation
			B cells	maturation & proliferation
			NK cells	activation
			macrophages, endothelium, other	inflammation, small amounts induce acute phase reaction, large amounts induce fever
IL-2	Th1-cells	CD25/IL2RA, CD122/IL2RB, CD132/IL2RG	activated T cells and B cells, NK cells, macrophages, oligodendrocytes	stimulates growth and differentiation of T cell response. Can be used in immunotherapy to treat cancer or suppressed for transplant patients. Has also been used in clinical trials (ESPRIT, Stalwart) to raise CD4 counts in HIV positive patients
IL-3	activated T helper cells, mast cells, NK cells, endothelium, eosinophils	CD123/IL3RA, CD131/IL3RB	hematopoietic stem cells	differentiation and proliferation of myeloid progenitor cells to e.g. erythrocytes, granulocytes
			mast cells	growth and histamine release
IL-4	Th2 cells, just activated naive CD4+ cell, memory CD4+ cells, mast cells, macrophages	CD124/IL4R, CD132/IL2RG	activated B cells	proliferation and differentiation, IgG1 and IgE synthesis. Important role in allergic response (IgE)
			T cells	proliferation
			endothelium	
IL-5	Th2 cells, mast cells, eosinophils	CD125/IL5RA, CD131/IL3RB	eosinophils	production
			B cells	differentiation, IgA production
IL-6	macrophages, Th2 cells, B cells, astrocytes, endothelium	CD126/IL6RA, CD130/IR6RB	activated B cells	differentiation into plasma cells
			plasma cells	antibody secretion
			hematopoietic stem cells	differentiation
			T cells, others	induces acute phase reaction, hematopoiesis, differentiation, inflammation
IL-7	Bone marrow stromal cells and thymus stromal cells	CD127/IL7RA, CD132/IL2RG	pre/pro-B cell, pre/pro-T cell, NK cells	differentiation and proliferation of lymphoid progenitor cells, involved in B, T, and NK cell survival, development, and homeostasis, ↑proinflammatory cytokines
IL-8 or	macrophages, lymphocytes, epithelial	CXCR1/IL8RA,	neutrophils, basophils,	...
IL-9	Th2 cells, specifically by CD4+ helper cells	CD129/IL9R	T cells, B cells	Potentiates IgM, IgG, IgE, stimulates mast cells
IL-10	monocytes, Th2 cells, CD8+ T cells, mast cells, macrophages, B cell subset	CD210/IL10RA, CDW210B/IL10RB	macrophages	cytokine production
			B cells	activation
			mast cells	
			Th1 cells	inhibits Th1 cytokine production (IFN-γ, TNF-β, IL-2)
			Th2 cells	Stimulation
IL-11	bone marrow stroma	IL11RA	bone marrow stroma	acute phase protein production, osteoclast formation
IL-12	dendritic cells, B cells, T cells, macrophages	CD212/IL12RB1, IR12RB2	activated T cells,	differentiation into Cytotoxic T cells with IL-2, ↑IFN-γ, TNF-α, ↓IL-10
			NK cells	↑IFN-γ, TNF-α
IL-13	activated Th2 cells, mast cells, NK cells	IL13R	TH2-cells, B cells, macrophages	Stimulates growth and differentiation of B cells (IgE), inhibits TH1-cells and the production of macrophage inflammatory cytokines (e.g. IL-1, IL-6), ↓IL-8, IL-10, IL-12
IL-14	T cells and certain malignant B cells		activated B cells	controls the growth and proliferation of B cells, inhibits Ig secretion
IL-15	mononuclear phagocytes (and some other cells), especially macrophages following infection by virus(es)	IL15RA	T cells, activated B cells	Induces production of Natural killer cells
IL-16	lymphocytes, epithelial cells, eosinophils, CD8+ T cells	CD4	CD4+ T cells (Th-cells)	CD4+ chemoattractant
IL-17	T helper 17 cells (Th17)	CDw217/IL17RA, IL17RB	epithelium, endothelium, other	osteoclastogenesis, angiogenesis, ↑inflammatory cytokines
IL-18	macrophages	CDw218a/IL18R1	Th1 cells, NK cells	Induces production of IFNγ, ↑NK cell activity
IL-19	-	IL20R		-
IL-20	-	IL20R		regulates proliferation and differentiation of keratinocytes
IL-21	activated T helper cells, NKT cells	IL21R	All lymphocytes, dendritic cells	costimulates activation and proliferation of CD8+ T cells, augment NK cytotoxicity, augments CD40-driven B cell proliferation, differentiation and isotype switching, promotes differentiation of Th17 cells
IL-22	-	IL22R		Activates STAT1 and STAT3 and increases production of acute phase proteins such as serum amyloid A, Alpha 1-antichymotrypsin and haptoglobin in hepatoma cell lines

FIGURE 9

B.

IL-22	-	IL22R		Activates STAT1 and STAT3 and increases production of acute phase proteins such as serum amyloid A, Alpha 1-antichymotrypsin and haptoglobin in hepatoma cell lines
IL-23	-	IL23R		Increases angiogenesis but reduces CD8 T-cell infiltration
IL-24	-	IL20R		Plays important roles in tumor suppression, wound healing and psoriasis by influencing cell survival
IL-25	-	LY6E		Induces the production IL-4, IL-5 and IL-13, which stimulate eosinophil expansion
IL-26	-	IL20R1		Enhances secretion of IL-10 and IL-8 and cell surface expression of CD54 on epithelial cells
IL-27	-	IL27RA		Regulates the activity of B lymphocytes and T lymphocytes
IL-28	-	IL28R		Plays a role in immune defense against viruses
IL-29	-			Plays a role in host defenses against microbes
IL-30	-			Forms one chain of IL-27
IL-31	-	IL31RA		May play a role in inflammation of the skin
IL-32	-			Induces monocytes and macrophages to secrete TNF-α, IL-8 and CXCL2
IL-33	-			Induces helper T cells to produce type 2 cytokine
IL-35	regulatory T cells			Suppression of T helper cell activation

FIGURE 10

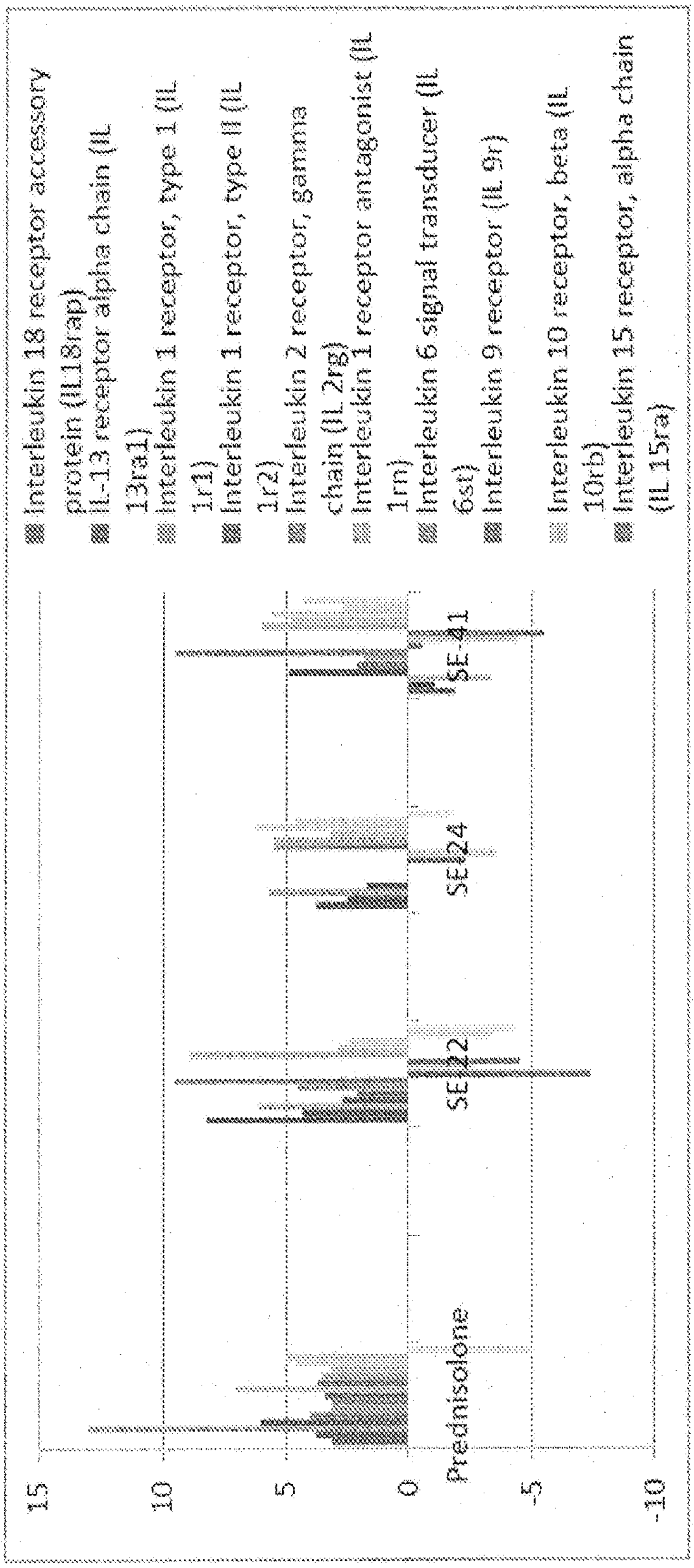
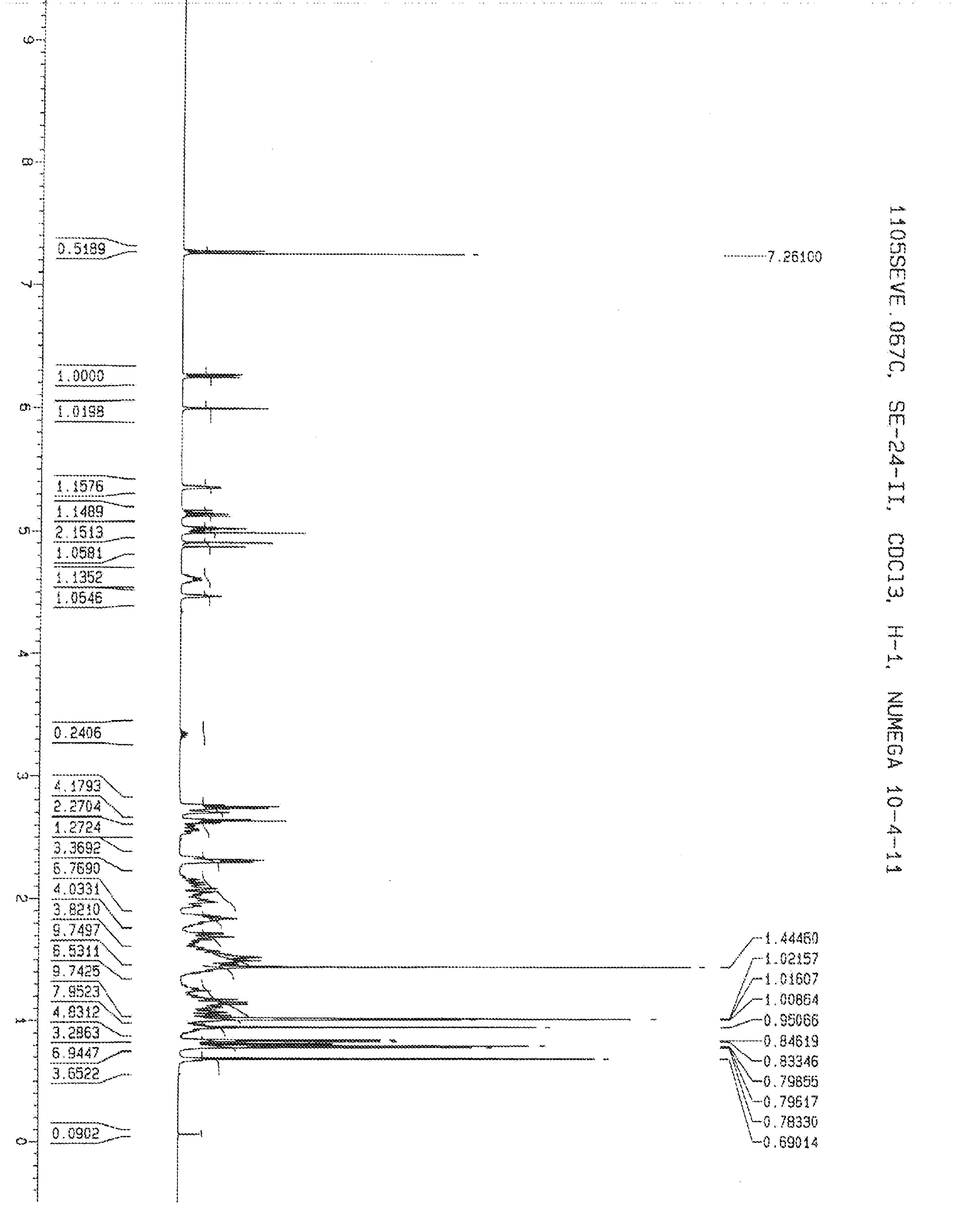

