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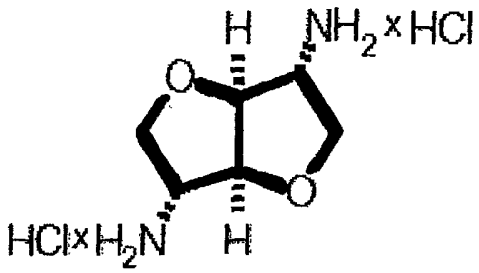
(54) Title: COMPOSITION COMPRISING A DENDRIMER AND THE USE THEREOF FOR BINDING PHOSPHATE



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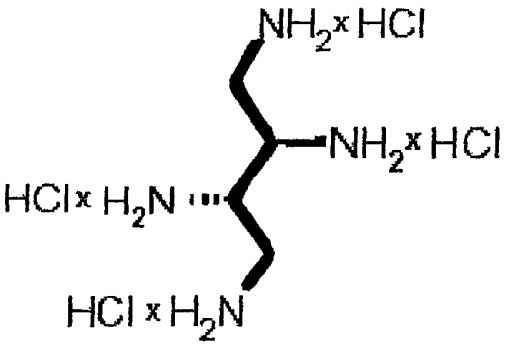

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(57) Abstract: The present invention provides improved methods and compositions for therapeutically controlling and/or reducing serum phosphate levels in animals and mammalian patients. The methods comprise administering to the patient an amount of a dendrimer composition effective to prevent absorption of substantial amounts of phosphate from the patient's GI tract. In a preferred version, a dose of between 2.5 and 15 grams per day is effective to prevent over 80% of phosphate present in the patient's GI tract from being absorbed. The dendrimer composition may comprise a hydrochloride, hydrobromide, hydroacetate or hydroanionic form.

WO 2007/033269 A1

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COMPOSITION COMPRISING A DENDRIMER AND THE USE THEREOF FOR BINDING PHOSPHAT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application no. 60/717,072, filed September 14, 2005, which is hereby incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

FIELD OF THE INVENTION

[0003] The invention relates generally to methods and compositions for therapeutic phosphate binding in a mammalian patient, preferably by use of dendrimers, as defined below. Most preferably, the methods and compositions of the present invention are used with dialysis patients and others who have an inability to excrete phosphate.

BACKGROUND

[0004] The kidney is essential not only for its ability to filter toxins and excess nutrients from the blood, but also for its ability to synthesize the active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. In patients with chronic kidney disease, both these functions are impaired. Consequently, levels of $1,25(OH)_2D_3$ decline, leading to hypocalcemia. Meanwhile, nutrients, particularly phosphorus, accumulate in the blood. Hypocalcemia and hyperphosphatemia are both potent stimulators of parathyroid hormone (PTH) secretion. Over time, hyperparathyroidism in the presence of even trace amounts of $1,25(OH)_2D_3$ cause excess bone resorption, leading to a condition known as renal osteodystrophy (1). In addition to dialysis treatment, it is essential to suppress excessive PTH levels and reduce phosphorus in the blood to prevent this condition.

[0005] Vitamin D analogs, such as 1,25-dihydroxy-19-nor-vitamin D_2 (19-nor-D_2, Zemplar®, Abbott Laboratories, Abbott Park, IL) and 1α-hydroxyvitamin D_2 [1α-(OH)D_2, Hectorol®, Genzyme Corporation/Bone Care International, Middleton, WI] are administered to patients to suppress hyperparathyroidism. Although these analogs are effective at suppressing PTH levels, they still retain some ability to stimulate intestinal calcium and phosphate absorption, which may be problematic when the analogs are administered at high doses or in conjunction with calcium-based oral phosphate binders (1).

[0006] Reducing the absorption of phosphorus from foods is also a challenging task. The current Recommended Dietary Allowance (RDA) for phosphorus is 700 mg per day (2), but most Americans consume 1000-1600 mg of phosphorus each day (3). Dietary phosphorus restriction is not very effective due to the richness of phosphorus in foods such as dairy products, meat, fish, eggs, nuts,

grains, baked goods, and soft drinks. Moreover, it is estimated that 65-75% of consumed phosphorus is absorbed (4). As a result, oral phosphate binders are often administered with meals to reduce the absorption of phosphorus.

[0007] In the 1970s, aluminum-based binders were extensively used to bind phosphate from foods, but the use was severely reduced after aluminum was shown to accumulate in patients causing toxic side-effects such as bone disease, encephalopathy, and anemia (5). Calcium acetate (PhosLo, Nabi Pharmaceuticals, Boca Raton, FL) was then developed as an alternative to aluminum-based binders, but must be administered at high levels to be effective. Furthermore, when administered in conjunction with $1,25(OH)_2D_3$ or a vitamin D analog, the oral calcium may contribute to hypercalcemia (5). Recently, lanthanum carbonate (Fosrenol®, Shire US Incorporated, Wayne, PA) was approved by the FDA for use as an oral phosphate binder. Although effective, its low rate of absorption raises some speculation that toxicity issues may arise with long-term use (6).

[0008] Sevelamer hydrochloride (Renagel®, Genzyme Corporation, Cambridge, MA), a phosphate-binding polymer, has been successfully used to reduce absorption of dietary phosphorus with fewer side effects than aluminum or calcium (7). Unfortunately, sevelamer hydrochloride is costly (average cost of $4400 per year in 2002) and must be taken in large quantities (average dose of 6.5 g per day) to be effective (8).

[0009] Dendrimers are well known therapeutic tools. Dendrimers have been used in applications including imaging agents, nano-scaffolds, antitumor drugs, gene transfection agents, nanoscale containers and biomimetic artificial proteins (14). However, therapeutic dendrimer compositions that bind phosphate, thereby treating hypocalcemia, hyperphosphatemia and chronic kidney disease, are not known.

[0010] Thus, a need exists for dendrimeric compositions containing varying amounts of free amines that can bind phosphate and inhibit its absorption *in vivo*.

BRIEF DESCRIPTION OF THE INVENTION

[0011] The present invention provides an improved method of controlling serum phosphate levels in mammals comprising administering to the mammal an amount of a dendrimer composition effective to prevent absorption of substantial amounts of phosphate from the mammal's GI tract, wherein the mammal's serum phosphate level is controlled. A dose of between 2.5 and 15 grams per day is effective to prevent at least 50% of phosphate present in the mammal's GI tract from being absorbed. In a preferred version at least 80% of the phosphate is prevented from being absorbed. The dendrimer composition may comprise a hydrochloride, hydrobromide, hydroacetate, or some hydro anion form.

[0012] In a preferred version the dendrimer is selected from the group consisting of erythro-1,2,3,4-tetraaminobutane tetrahydrochloride or diaminobutane. In a further preferred version the dendrimer composition comprises a dendrimer according to Structures 4, 5 or 6 (Figures 1D-1F).

[0013] In another version, the present invention provides a method of reducing intestinal phosphate absorption in animals by administering to the animal an amount of a dendrimer composition effective to prevent absorption of substantial amounts of phosphate from the animal's GI tract, wherein the animals serum phosphate level is reduced. In a preferred version, a daily dose of between 2.5 and 15 grams per day is effective to prevent at least 50% of phosphate present in the animal's GI tract from being absorbed. In a preferred version at least 80% of the phosphate is prevented from being absorbed. The dendrimer composition may comprise a hydrochloride, hydrobromide or hydroacetate or other hydroanionic forms.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 shows therapeutic phosphate binders of the present invention. Figure 1A) Structure 1: 1,4:3,6-Dianhydro-2,5-diamino-2,5-dideoxy-D-iditol dihydrochloride (FC). Figure 1B) Structure 2: erythro-1,2,3,4-tetraaminobutane tetrahydrochloride (KB-54). Figure 1C) Structure 3: Diaminobutane dendrimer Generation 1 (DAB-4-Cl). Figure 1D) Structure 4: Diaminobutane dendrimer Generation 2 (DAB-8-Cl). Figure 1E) Structure 5: Diaminobutane dendrimer Generation 3 (DAB-16-Cl). Figure 1F) Structure 6: Diaminobutane dendrimer Generation 5 (DAB-64-Cl). Figure 1G) Structure 7: DAB-8-AcOH.

[0015] Figure 2 shows that Calcium or Renagel® bind phosphate *in vivo*. Fasted rats were administered 0.5 mL water or 20 mg calcium (as calcium acetate) or 14.4 mg Renagel® dissolved in water via gastric gavage. Rats were immediately administered a dose of 3 μCi ^{33}P in 0.5 mL buffer containing 10, 50, or 100 mM KH_2PO_4, and killed after 60 minutes. Figure 2A) Percent of oral ^{33}P dose remaining in the digestive tract. *Significantly different from rats administered water prior to ^{33}P in same level of unlabeled phosphate ($p<0.05$). **Significantly different from rats administered 14.4 mg Renagel® prior to ^{33}P in same level of unlabeled phosphate ($p<0.05$). Figure 2B) Percent of oral ^{33}P dose detected in serum. *Significantly different from rats administered water prior to ^{33}P in same level of unlabeled phosphate ($p<0.05$).

[0016] Figure 3 compares the novel oral phosphate binders disclosed herein. Fasted rats were administered 0.5 mL water or 10 mg calcium (as calcium acetate), 14.4 mg Renagel®, or a novel phosphate binder (described in Table 1) dissolved in water via gastric gavage. Rats were immediately administered a second dose of 3 μCi ^{33}P in 0.5 mL buffer containing 10 mM KH_2PO_4, and killed after 60 minutes. Figure 3A) Percent of oral ^{33}P dose remaining in the digestive tract. Figure 3B) Percent

of oral ^{33}P dose detected in serum. *Significantly different from rats administered water prior to ^{33}P ($p<0.05$). **Significantly different from rats administered Renagel® prior to ^{33}P ($p<0.05$).

[0017] Figure 4 illustrates the dose response to dendrimer compounds. Fasted rats were administered 0.5 mL water or 14.4 mg Renagel® or a novel phosphate binder dissolved in water via gastric gavage. Rats were immediately administered a dose of 3 μCi ^{33}P in 0.5 mL buffer containing 10 mM KH_2PO_4, and killed after 60 minutes. Figure 4A) Percent of oral ^{33}P dose remaining in the digestive tract. Figure 4B) Percent of oral ^{33}P dose detected in serum. *Significantly different from rats administered water prior to ^{33}P ($p<0.05$). **Significantly different from rats administered Renagel® prior to ^{33}P ($p<0.05$).

[0018] Figure 5 illustrates the mechanism underlying the dendrimer's ability to bind phosphate. Fasted rats were administered 0.5 mL water or 14.4 mg Renagel® or a novel phosphate binder dissolved in water via gastric gavage. Rats were immediately administered a dose of 3 μCi ^{33}P in 0.5 mL buffer containing 10 mM KH_2PO_4, and killed after 60 minutes. Figure 5A) Percent of oral ^{33}P dose remaining in the digestive tract. Figure 5B) Percent of oral ^{33}P dose detected in serum. *Significantly different from rats administered water prior to ^{33}P ($p<0.05$). **Significantly different from rats administered Renagel® prior to ^{33}P ($p<0.05$). ND = none detectable.

[0019] Figure 6 illustrates the synthesis of Structure 1, FC.

[0020] Figure 7 illustrates the synthesis of Structure 2, KB-54.

[0021] Figure 8 illustrates the synthesis of Structure 3, DAB-4-Cl.

[0022] Figure 9 illustrates the synthesis of Structure 4, DAB-8-Cl.

[0023] Figure 10 illustrates the synthesis of Structure 5, DAB-16-Cl.

[0024] Figure 11 illustrates the synthesis of Structure 6, DAB-64-Cl.

[0025] Figure 12 illustrates the synthesis of Structure 7, DAB-8-AcOH.

[0026] Figure 13 illustrates the effect of hydroacetate dendrimers on intestinal 33P absorption.

[0027] Figure 14 illustrates the effect of hydroacetate dendrimers on absorption of 33P into serum.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention provides an improved method of therapeutic phosphate binding in an animal or mammalian patient, preferably by use of a dendrimer, as defined below. Most preferably, the method of present invention is used with dialysis patients and others who have an inability to excrete phosphate.

[0029] The present invention also provides therapeutic dendrimeric compositions. Preferably, the present invention is a hydrochloride, hydrobromide or hydroacetate form of dendrimers described

in U.S. Patents 5,530,092 and 5,610,268, incorporated herein. Most preferably, the present invention is the hydrochloride form of DAB-16 and DAB-64 (Figures 1E and 1F).

[0030] The present invention involves treating a patient with an amount of dendrimer composition effective to control serum phosphate levels in the patient. By "control," we mean increase and/or, more preferably, decrease the amount of phosphate absorbed by the patient's GI tract according to the dose and composition of the dendrimer administered to the patient. For instance, when a patient requires reduced levels of serum phosphate, the present invention prevents the absorption of substantial amounts of phosphate from the GI tract. By "substantial," we mean the present invention prevents at least 50% of phosphate from being absorbed in the GI tract. Most preferably, the present invention prevents at least 80% of the phosphate from being absorbed in the GI tract.

[0031] The effectiveness of this invention is determined by measuring the serum phosphate levels of the patient by any conventional test known to the art. The present invention is effective when the patient's serum phosphate levels are reduced by at least 10%, but more preferably, when the patient's serum phosphate levels are reduced by at least 20%.

[0032] The dendrimer is administered in an amount ranging between 2.5 and 15 grams per day. This dose is preferably equally divided among two or more meals. A preferable route of administration is in liquid form, such as a drink or a capsule. It is an advantage of the present invention that the dendrimer composition is soluble.

[0033] The invention also may include a pharmaceutical composition comprising a dendrimer composition combined with a pharmaceutically acceptable carrier intended to reduce and/or control serum phosphate levels in mammals. The composition may be administered to a mammal, a cell, or an isolated organ.

[0034] Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions and the like. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the mammal at a suitable dose.

[0035] Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal injection, or by inhalation or intracranial injection.

[0036] By "dendrimer composition" we mean to include the molecules described in U.S. Patents 5,530,092 and 5,610,268, incorporated by reference herein. These molecules include macromolecules comprising a core and branches emanating from the core, wherein the branches are based on vinyl

cyanide and/or fumaryl dinatrile units. Most preferably, the dendrimer comprises a diaminobutane (DAB) dendrimer.

[0037] By "dendrimer composition" we also mean to include neutralized versions of dendrimers described in the patents listed above. Most preferably, the diaminobutane dendrimer is in the hydrochloride form, as described below. Other preferable neutralized forms include the hydrobromide (or any halide or organic acid) form and the hydroacetate form.

[0038] Dendrimer compositions of this kind may be synthesized according to conventional techniques, including those described in U.S. Patents 5,530,092 and 5,610,268, incorporated by reference herein, and Buhleier, "Cascade" and "Non-skid-Chain-Like" Synthesis of Molecular Cavity Topologies, Synthesis, 155-158 (Feb. 1978).

EXAMPLES

[0039] The following examples set forth preferred aspects of the present invention. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

Materials and Methods

[0040] **Animals.** Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) weighing approximately 120 grams were used in all experiments.

[0041] In experiments to measure ^{33}P absorption, the rats were fed a laboratory chow diet (Lab Diet 5012, Richmond, IN) containing 1% calcium and 0.7% phosphorus *ad libitum* for less than one week prior to the experiment.

[0042] In experiments to measure fecal calcium and phosphorus levels, rats were fed purified diet described previously (9) for 9 days. This diet was mixed with egg white protein (Harlan Teklad, Madison, WI) and contained 0.20% inorganic phosphorus and 0.47% calcium. The purified diet was supplemented with 100 μL soybean oil (Wesson oil, ConAgra Foods, Irvine, CA) containing 500 μg α-tocopherol, 60 μg menadione, 40 μg β-carotene, and 1.875 μg cholecalciferol three times each week.

[0043] All rats were housed in hanging-wire cages under a 12-hour light/12-hour dark cycle and had free access to distilled water. All experimental methods were approved by the Research Animal Resources Center at the University of Wisconsin-Madison.

[0044] **Intestinal Phosphate Absorption.** Following an overnight fast, rats were administered 0.5 mL water or an oral phosphate binder dissolved in water via gastric gavage. A second dose of 0.5 mL containing 3 μCi ^{33}P (as H_3PO_4, specific activity 155.8 Ci/mg, New England Nuclear/Perkin Elmer, Boston, MA) in a 10, 50, or 100 mM KH_2PO_4 buffer at pH 7.4 was immediately administered via gastric gavage. Rats were killed by CO_2 asphyxiation immediately or

60 minutes after the oral dose. The rats killed immediately (labeled "0 min control" in figures) were used to determine if the oral ^{33}P dose was properly administered and completely recovered.

[0045] Blood was collected via heart puncture and centrifuged at 1500 x g for 15 minutes at 22°C to yield serum. A suture was tied to the cranial end of the esophagus to contain liquid inside the stomach. The entire digestive tract was then removed and allowed to dissolve for several days in concentrated HNO_3 (approximately 1 mL HNO_3 per gram tissue). The exact volume of the dissolved digestive tract was determined by diluting the dissolved tissues to equal volumes with water.

[0046] The amount of radioactivity in total body serum and total volume of dissolved tissue was determined following liquid scintillation counting of 50 µL aliquots in triplicate (Tri-Carb Liquid Scintillation Analyzer, Perkin-Elmer/Packard, Boston, MA). Total body serum was estimated to be 40 mL serum/kg body weight (10).