15
10
5
0
-5
-10
Prednisolone
SE-22
SE-24
SE-41
Interleukin 18 receptor accessory protein (IL18rap)
IL-13 receptor alpha chain (IL 13ra1)
Interleukin 1 receptor, type 1 (IL 1r1)
Interleukin 1 receptor, type II (IL 1r2)
Interleukin 2 receptor, gamma chain (IL 2rg)
Interleukin 1 receptor antagonist (IL 1rn)
Interleukin 6 signal transducer (IL 6st)
Interleukin 9 receptor (IL 9r)
Interleukin 10 receptor, beta (IL 10rb)
Interleukin 15 receptor, alpha chain (IL 15ra)

FIGURE 11

A


1105SEVE.067C, SE-24-II, CDCl3, H-1, NUMEGA 10-4-11
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1.01607
1.00864
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0.83346
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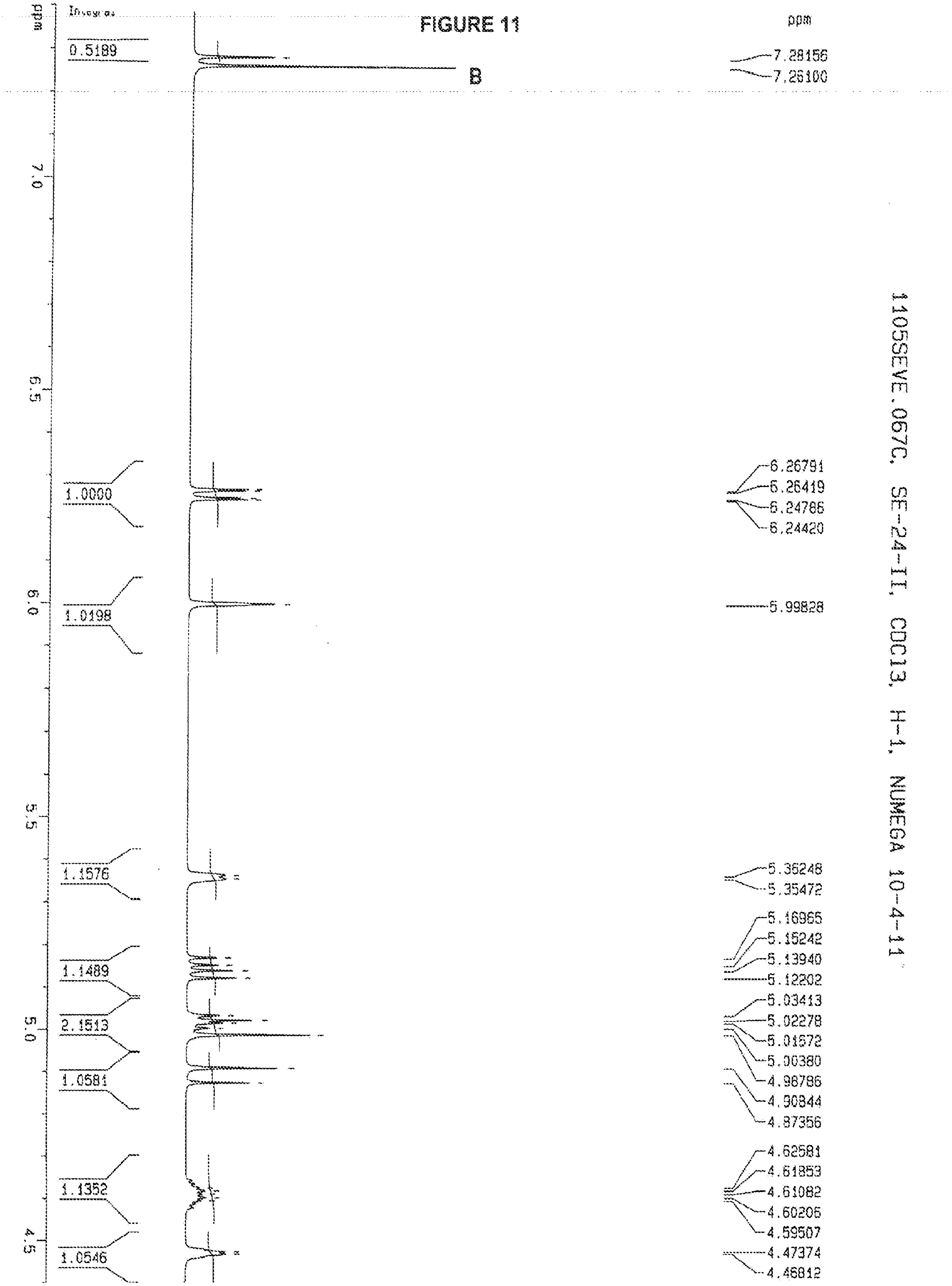
0.79617
0.78330
0.69014
0.5189
1.0000
1.0198
1.1576
1.1489
2.1513
1.0581
1.1352
1.0546
0.2406
4.1793
2.2704
1.2724
3.3692
6.7690
4.0331
3.8210
9.7497
6.5311
9.7425
7.9523
4.8312
3.2863
6.9447
3.6522
0.0902

FIGURE 11


B
1105SEVE.067C, SE-24-II, CDCl3, H-1, NUMEGA 10-4-11
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7.26100
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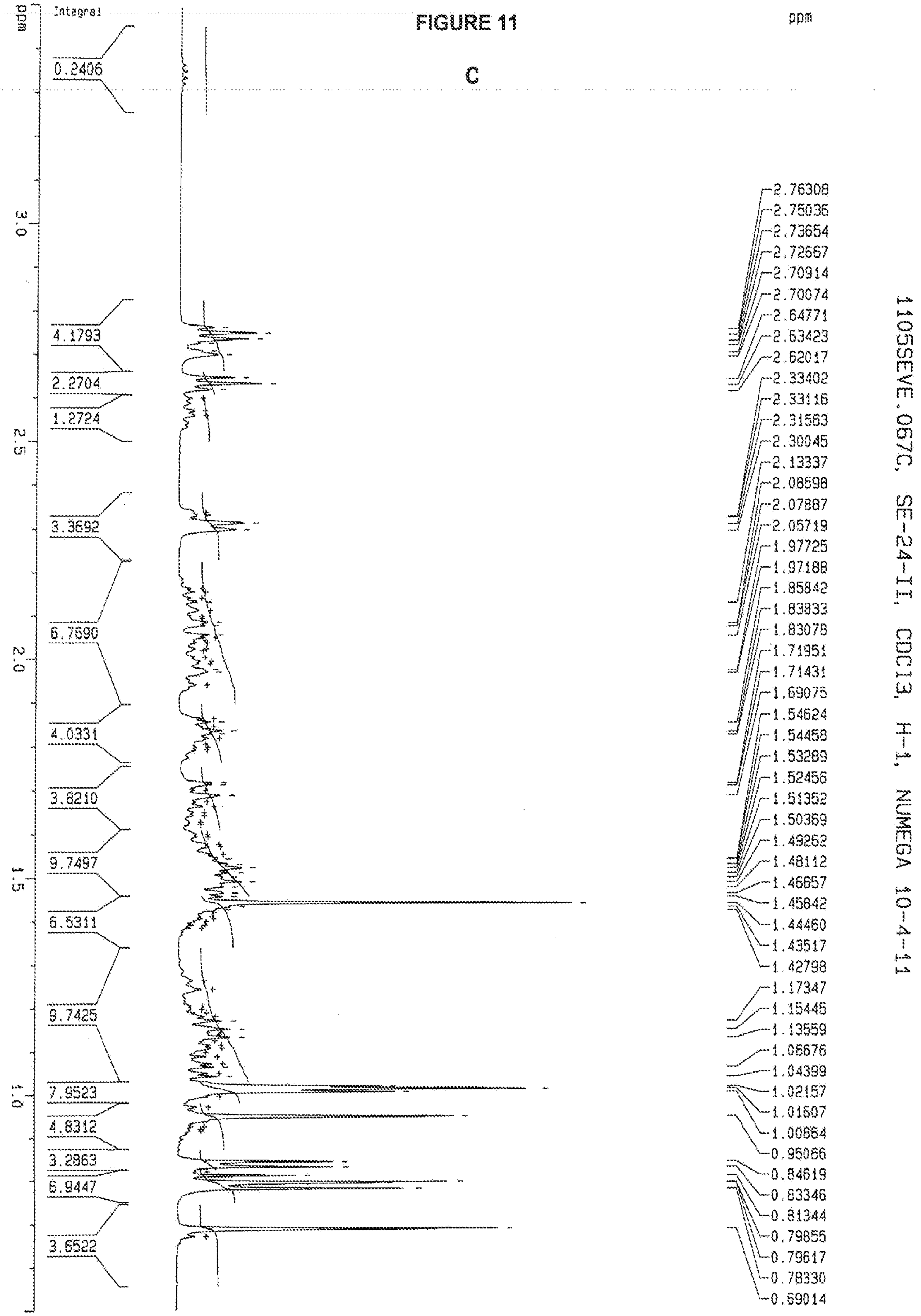
6.26419
6.24786
6.24420
5.99828
5.36248
5.35472
5.16965
5.15242
5.13940
5.12202
5.03413
5.02278
5.01672
5.00380
4.98786
4.90844
4.87356
4.62581
4.61853
4.61082
4.60205
4.59507
4.47374
4.46812
0.5189
1.0000
1.0198
1.1576
1.1489
2.1513
1.0581
1.1352
1.0546
7.0
6.5
6.0
5.5
5.0
4.5