[0047] **Fecal calcium and phosphorus measurements.** Rats were fed the purified diet described above or the same diet with 1.2% calcium, 0.15% Renagel®, or 0.15% DAB-4, DAB-8, or DAB-16 for 7days. Rats were then moved to metabolic cages and fecal matter was collected for 48 hours. Fecal samples were frozen, lyophilized, and heated to over 600°C overnight in a muffle oven. Remaining ash was then dissolved overnight in 25 mL 6 N HCl. The calcium concentration of the acid was determined by flame atomic absorption spectroscopy (Model 3110, Perkin Elmer, Norwalk, CT) using an aliquot of the dissolved ash diluted 1:40 with 1 g/L $LaCl_3$. The phosphorus concentration of an aliquot of the dissolved ash was determined by a colorimetric assay described previously (11).

[0048] **Statistical analysis.** Data are presented as means ± standard error of the means (SEM). Treatment groups were compared by a fully factorial analysis of variance (ANOVA) and means were subjected to Tukey, Scheffe, and Fisher's Least-Significant-Difference (LSD) tests (Systat 5.2.1, Systat Software, Inc., Point Richmond, CA). Differences were considered significant if at least two of the tests detected significance with a p-value < 0.05, unless specified otherwise.

[0049] **Synthesis of Structure 1, FC.** As seen in Figure 6, the synthesis of Structure 1, FC (compound 17 in Figure 6) is a three-step process. In step 1, a suspension of 174mg (0.95mmol) D-mannitol (compound 14) in 4mL (49.4mmol) dry pyridine was stirred under argon at room temperature for 0.5h. Then, dry dichloromethane (14mL) was added, the mixture was cooled down to -10°C (salt-ice bath) and triflic anhydride (1.15mL, 6.86mmol) was added dropwise over a 0.5h period. Stirring was continued at 4°C (cold room) for 12h. The solution was diluted with dichloromethane (20mL) and washed with water (6x7mL), saturated aqueous solution of $CuSO_4$ (7mL), again water (3x7mL) and dried over anhydrous Na_2SO_4, filtered. Evaporation of the solvents, then very fast column chromatography (30% hexane/ethyl acetate) afforded an unstable, creamy semisolid, compound 15 (139mg, 0.14mmol, 15% yield). $[\alpha]_D$ +97.9 (c.1.0, $CHCl_3$); ^1H NMR

(400MHz, $CDCl_3$): δ 4.15 (m, 2H), 4.77 (dd, 2H, J = 4.1 Hz, J = 8.1 Hz), 5.21 (dd, 2H, J = 4.3 Hz, J = 9.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 70.9, 80.4, 80.45, 118.5 (q, $J_{C,F}$ = 318.98Hz).

[0050] In step 2, 120mg (0.123mmol) compound 15 and 72 mg(1.107mmol) NaN3 were dissolved in dry benzene (2mL). The 18-crown-6 (0.956g, 0.36mmol) was added and the reaction mixture was stirred under argon at 40°C for 3h, then cooled down to room temperature, diluted with CH2CL2 (10mL) and washed with water (6x4mL). Organic layer was dried over anhydrous Na2SO4, filtered and very carefully concentrated under reduced pressure. The residue was purified by column chromatography (20% ethylacetate/.hexane). After chromatography, solvents were removed under reduced pressure and finally by purging a stream of argon for 1h to give compound 16 as a colorless oil (20mg, 0.102mmol, 83% yield). ^1H NMR (400MHz, $CDCl_3$): δ 3.89 (dd, 2H, J = 4.0 Hz, J = 10.2 Hz), 3.93 (dd, 2H, J = 1.5Hz, J = 10.1 Hz), 4.6 (dd, 2H, J = 1.2Hz, J = 3.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.7, 71.9, 86.0.

[0051] In step 3, 20mg (0.102mmol) of compound 16 was dissolved in 2mL ethanol and 10mg of 10% Pd/.C was added. Air was removed by purging with argon for 15 min. The mixture was hydrogenated using a slow stream of hydrogen at room temperature for 3h (TLC control, 20% ethyl acetate/hexane). After that, the mixture was filtered through celite. Flask and celite were washed with ethanol (10mL). Filtrate containing crude 1,4:3,6-dianhydro-2,5-diamino-2,5-dideoxy-D-iditol was treated with a solution of HCl (aqueous HCl-37.3%: 35μL, 0.432mmol; ethanol: 1.2mL) and stirred at room temperature for 2h. Precipitate was then filtered off, washed with ethanol (15mL), dried on air for 12h and next in a vacuum oven at 60°C for 48h to give 13mg (0.06mmol, after two steps 59% yield) of compound 17 as a white crystal (m.p. above 270°C; at 250°C the compound turns dark grey. $[\alpha]_D$ +55.2 (c.1.1, H_2O); ^1H NMR (400MHz, D_2O): δ 4.01-4.07 (m, 4H) 4.22 (dd, 2H, J = 5.1 Hz, J = 10.9 Hz), 5.01 (s, 2H); , J = 1.5Hz, J = 10.1 Hz), ^1H NMR (400MHz, DMSO-d_6): δ 3.68 (br s, 2H), 3.88 (dd, 2H, J=2.6Hz, J=10.3Hz), 3.98 (dd, 2H, J= 5.2Hz, J=10.4Hz), 4.85 (s, 2H), 8.71 (br s, 6H); ^{13}C NMR (100 MHz, D_2O): δ 55.9, 70.1, 84.6; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.5, 70.2, 84.7; Elemental analysis calculated for $C_6H_{14}O_2N_2Cl_2$: C 33.52%, H 6.56%, N 13.03%, Cl 32.03%; found C 33.24%, H 6.43%, N 12.72%, Cl 33.98%.

[0052] **Synthesis of Structure 2, KB-54.** As seen in Figure 7, the synthesis of Structure 2, KB-54 (compound 4 in Figure 7) is a three-step process. Step 1 involves the synthesis of 1,2,3,4-Tetra-*O*-benzenesulfonyl-*meso*-erythritol (compound 2). Compound 3 is synthesized by dissolving 13g (106mmol) of *meso*-erythritol (compound 1) in dry pyridine (400ml). The solution was cooled to -10°C (salt ice bath) and benzenesulfonyl chloride (81.5mL, 640mmol) was added dropwise over a 1h period. The cooling bath was removed and the mixture was stirred at room temperature for 5 h. The precipitate was collected and washed with ethyl acetate (250ml), water (1L0 and again with ethyl acetate (200ml). Then the produce was dried with air for 12h and then in vacuum oven at 50°C for

30h to yield 27g (39mmol, 37% yield) of white crystals (compound 2) (m.p. 184-186°C, lit. m.p. 184-185.5°C -R.L. Willer, *J.Org.Chem.*, 1984, 49, 5150-5154). The organic filtrates were combined, concentrated to 200mL and allowed to stand at room temperature to give the next portion of the crystalline product. Washing and drying procedures were repeated, yielding 30g (44mmol, 41% yield) of a second portion of compound 2 (m.p. 183-185°C). Total yield was 57g (83mmol, 78% yield). ^{1}H NMR (400MHz, D_2O): δ 4.03 (dd, 2H, J = 6.6 Hz, J = 11.5 Hz), 4.31 (d, 2H, J = 11.5 Hz), 5.03 (d, 2H, J = 6.7 Hz), 7.60-7.81 (m, 20H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 66.7, 76.7, 127.56, 129.7, 129.8, 134.3, 134.6, 134.7, 134.8; MS (ESI) exact mass calculated for $C_{28}H_{26}O_{12}S_4Na([M+Na]^+)$ 705.0205, found 705.175.

[0053] Step 2 involves the synthesis of erythro-1,2,3,4-tetraazidobutane (compound 3). This is accomplished by combining 27g (39mmol) of compound 2 with 17.17g (264mmol) NaN_3, 0.5g (1.89mmol) 18-crown-6 and 220mL dry DMF in a flask equipped with a refluxing condenser. The reaction mixture was stirred at 100°C for 48h and then cooled to room temperature, diluted with water (0.5L) and washed with CH_2Cl_2 (7X200mL). Organic layers were combined, washed with water (8x100mL) and saturated aqueous solution of NaCl (3x100mL) dried over anhydrous Na_2SO_4, filtered and very carefully concentrated under reduced pressure. The dark brown residue (containing small amounts of DMF) was purified by column chromatograph (Hexane, 5-10% ethyl acetate/hexane) to give 6.36g (0.028mmol, 72% yield) of compound 3, a colorless liquid. Because of well known hazards of polyazido compounds, the product was partially concentrated under reduced pressure after chromatography and the residue of solvents was removed by purging a stream of argon for 2h (R.L. Willer, *J.Org.Chem.*, 1984, 49, 5150-5154). ^{1}H NMR (400MHz, $CDCl_3$): δ 3.52-3.8 (m, 4H) 3.67 (d, 2H, J=10.2Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.0, 61.5.

[0054] In step 3, 5.93g (26.7mmol) compound 3 was dissolved in 130mL ethanol and 1.5g 10% Pd/C was added. Air was removed by purging with argon for 15 min. The mixture was hydrogenated using a slow stream of hydrogen at room temperature for 5 h (TLC control, 10% ethylacetate/hexane). After that the mixture was filtered through celite. Flask and celite were washed with methanol (12mL). Filtrate containing crude erythro-1,2,3,4-tetraaminobutane was treated with solution of HCl (aqueous HCl-36.3%: 9.73ml, 117.4 mmol; methanol: 34 mL) and stirred at room temperature for 12h. Pale pink precipitated was filtered off, washed with methanol (300mL), dried on air for 12h and then in vacuum oven at 60°C for 48h to give 4.84g(8.3mmol, after two steps, 68%yield) of compound 4 (m.p. 255°C; at 150°C compound 4 turns brown). ^{1}H NMR (400MHz, D_2O): δ 3.20 (dd, 2H, J=8.6Hz, J=14.0Hz), 3.35(dd, 2H, J=3.0Hz, J=14.0Hz), 3.75 (br d, 2H, J=9.3Hz); ; ^{1}H NMR (400MHz, DMSO-d_6): δ 3.31 (dd, 2H, J=7.2Hz, J=14.2Hz), 3.47 (dd, 2H, J=3.8Hz, J=14.3Hz), 4.08 (br d, 2H, J=8.9Hz), 8.98 (br s, 12H); ^{13}C NMR (100 MHz, D_2O): δ 39.0,

50.3; ^{13}C NMR (100 MHz, DMSO-d_6): δ38.2, 49.9; Elemental analysis calculated for $C_4H_{18}N_4Cl_4$: C18.19%, H6.87%, N21.21%, Cl 53.71%; found C18.37%, H7.01%, N21.29%, Cl 53.46%.

[0055] **Synthesis of Structure 3, DAB-4-Cl.** As seen in Figure 8, the conversion of DAB-Am-4 dendrimer into hydrochloride (compound 6) is accomplished by dissolving 8.47g (26.76 mmol) of DAB-Am-4, Polypropylenimine tetraamine Dendrimer, Generation 1.0 (DSM product) (compound 5) in deionized water (200 mL). Air was removed by purging with argon for 15 min and solution of HCl (aqueous HCl – 37.0%: 15.85 mL, 193.02 mmol; deionized water: 30 mL) was added dropwise. Reaction mixture was stirred at room temperature for 1 h and then solvents were removed under reduced pressure. Residue was dissolved in 100 mL of deionized water and evaporated (procedure was repeated five times), dried on vacuum pump (48 h) and finally in vacuum oven at 60°C for 2 days to yield 14.32 g (26.75 mmol, quantitative yield) of beige crystal (compound 6) (m.p. 242-245°C). ^1H NMR (400MHz, D_2O): δ 1.89 (s, 4H), 2.14 – 2.24 (m, 8H), 3.15 (t, 8H, J = 7.5 Hz), 3.36–3.40 (m, 12H); ^{13}C NMR (100 MHz, D_2O): δ 20.5, 21.6, 36.4, 49.9, 52.3; Elemental analysis calculated for $C_{16}H_{46}N_6Cl_6$: C 35.90%, H 8.66%, N 15.69%, Cl 39.73%; found C 35.88%, H 8.73%, N 15.28%, Cl 39.25%.

[0056] **Synthesis of Structure 4, DAB-8-Cl.** As seen in Figure 9, the conversion of DAB-Am-8 Dendrimer, Generation 2.0 into hydrochloride (compound 8) is accomplished by dissolving (10 g, 12.93 mmol) of DAB-Am-8 Polypropylenimine octaamine Dendrimer, Generation 2.0 (DSM product) in deionized water (300 mL). Air was removed by purging with argon for 15 min and solution of HCl (aqueous HCl – 37.0%: 19.3 mL, 235.44 mmol; deionized water: 40 mL) was added dropwise. Reaction mixture was stirred at room temperature for 1 h and then solvents were removed under reduced pressure. Residue was dissolved in 100 mL of deionized water and evaporated (procedure was repeated five times), dried on vacuum pump (24 h) and finally in vacuum oven at 60 °C for 3 days to yield 16.44 g (12.81 mmol, 99%) of white crystalline compound 8 (m.p. 153 – 155 °C). ^1H NMR (400 MHz, D_2O): δ 1.94 (s, 4H), 2.18 – 2.23 (m, 16H), 2.26 – 2.34 (m, 8H), 3.16 (t, 16H, J = 7.8 Hz), 3.41 – 3.43 (m, 36H); ^{13}C NMR (100 MHz, D_2O): δ 19.0, 20.6, 21.6, 36.4, 49.8, 49.9, 50.0, 52.6; Elemental analysis calculated for $C_{40}H_{110}N_{14}Cl_{14}$: C 37.42%, H 8.63%, N 15.27%, Cl 38.66%; found C 35.81%, H 9.22%, N 14.52%, Cl 37.66%. Ratio of elements indicates full conversion of amino groups into hydrochlorides: calculated Cl/C 1.03, Cl/N 2.53, C/N 2.45; found Cl/C 1.05, Cl/N 2.59, C/N 2.46.

[0057] **Synthesis of Structure 5, DAB-16-Cl.** As seen in Figure 10, the conversion of DAB-Am-16 dendrimer, Generation 3.0 into hydrochloride (10) is accomplished by dissolving 5g (2.96mmol) DAB-Am-16 dendrimer, polypropylenimine hexadecaamine dendrimer (9) in deionized water (150mL). Air was removed by purging with argon for 15min and HCl solution (aqueous HCl-37%: 9.5mL, 115.56 mmol deionized water: 20 mL) was added dropwise. Reaction mixture was

stirred at room temperature for 1 h and then solvents were removed under reduced pressure. Residue was dissolved in 150mL deionized water and evaporated (procedure was repeated five times), dried on vacuum pump (24h) and finally in vacuum oven at 60°C for 3 days to yield 8.24g (2.96mmol, quantitative yield) of creamy crystalline compound (10) (m.p. 266°C). ^{1}H NMR (600MHz, D_2O): δ 1.81 (s, 4H), 2.07 – 2.10 (m, 32H), 2.14-2.19 (m, 24H), 3.06(t, 32H, *J*=7.7Hz), 3.28 – 2.37 (m, 84H); ^{13}C NMR (100 MHz, D_2O): δ 19.0, 19.1, 20.7, 21.6, 36.4, 49.8, 49.9, 50.1, 50.3, 52.9; Elemental analysis calculated for $C_{88}H_{238}N_{30}Cl_{30}$: C 38.01%, H 8.63%, N 15.11%, Cl 38.25%; found C 38.25%, H 9.13%, N 15.11%, Cl 38.25%. Ratio of elements indicates full conversion of amino groups into hydrochlorides: calculated Cl/C 1.01, Cl/N 2.53, C/N 2.51; found Cl/C 1.03, Cl/N 2.60, C/N 2.52.

[0058] **Synthesis of Structure 6, DAB-64-Cl.** As seen in Figure 11, the conversion of DAB-Am-64 dendrimer, Generation 5.0 into hydrochloride (12) is accomplished by dissolving 1.06 g(0.14mmol) of DAB-Am-64, polypropylenimine tetrahexacontaamine dendrimers in CH_3Cl (25mL). Air was removed by purging with argon for 15 min and concentrated solution of HCl (aqueous HCl-37.3%: 1.66mL, 19.99mmol) was added dropwise. Reaction mixture was stirred at room temperature for 1 h and then solvents were removed under reduced pressure. The residue was dissolved in 20mL of deionized water and evaporated (procedure was repeated five times), dried on vacuum pump (5h) and finally in vacuum oven at 60°C for 3 days to yield 1.525g (0.13mmol, 95% yield) of yellow crystalline compound (12) (m.p. 274-276 °C). ^{1}H NMR (600MHz, D_2O): δ 1.793 (s, 4H), 2.11 – 2.19 and 2.23-2.34 (2x m, 248H), 3.09 (t, 128H, *J*=7.6Hz), 2.32 – 2.47 (m, 372H); ^{13}C NMR (100 MHz, D_2O)-only easy visible signals: δ 19.2, 19.3, 20.9, 21.8, 36.7, 49.3, 49.7, 49.9, 50.3, 51.0; Elemental analysis calculated for $C_{376}H_{1006}N_{126}Cl_{126}$: C 38.39%, H 8.62%, N 15.00%, Cl 37.97%; found C 38.43%, H 9.25%, N 15.05%, Cl 38.35%. Ratio of elements indicates full conversion of amino groups into hydrochlorides: calculated Cl/C 0.99, Cl/N 2.53, C/N 2.55; found Cl/C 0.99, Cl/N 2.54, C/N 2.55.