FIGURE 11

C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9.7425
7.9523
4.8312
3.2863
6.9447
3.6522
3.0
2.5
2.0
1.5
1.0
ppm
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2.63423
2.62017
2.33402
2.33116
2.31563
2.30045
2.13337
2.08598
2.07887
2.05719
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1.97188
1.85842
1.83833
1.83078
1.71951
1.71431
1.69075
1.54624
1.54458
1.53289
1.52456
1.51352
1.50369
1.49252
1.48112
1.46657
1.45842
1.44460
1.43517
1.42798
1.17347
1.15445
1.13559
1.06676
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1.02157
1.01607
1.00664
0.95066
0.84619
0.83346
0.81344
0.79855
0.79617
0.78330
0.69014
1105SEVE.067C, SE-24-II, CDCl3, H-1, NUMEGA 10-4-11

FIGURE 11

D

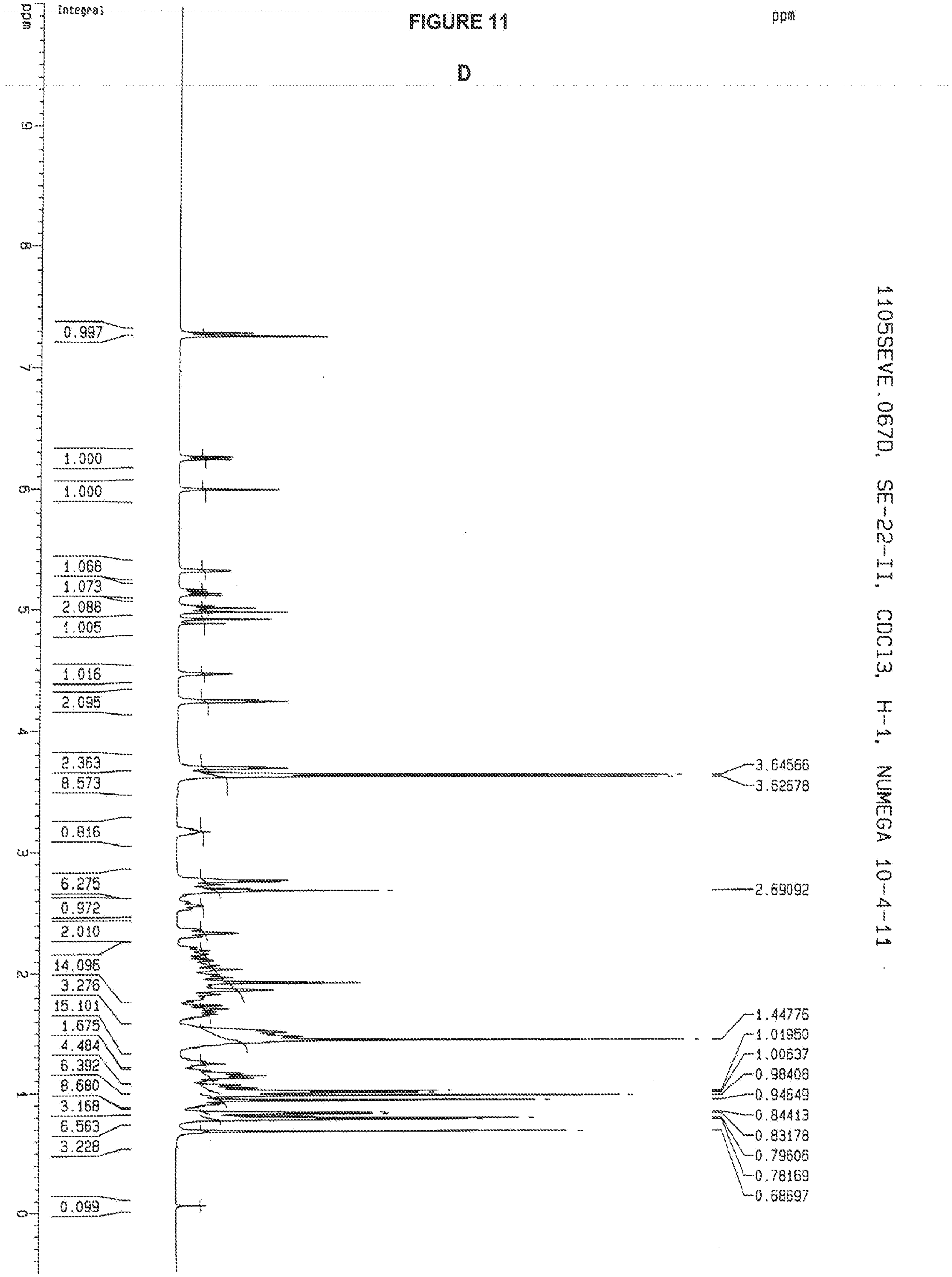

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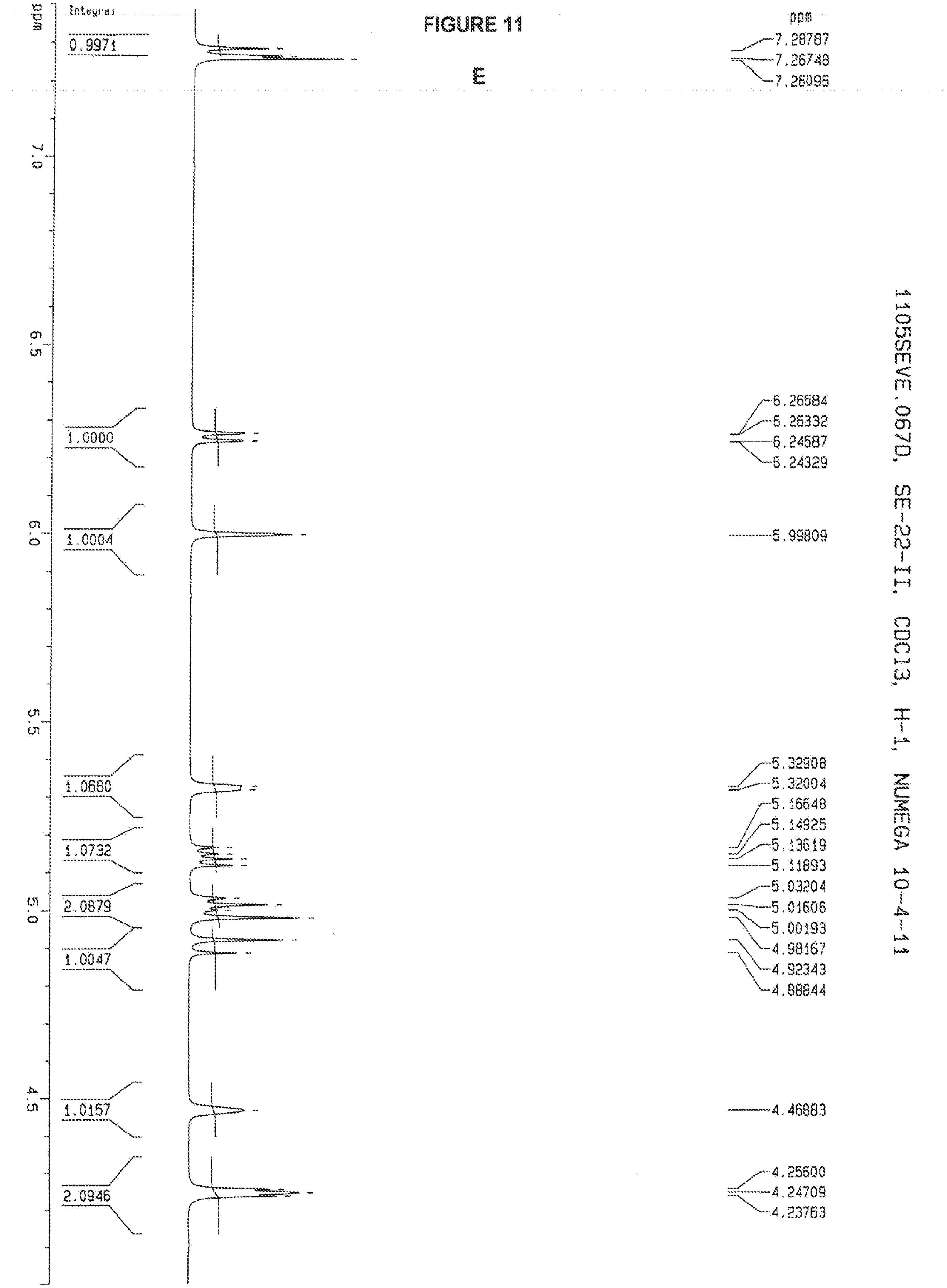
FIGURE 11