[0059] **Synthesis of Structure 7, DAB-8-AcOH.** As seen in Figure 12, the conversion of DAB-Am-8 dendrimer, Generation 2.0 into decahydroacetate is accomplished by dissolving 6.92 g (8.95 mmol) of DAB-Am-8, Polypropylenimine octaamine Dendrimer, Generation 2.0 (DSM product) 1 in deionized water (260 mL). Air was removed by purging with argon for 15 min and solution of AcOH (glacial AcOH: 8.0 mL, 137.86 mmol; deionized water: 160 mL) was added dropwise. Reaction mixture was stirred at room temperature for 12 h and then solvents were removed under reduced pressure. Residue was dissolved in 250 mL of deionized water and evaporated (procedure was repeated sixteen times). Finally sample was dissolved in 100 mL of deionized water, frozen and lyophilized (48 h) to yield 11.78 g (8.57 mmol, 96%) of compound 3 as the very sticky pale orange oil. ^{1}H NMR (400 MHz, D_2O): δ 1.63 (s, 4H), 1.74 – 1.85 (m, 24H), 1.88 (s, 30 H), 2.52 – 2.62 (m, 24H), 2.86 – 3.02 (m, 28H); ^{13}C NMR (100 MHz, D_2O): δ 20.9, 21.7, 23.2, 23.4, 37.7,

49.7, 50.3, 50.8, 52.6, 181.3; Elemental analysis calculated for $C_{60}H_{136}N_{14}O_{20}$: C 52.45%, H 9.97%, N 14.27%; found C 52.05%, H 10.28%, N 14.43%.

RESULTS

[0060] **Calcium or Renagel® bind phosphate *in vivo*.** In previous measurements of intestinal phosphate absorption, ^{33}P was administered in a 0.5 mM KH_2PO_4 buffer. However, when 0.5 mM KH_2PO_4 was mixed with 100 mM $CaCl_2$, that concentration of phosphate did not precipitate. This suggests that a higher concentration of KH_2PO_4 is needed for calcium to bind phosphate. In fact, to detect precipitation of phosphate by excess calcium, the level of phosphate needed to be raised to 10 mM (data not shown). Thus, to determine optimal conditions for testing oral phosphate binders *in vivo*, water, 20 mg calcium (as calcium acetate), or 14.4 mg Renagel® were administered to fasted rats. Rats were immediately administered an oral dose of ^{33}P in a buffer containing 10, 50, or 100 mM KH_2PO_4 and killed after 60 minutes.

[0061] As shown in Figure 2A, rats administered 20 mg calcium or 14.4 mg Renagel® prior to ^{33}P had significantly more ^{33}P remaining in the intestine after 60 minutes than rats administered water prior to ^{33}P regardless of the level of unlabeled phosphate in the oral dose. Moreover, 20 mg calcium bound more ^{33}P in the intestine than did 14.4 mg Renagel®, and this difference reached significance when ^{33}P was administered in 10 or 100 mM phosphate. A significant decrease in serum ^{33}P levels was also detected in rats dosed with 10 mg calcium, but the decrease in serum ^{33}P levels observed in rats dosed with 14.4 mg Renagel® was not statistically significant (Figure 2B).

[0062] **Comparison of novel oral phosphate binders.** The binding ability of novel oral phosphate binders shown in Figure 1 was compared to calcium and Renagel®. Table 1 lists the weight and molar amounts of all compounds used in this and subsequent experiments. Rats were first administered 0.5 mL water or 0.5 mL water containing 10 mg calcium (as calcium acetate), 14.4 mg Renagel®, or a novel phosphate binder. An oral dose of ^{33}P in a 10 mM KH_2PO_4 buffer was immediately administered and rats were killed after 60 minutes. Both 10 mg calcium and 14.4 mg Renagel® significantly increased the amount of ^{33}P remaining in the digestive tract (Figure 4A), and significantly reduced serum ^{33}P levels (Figure 4B).

[0063] The novel binders KB-54 (Structure 2, Figure 1B), DAB-4 (Structure 3, Figure 1C), DAB-8 (Structure 4, Figure 1D), DAB-16 (Structure 5, Figure 1E) and DAB-64 (Structure 6, Figure 1F) also significantly increased the amount of ^{33}P remaining in the digestive tract and significantly reduced serum ^{33}P levels. Furthermore, DAB-8-Cl (Structure 4, Figure 1D) and DAB-16-Cl (Structure 5, Figure 1E) significantly increased the amount of ^{33}P remaining in the digestive tract and significantly reduced serum ^{33}P levels compared to a comparable amount of Renagel®. FC (Structure

1, Figure 1A) did not affect the amount of ^{33}P that remained in the digestive tract, but caused a slight, but significant, decrease in serum ^{33}P levels.

Table 1: Summary of solutions used to bind oral ^{33}P dose. 0.5 mL of solution containing an oral phosphate binder was administered to rats prior to the oral ^{33}P dose. NA = not available because structural information is proprietary.

Oral phosphate binder	mg/rat	moles/Liter (M)	Moles NH_2/Liter
Calcium acetate	10	0.5	0
Renagel®	14.4	NA	NA
FC	10.8	0.1	0.2
KB-54	66	0.5	1.0
DAB-4	1.8	0.00675	0.027
	3.6	0.0135	0.054
	7.2	0.027	0.108
	10.7	0.04	0.160
	14.4	0.054	0.216
	28.8	0.108	0.432
DAB-8	1.8	0.0028	0.0224
	3.6	0.0056	0.0448
	7.2	0.01	0.08
	10.7	0.011	0.088
	14.4	0.022	0.176
	28.8	0.045	0.36
DAB-16	6.95	0.005	0.08
	13.9	0.01	0.16
	69.5	0.05	0.8
DAB-64	6.64	0.00115	0.0736
	13.28	0.0023	0.1472
	132.8	0.023	1.472

[0064] **Dose response to dendrimer compounds.** The ability of DAB-4, DAB-8, DAB-16, and DAB-64 to bind phosphate was compared in a dose response study. Rats were first administered 0.5 mL water , 0.5 mL water containing 14.4 mg Renagel®, or a dendrimer. An oral dose of ^{33}P in a

10 mM KH_2PO_4 buffer was immediately administered and rats were killed after 60 minutes. All dendrimer compounds increased ^{33}P remaining in the digestive tract (Figure 4A) and correspondingly decreased serum ^{33}P (Figure 4B) levels in a dose-dependent manner.

[0065] Nearly all the increases in ^{33}P remaining in the digestive tract, and many of the decreases in serum ^{33}P levels, were statistically significant. In addition, the two highest levels of DAB-8 and DAB-16, and the highest level of DAB-64, significantly increased ^{33}P remaining in the intestine and significantly reduced serum ^{33}P levels compared to Renagel®.

[0066] **Mechanism underlying the dendrimer compound's ability to bind phosphate.** To determine if the number of free amino groups in the dendrimer compound is responsible for its phosphate binding ability, rats were administered equal numbers of moles or free amino groups from DAB-4, DAB-8 and DAB-16. Rats were immediately administered an oral dose of ^{33}P in a 10 mM KH_2PO_4 buffer and killed after 60 minutes.

[0067] As seen in Figure 5A, Renagel® and all levels of the dendrimers were able to increase the amount of ^{33}P remaining in the digestive tract to a significant degree. However, 13.9 mg DAB-16-Cl was the only level of binder able to significantly reduce serum ^{33}P levels (Figure 5B). Interestingly, when an equivalent amount of free amino groups were added from DAB-4 and DAB-16, DAB-16 was able to retain significantly more ^{33}P in the digestive tract. In addition, when equimolar amounts of DAB-8-Cl and DAB-16-Cl were administered to rats, DAB-16-Cl retained significantly more ^{33}P in the digestive tract.

[0068] Rats were fed a purified control diet containing 0.47% calcium and 0.20% phosphorus or the same diet with added calcium, or 0.15% Renagel®, DAB-4, DAB-8, or DAB-16 for one week. Fecal samples were then collected for 48 hours, dried, and ashed. Ash was dissolved in acid to determine calcium and phosphorus levels. Fecal calcium levels were significantly increased in rats fed a 1.20% calcium diet, confirming that diets were mixed and administered correctly. Fecal phosphorus was increased, though not significantly, in rats fed a diet containing 1.20% calcium or 0.15% DAB-4. As shown in Table 2, rats fed 0.15% DAB-8 or DAB-16 had significantly increased fecal phosphorus levels compared to rats fed a control diet or a diet with 0.15% Renagel® according to Fisher's LSD test only.