E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7.26098
0.9971
7.0
6.5
1.0000
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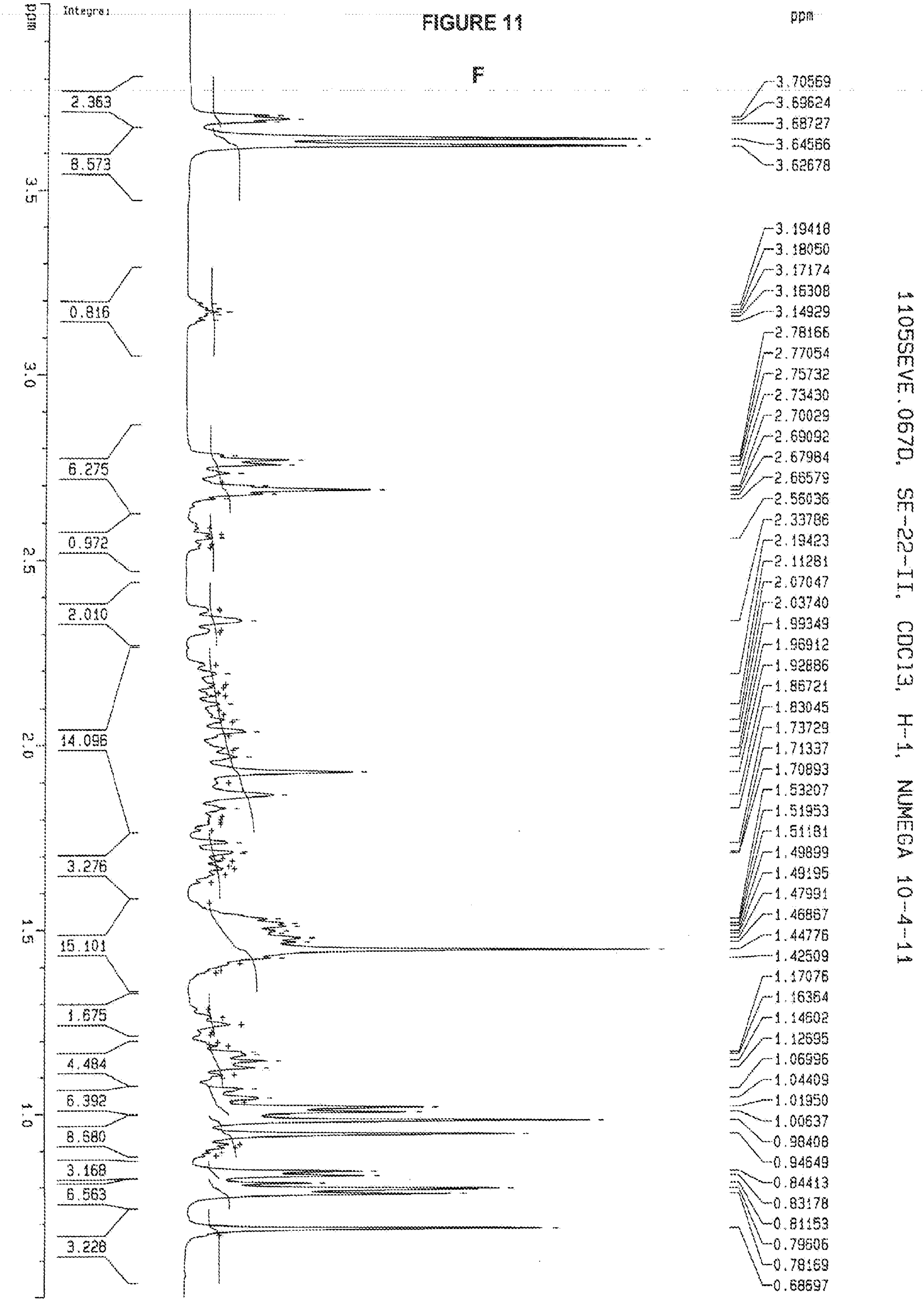
6.26332
6.24587
6.24329
6.0
1.0004
5.99809
5.5
1.0680
1.0732
2.0879
1.0047
5.32908
5.32004
5.16648
5.14925
5.13619
5.11893
5.03204
5.01606
5.00193
4.98167
4.92343
4.88644
5.0
4.5
1.0157
4.46883
2.0946
4.25600
4.24709
4.23763
1105SEVE.067D, SE-22-II, CDCl3, H-1, NUMEGA 10-4-11

FIGURE 11

F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6.275
0.972
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14.096
3.276
15.101
1.675
4.484
6.392
8.680
3.168
6.563
3.228
3.5
3.0
2.5
2.0
1.5
1.0
3.70569
3.69624
3.68727
3.64566
3.62678
3.19418
3.18050
3.17174
3.16308
3.14929
2.78166
2.77054
2.75732
2.73430
2.70029
2.69092
2.67984
2.66579
2.56036
2.33786
2.19423
2.11281
2.07047
2.03740
1.99349
1.96912
1.92886
1.86721
1.83045
1.73729
1.71337
1.70893
1.53207
1.51953
1.51181
1.49899
1.49195
1.47991
1.46867
1.44776
1.42509
1.17076
1.16364
1.14602
1.12695
1.06996
1.04409
1.01950
1.00637
0.98408
0.94649
0.84413
0.83178
0.81153
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0.78169
0.68697
1105SEVE.067D, SE-22-II, CDCl3, H-1, NUMEGA 10-4-11

FIGURE 11

G

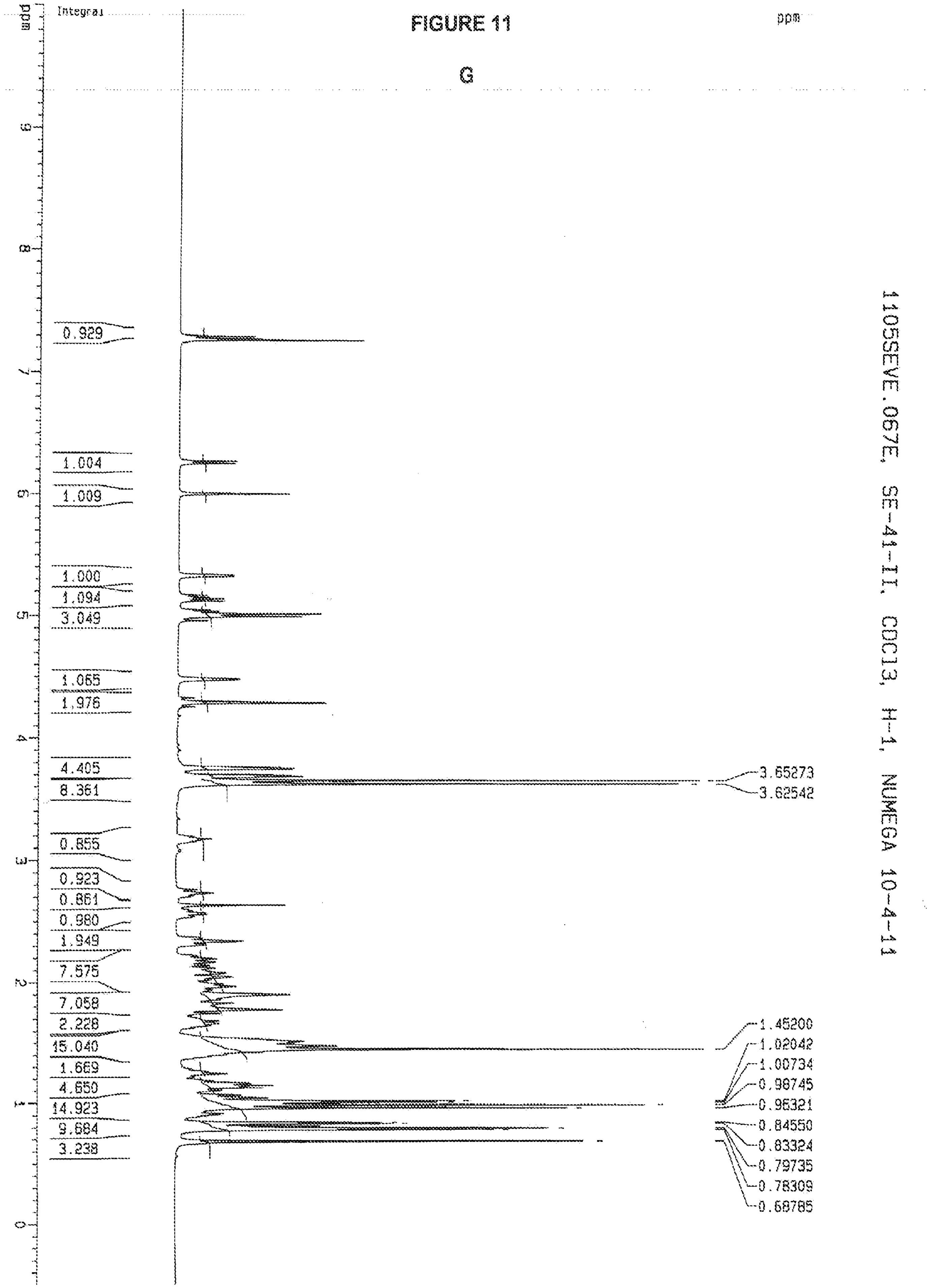
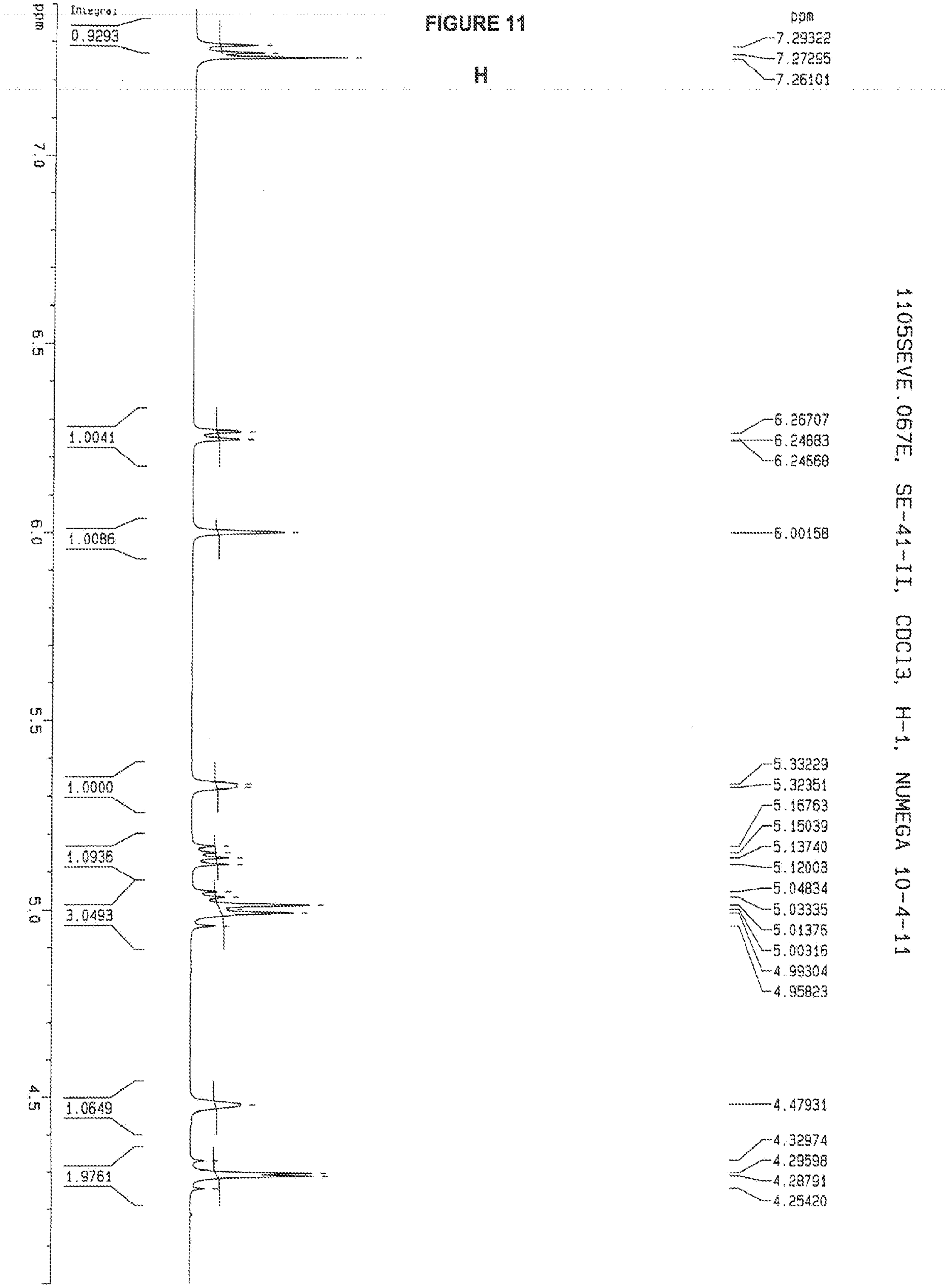


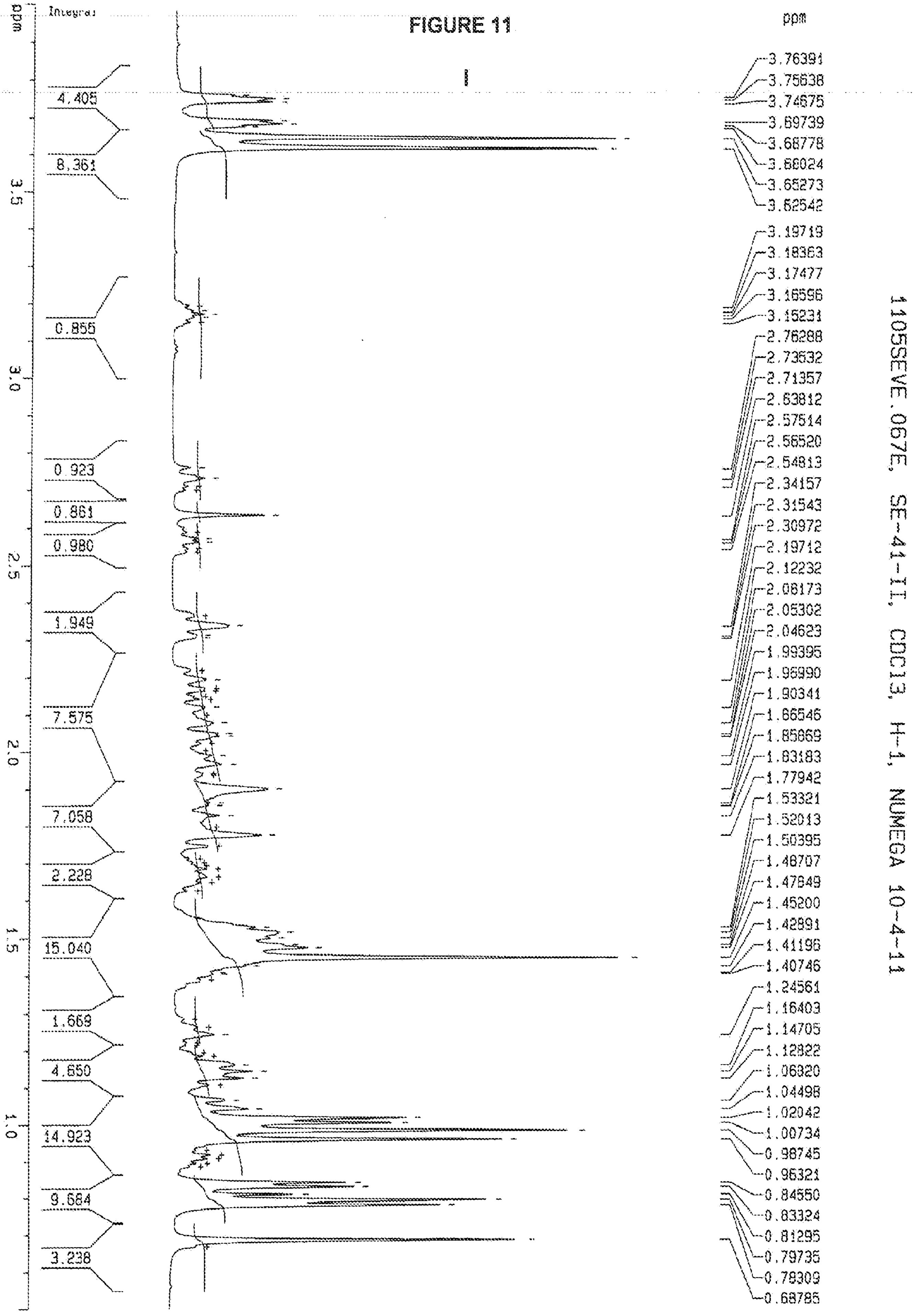
FIGURE 11

H



SUBSTITUTE SHEET (RULE 26)

FIGURE 11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Integral
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0.855
0.923
0.861
0.980
1.949
7.575
7.058
2.228
15.040
1.669
4.650
14.923
9.684
3.238
3.5
3.0
2.5
2.0
1.5
1.0
3.76391
3.75638
3.74675
3.69739
3.68778
3.68024
3.65273
3.62542
3.19719
3.18363
3.17477
3.16596
3.15231
2.76288
2.73632
2.71357
2.63812
2.57514
2.56520
2.54813
2.34157
2.31543
2.30972
2.19712
2.12232
2.08173
2.05302
2.04623
1.99395
1.96990
1.90341
1.86546
1.85669
1.83183
1.77942
1.53321
1.52013
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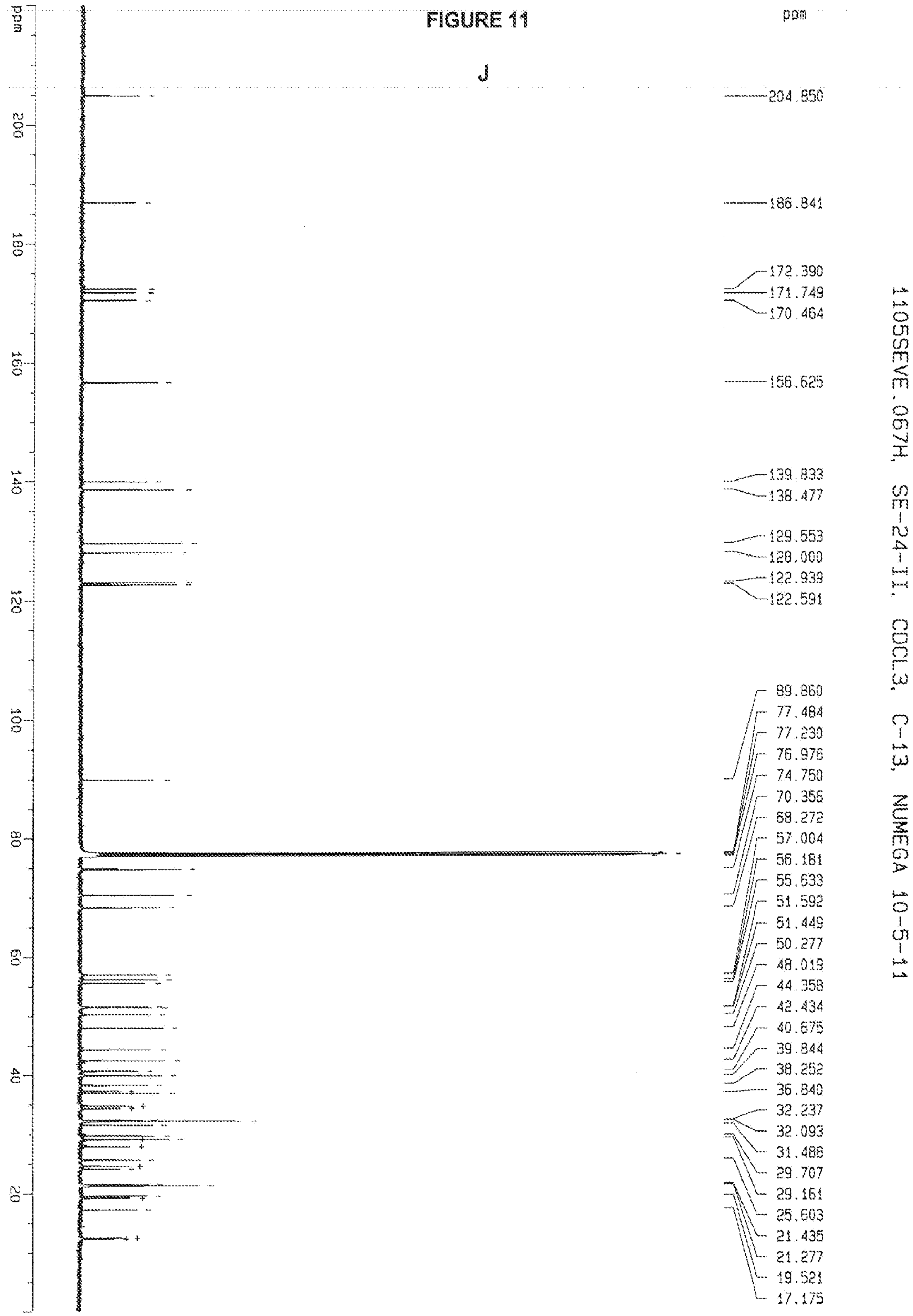
1.47849
1.45200
1.42891
1.41196
1.40746
1.24561
1.16403
1.14705
1.12822
1.06320
1.04498
1.02042
1.00734
0.98745
0.96321
0.84550
0.83324
0.81295
0.79735
0.78309
0.68785
1105SEVE.067E, SE-41-II, CDCl3, H-1, NUMEGA 10-4-11