[0069] As seen in Figures 13 and 14, hydroacetate dendrimers (such as structure 7, Figure 1G) work just as effectively as the hydrochloride dendrimers.

Table 2: Dendrimers increase fecal phosphorus levels. Fecal calcium and phosphorus levels from rats fed a control diet containing 0.47% Ca and 0.2% P, or control diet with added calcium, Renagel®, DAB-4, DAB-8, or DAB-16. Data are presented as means ± standard error of the means (SEM). *Significantly different from amount of calcium in feces from rats fed control diet ($p<0.05$). **Significantly different from amount of calcium in feces from rats fed control diet as detected by Fisher's LSD test only ($p<0.05$).

Group	**mg Ca per gram feces**	**mg P per gram feces**
Control	11.23 ± 0.84	3.79 ± 0.18
1.20% Ca	80.71 ± 4.60 *	4.39 ± 0.23
0.15% Renagel®	13.06 ± 1.08	3.69 ± 0.21
0.15% DAB-4	14.28 ± 2.01	4.30 ± 0.13
0.15% DAB-8	16.06 ± 1.44	4.71 ± 0.53 **
0.15% DAB-16	15.82 ± 1.52	4.78 ± 0.30 **

CONCLUSION

[0070] Managing blood phosphate is a challenging, but essential, element in the treatment of secondary hyperparathyroidism in chronic kidney disease patients. In addition to dialysis treatment, patients are often administered vitamin D analogs to suppress PTH levels and oral phosphate binders to reduce the absorption of phosphate from foods. Although several types of oral phosphate binders have been developed, all have limited effectiveness due to potential toxicity, low binding ability, or high cost.

[0071] The present document compares a variety of novel compounds containing free amino groups for the potential to bind phosphate when administered orally in rats. One of these compounds, FC, does not appear to bind an oral ^{33}P dose. However, KB-54 and the first, second, third and fifth generations of a DAB dendrimer reduced the absorption of an oral ^{33}P dose. Each generation of the dendrimer compound bound oral ^{33}P in a dose dependent manner, and DAB-8 and DAB-16 bound significantly more ^{33}P than did an equivalent amount of Renagel®.

[0072] The mechanism by which dendrimer compounds bind phosphate was investigated by measuring the ability of equal number of moles and equal number of free amino groups from DAB-4, DAB-8, and DAB-16 to reduce the absorption of ^{33}P. When an equivalent number of free amino groups was administered in the form of DAB-4 and DAB-16, DAB-16 bound significantly more ^{33}P, suggesting free amino groups are not exclusively responsible for the dendrimer's ability to bind

phosphate. However, when an equimolar amount of DAB-8 and DAB-16 were administered to rats, DAB-16 retained significantly more ^{33}P in the digestive tract, implying that the number of free amino groups may be, in part, responsible for the dendrimer compound's ability to bind phosphate.

[0073] Tolerable levels of the DAB-4, DAB-8, and DAB-16 dendrimers were then fed to rats and were found to increase fecal phosphorus levels. Although the differences were significant by the Fisher's LSD test only, the increase in fecal phosphorus by DAB-8 and DAB-16 was significantly higher then the increase from excess calcium or an equivalent amount of Renagel®.

[0074] Unfortunately, little is known regarding the toxicity of DAB dendrimers when administered orally. Previous research has shown DAB dendrimers to be cytotoxic *in vitro* (12), and when administered intravenously, the DAB dendrimers are lethal (13). In our experiments, however, the DAB dendrimers were well tolerated by rats when administered orally. DAB-4, DAB-8, and DAB-16 as hydrochlorides were tolerated at 0.15% of the diet, but when fed at levels as high as 0.3% or 0.6%, only softened stool was observed after 5 days (data not shown).

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CLAIMS

We claim:

1. A method of controlling serum phosphate levels in mammals comprising administering to the mammal an amount of a dendrimer composition effective to prevent absorption of substantial amounts of phosphate from the mammal's GI tract, wherein the mammal's serum phosphate level is controlled.

2. The method of claim 1 wherein at least 50% of phosphate in the mammal's GI tract is prevented from being absorbed.

3. The method of claim 1 wherein at least 80% of phosphate in the mammal's GI tract is prevented from being absorbed.

4. The method of claim 1 wherein the amount of dendrimer composition administered to the mammal is between 2.5 and 15 grams per day.

5. The method of claim 1 wherein the dendrimer composition comprises a dendrimer in hydroanionic form.

6. The method of claim 1 wherein the dendrimer composition comprises a dendrimer in hydrochloride form.

7. The method of claim 1 wherein the dendrimer composition comprises a dendrimer in hydrobromide form.

8. The method of claim 1 wherein the dendrimer composition comprises a dendrimer in hydroacetate form.

9. A dendrimer composition comprising a dendrimer in a hydrochloride, hydrobromide or hydroacetate form.

10. The composition of claim 9 wherein the dendrimer composition is selected from the group consisting of erythro-1,2,3,4-tetraaminobutane tetrahydrochloride or diaminobutane.

11. The composition of claim 9 wherein the composition comprises the structure according to Structure 4

12. The composition of claim 9 wherein the composition comprises the structure according to Structure 5.

13. The composition of claim 9 wherein the composition comprises the structure according to Structure 6.

14. A method of reducing intestinal phosphate absorption in animals by administering to the animal an amount of a dendrimer composition effective to prevent absorption of substantial amounts of phosphate from the animal's GI tract, wherein the animal's intestinal phosphate absorption is reduced.

15. The method of claim 14 wherein at least 50% of phosphate in the animal's GI tract is prevented from being absorbed.

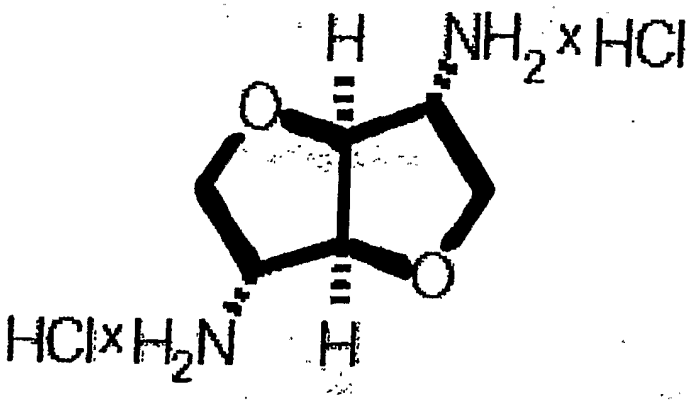
16. The method of claim 14 wherein at least 80% of phosphate in the animal's GI tract is prevented from being absorbed.

17. The method of claim 14 wherein the amount of dendrimer composition administered to the animal is between 2.5 and 15 grams per day.

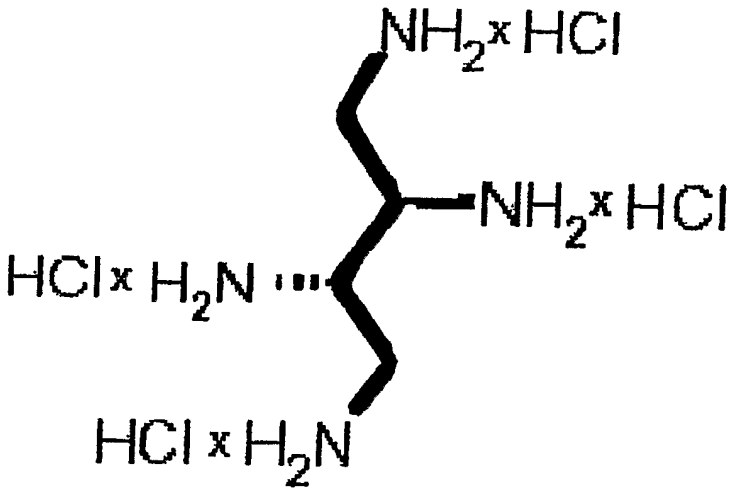
18. The method of claim 14 wherein the dendrimer composition comprises a dendrimer in hydrochloride form.

19. The method of claim 14 wherein the dendrimer composition comprises a dendrimer in hydrobromide form.

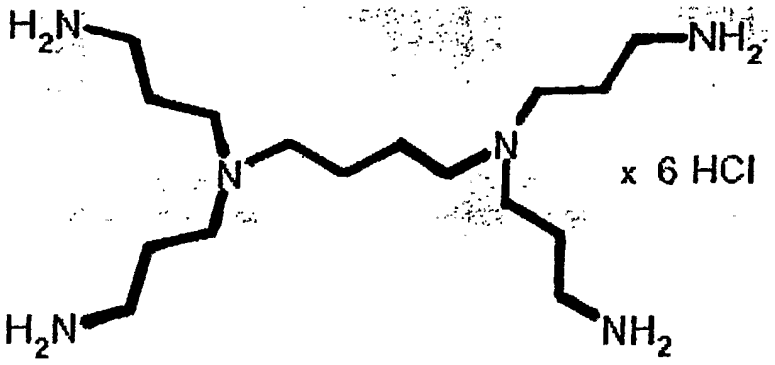
20. The method of claim 14 wherein the dendrimer composition comprises a dendrimer in hydroacetate form.