FIGURE 11

J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140
120
100
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139.833
138.477
129.553
128.000
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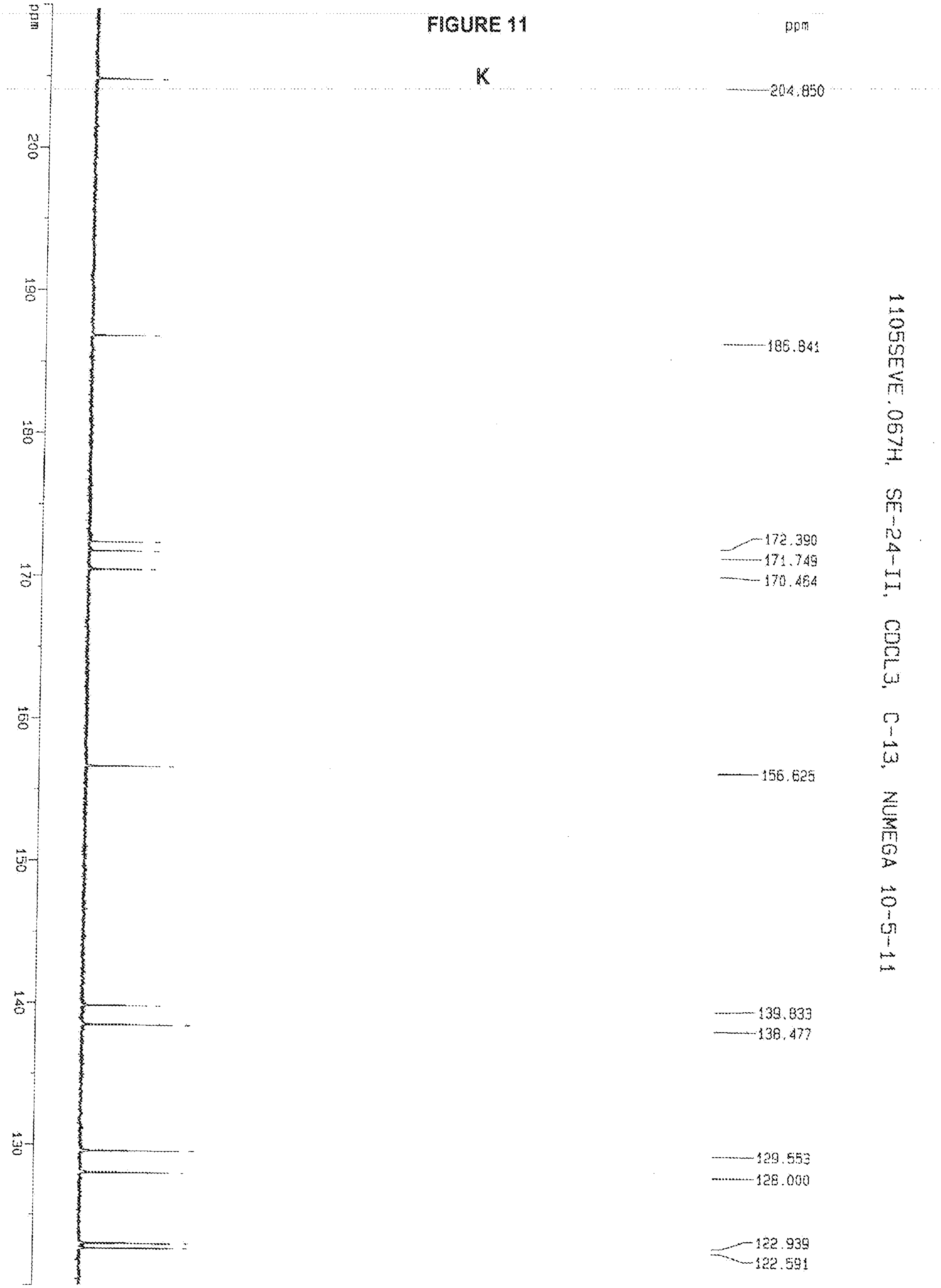
89.860
77.484
77.230
76.976
74.750
70.356
68.272
57.004
56.181
55.633
51.592
51.449
50.277
48.019
44.358
42.434
40.675
39.844
38.252
36.840
32.237
32.093
31.488
29.707
29.161
25.603
21.435
21.277
19.521
17.175
1105SEVE.067H, SE-24-II, CDCL3, C-13, NUMEGA 10-5-11

FIGURE 11

K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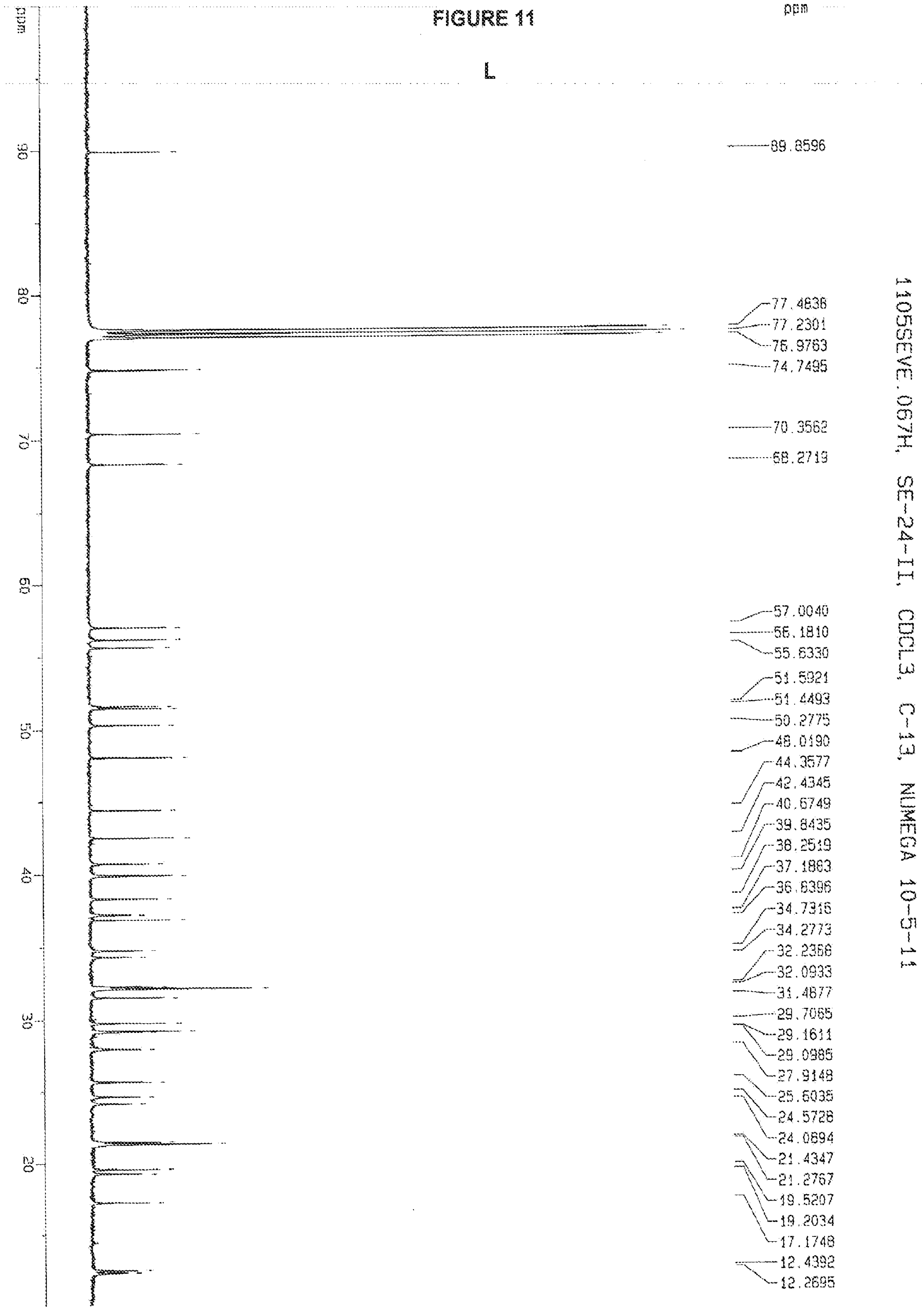
138.477
129.553
128.000
122.939
122.591
200
190
180
170
160
150
140
130
1105SEVE.057H, SE-24-II, CDCL3, C-13, NUMEGA 10-5-11

FIGURE 11

L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80
70
60
50
40
30
20
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77.4838
77.2301
76.9763
74.7495
70.3562
68.2719
57.0040
56.1810
55.6330
51.5921
51.4493
50.2775
48.0190
44.3577
42.4345
40.6749
39.8435
38.2519
37.1863
36.6396
34.7316
34.2773
32.2368
32.0933
31.4677
29.7065
29.1611
29.0985
27.9148
25.6035
24.5728
24.0894
21.4347
21.2767
19.5207
19.2034
17.1748
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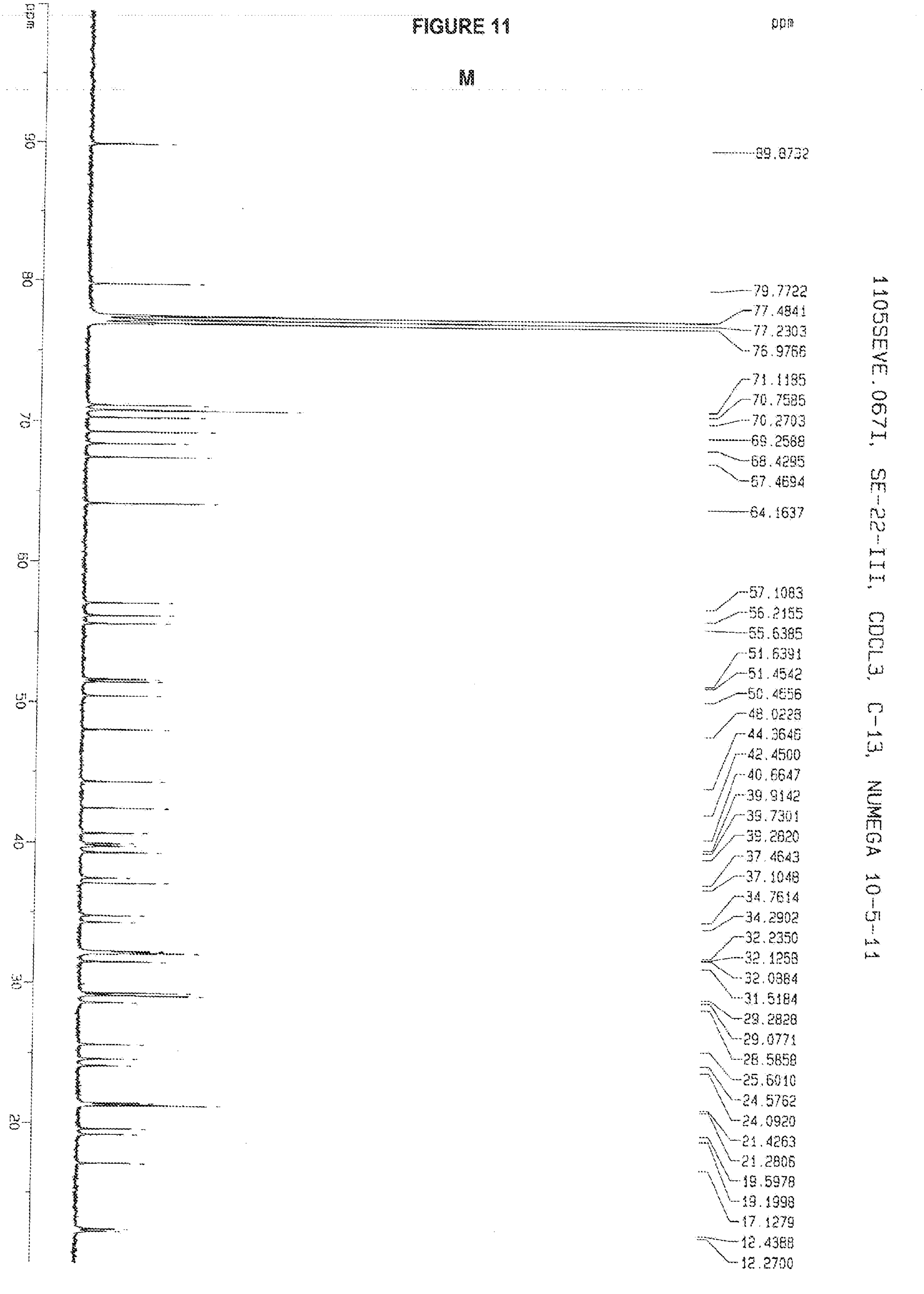
12.2695
1105SEVE.067H, SE-24-II, CDCL3, C-13, NUMEGA 10-5-11

FIGURE 11

M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67.4694
64.1637
57.1083
56.2155
55.6385
51.6391
51.4542
50.4656
48.0228
44.3646
42.4500
40.6647
39.9142
39.7301
39.2820
37.4643
37.1048
34.7614
34.2902
32.2350
32.1258
32.0384
31.5184
29.2828
29.0771
28.5858
25.6010
24.5762
24.0920
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21.2806
19.5978
19.1998
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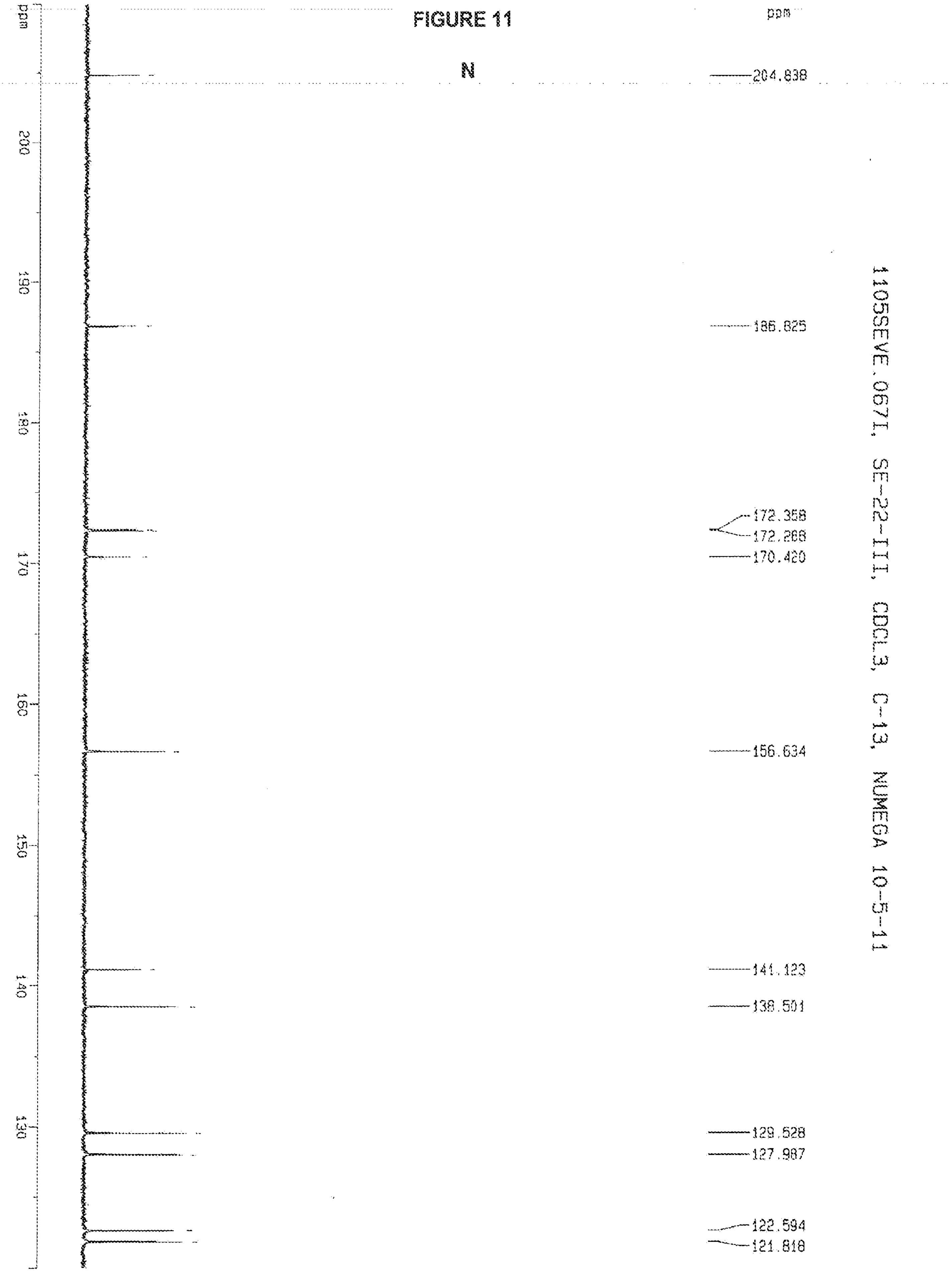
12.2700
1105SEVE.067I, SE-22-III, CDCL3, C-13, NUMEGA 10-5-11

FIGURE 11

N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138.501
129.528
127.987
122.594
121.818
200
190
180
170
160
150
140
130
1105SEVE.067I, SE-22-III, CDCL3, C-13, NUMEGA 10-5-11