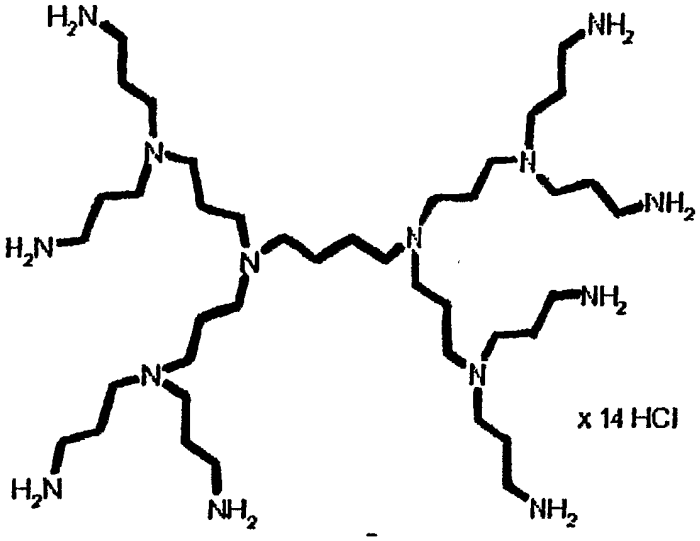
Structure 1
Figure 1A



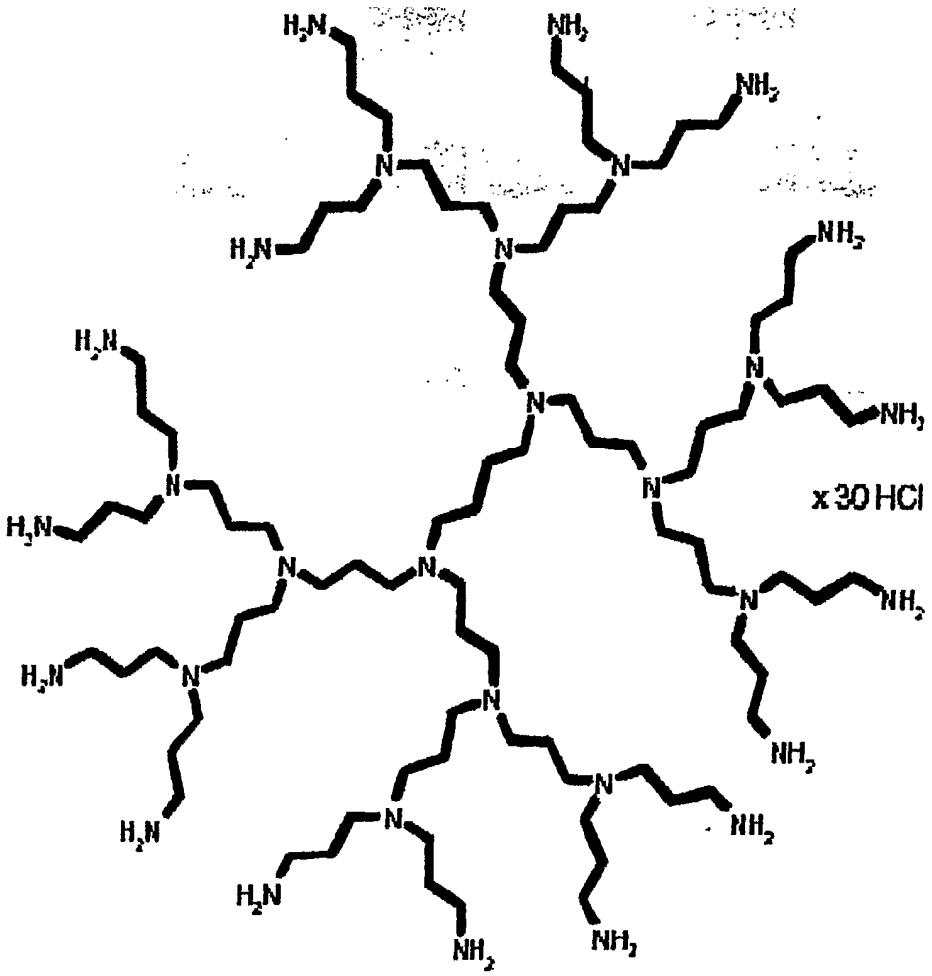
Structure 2
Figure 1B



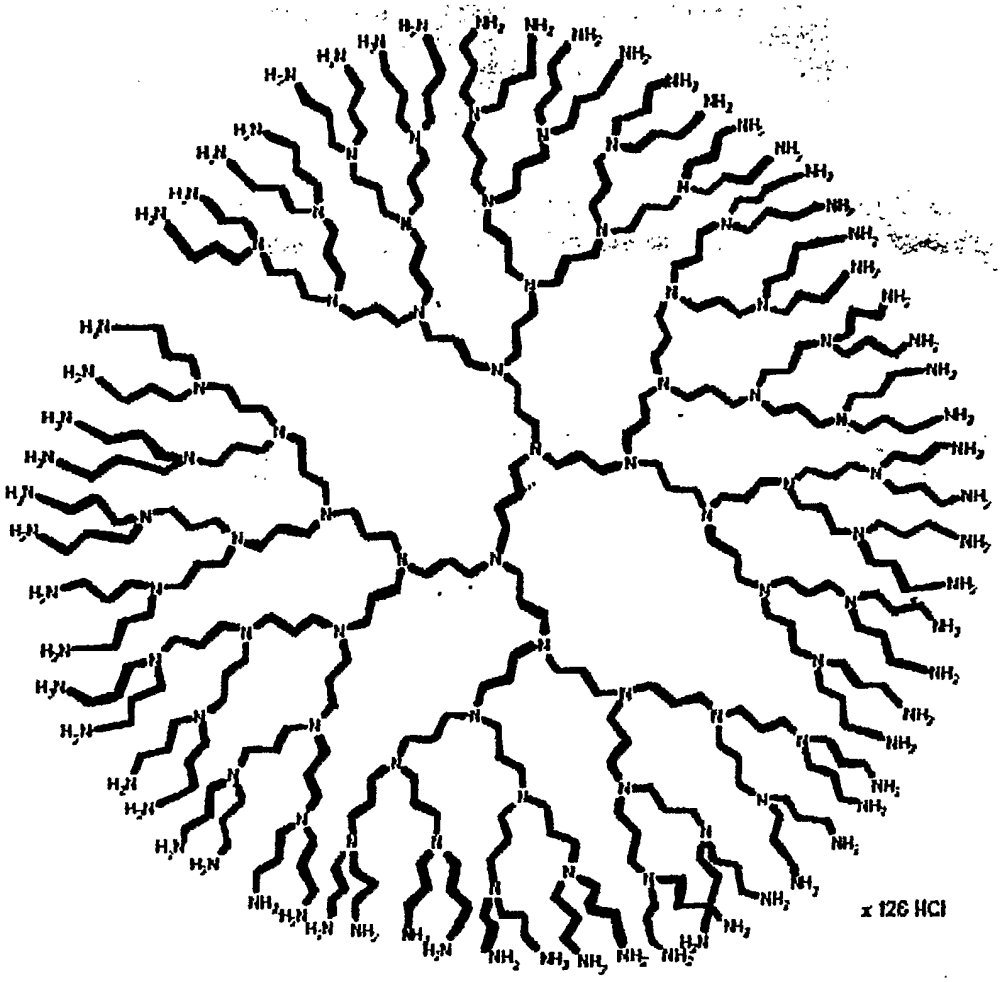
Structure 3
Figure 1C



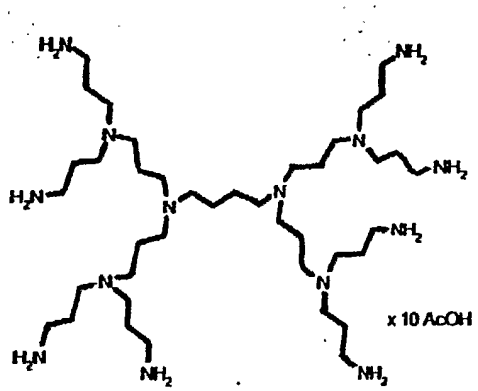
Structure 4
Figure 1D



Structure 5
Figure 1E



Structure 6
Figure 1F



Structure 7
Figure 1G

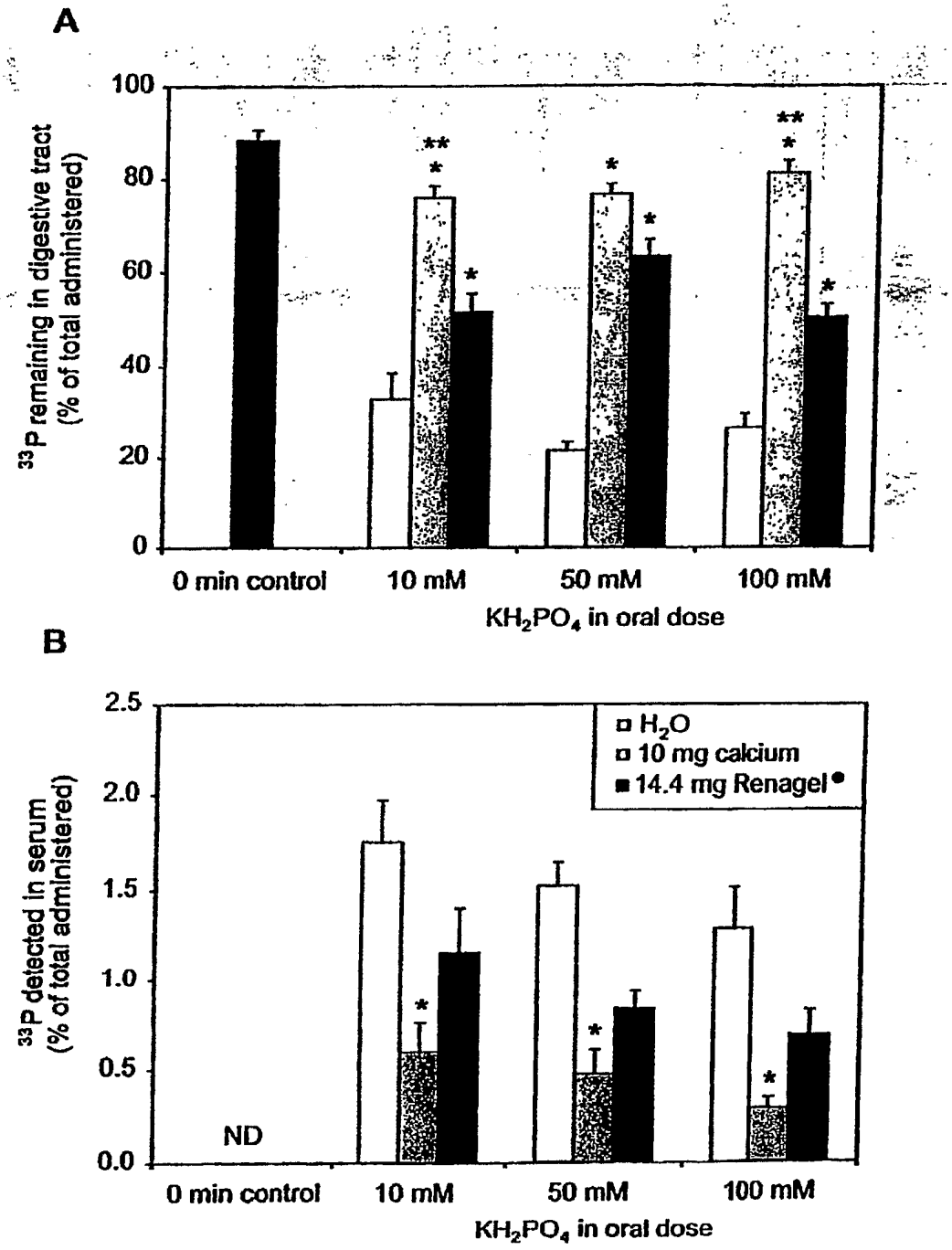

A
100
80
60
40
20
0
^{33}P remaining in digestive tract (% of total administered)
0 min control
10 mM
50 mM
100 mM
KH_2PO_4 in oral dose
B
2.5
2.0
1.5
1.0
0.5
0.0
^{33}P detected in serum (% of total administered)