FIGURE 11

O

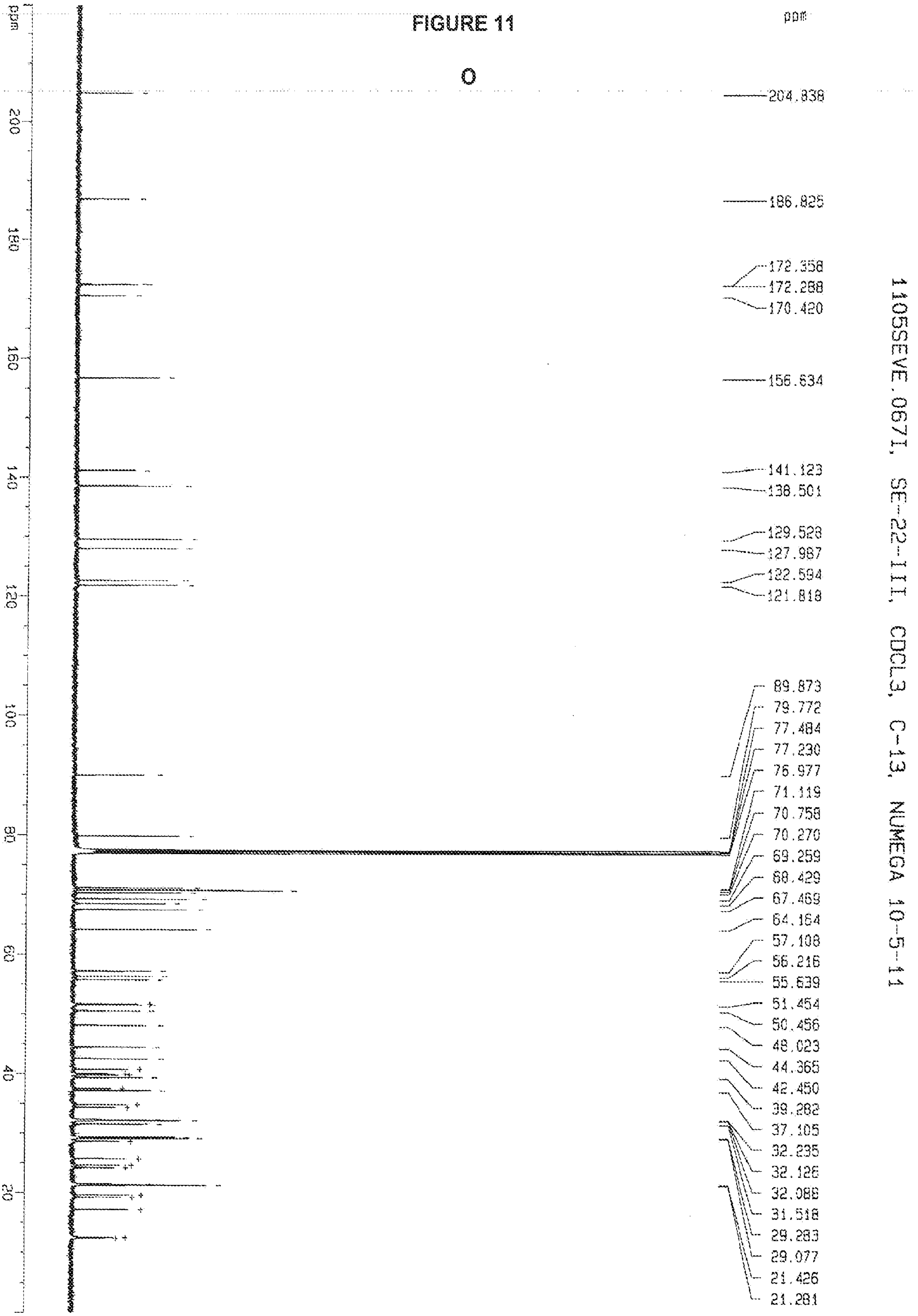

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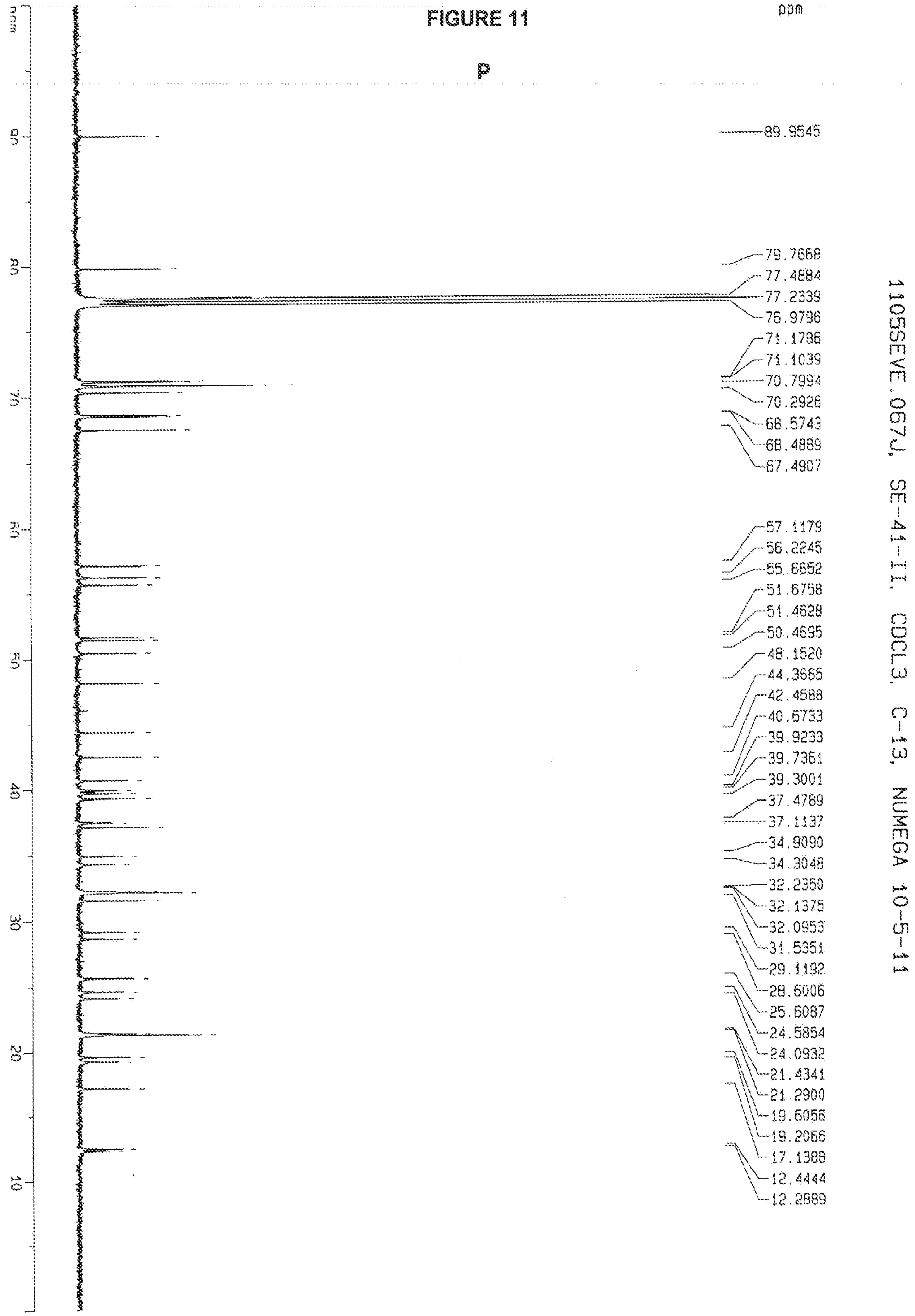
FIGURE 11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70.7994
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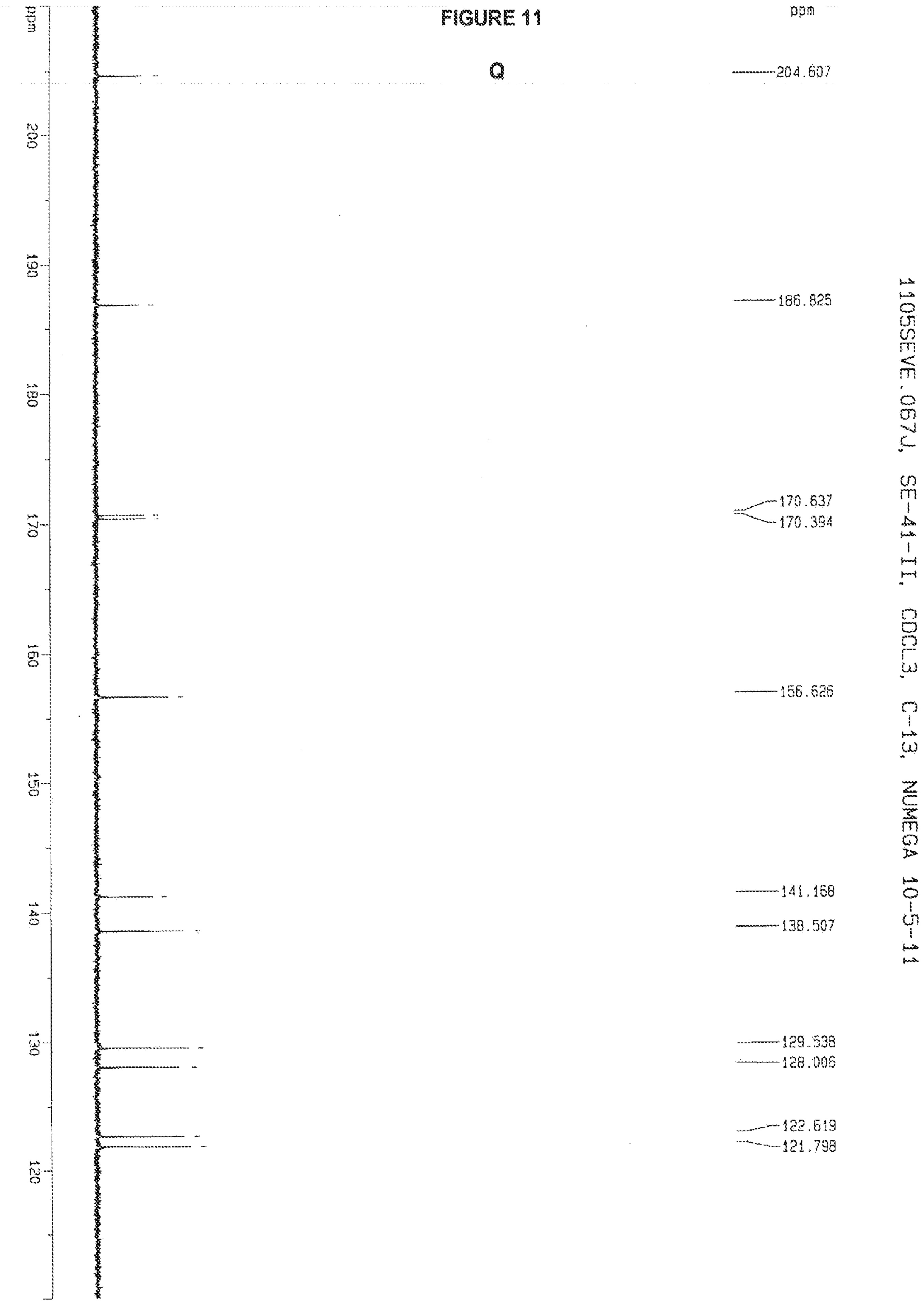
68.5743
68.4889
67.4907
57.1179
56.2245
55.6652
51.6758
51.4628
50.4695
48.1520
44.3665
42.4588
40.6733
39.9233
39.7361
39.3001
37.4789
37.1137
34.9090
34.3048
32.2350
32.1375
32.0953
31.5351
29.1192
28.6006
25.6087
24.5854
24.0932
21.4341
21.2900
19.6056
19.2066
17.1388
12.4444
12.2889
1105SEVE.067J, SE-41-II, CDCL3, C-13, NUMEGA 10-5-11
90
80
70
60
50
40
30
20
10

FIGURE 11

Q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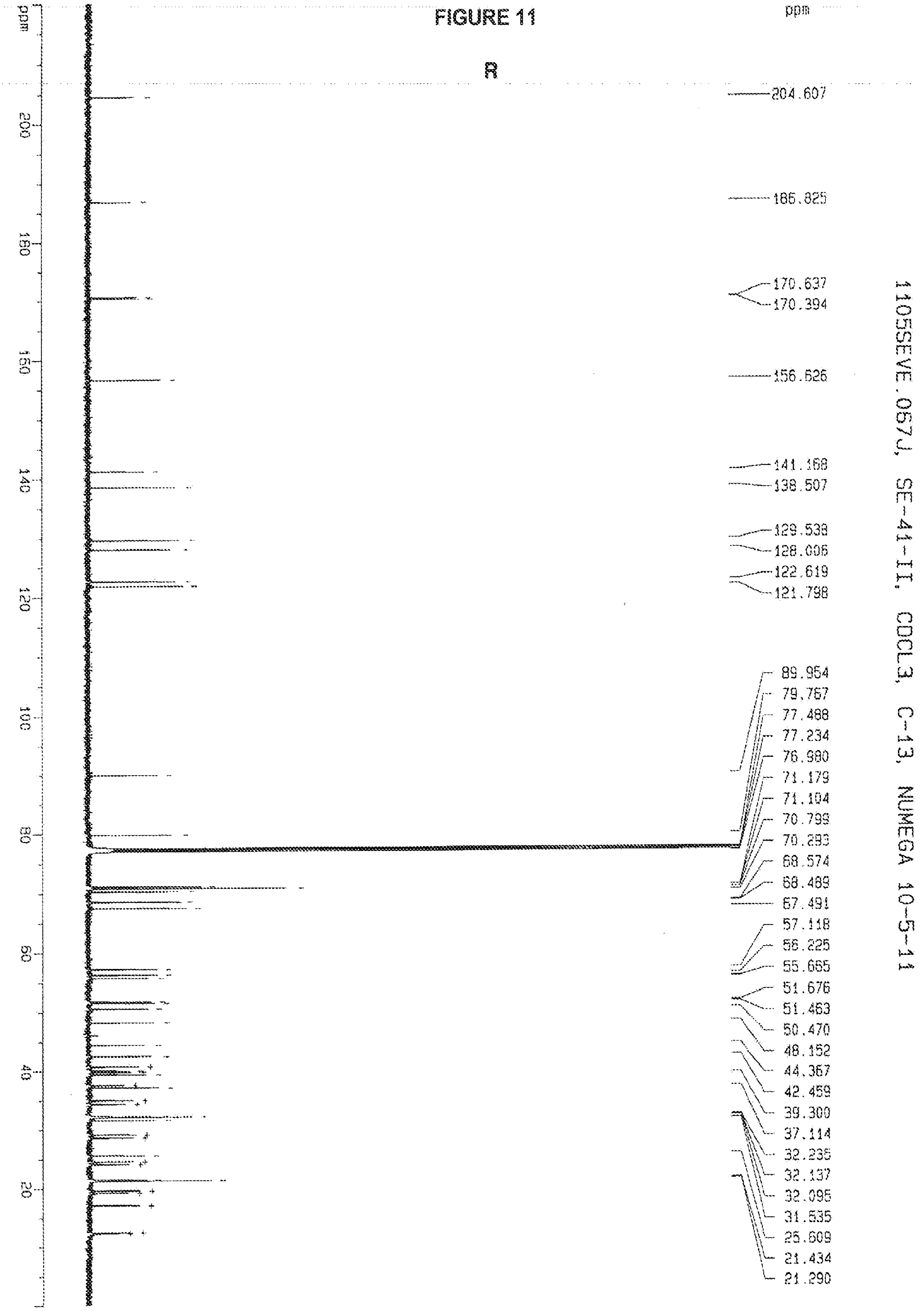
129.538
128.006
122.619
121.798
1105SEVE.067J, SE-41-II, CDCL3, C-13, NUMEGA 10-5-11
200
190
180
170
160
150
140
130
120

FIGURE 11

R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170.637
170.394
156.626
141.168
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129.538
128.006
122.619
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77.488
77.234
76.980
71.179
71.104
70.799
70.293
68.574
68.489
67.491
57.118
56.225
55.665
51.676
51.463
50.470
48.152
44.367
42.459
39.300
37.114
32.236
32.137
32.095
31.535
25.609
21.434
21.290
1105SEVE.067J, SE-41-II, CDCL3, C-13, NUMEGA 10-5-11
ppm
200
180
160
140
120
100
80
60
40
20