H_2O
10 mg calcium
14.4 mg Renagel®
ND
0 min control
10 mM
50 mM
100 mM
KH_2PO_4 in oral dose

Figure 2

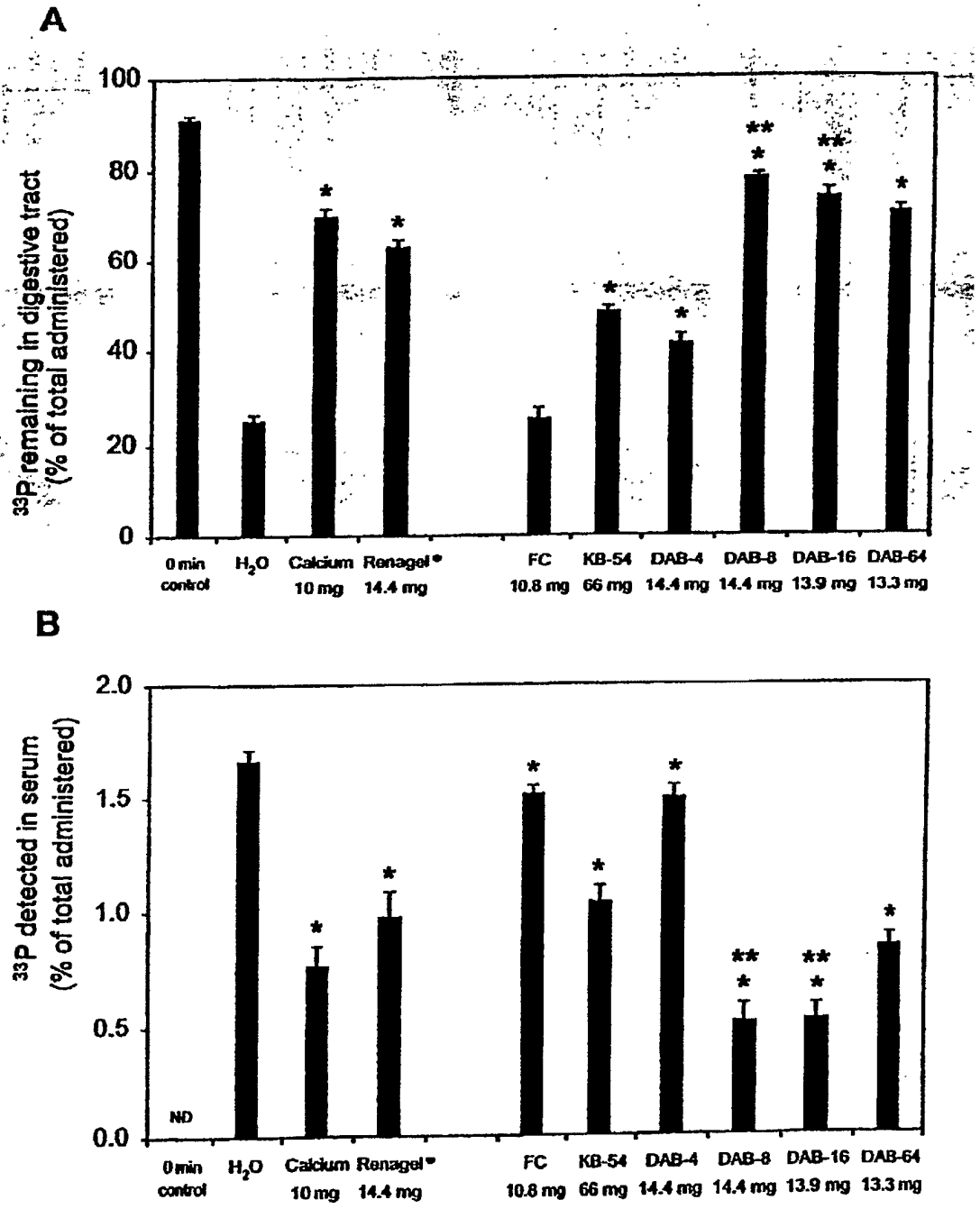

A
^{33}P remaining in digestive tract (% of total administered)
100
80
60
40
20
0
0 min control
H_2O
Calcium 10 mg
Renagel® 14.4 mg
FC 10.8 mg
KB-54 66 mg
DAB-4 14.4 mg
DAB-8 14.4 mg
DAB-16 13.9 mg
DAB-64 13.3 mg
B
^{33}P detected in serum (% of total administered)
2.0
1.5
1.0
0.5
0.0
ND
0 min control
H_2O
Calcium 10 mg
Renagel® 14.4 mg
FC 10.8 mg
KB-54 66 mg
DAB-4 14.4 mg
DAB-8 14.4 mg
DAB-16 13.9 mg
DAB-64 13.3 mg

Figure 3

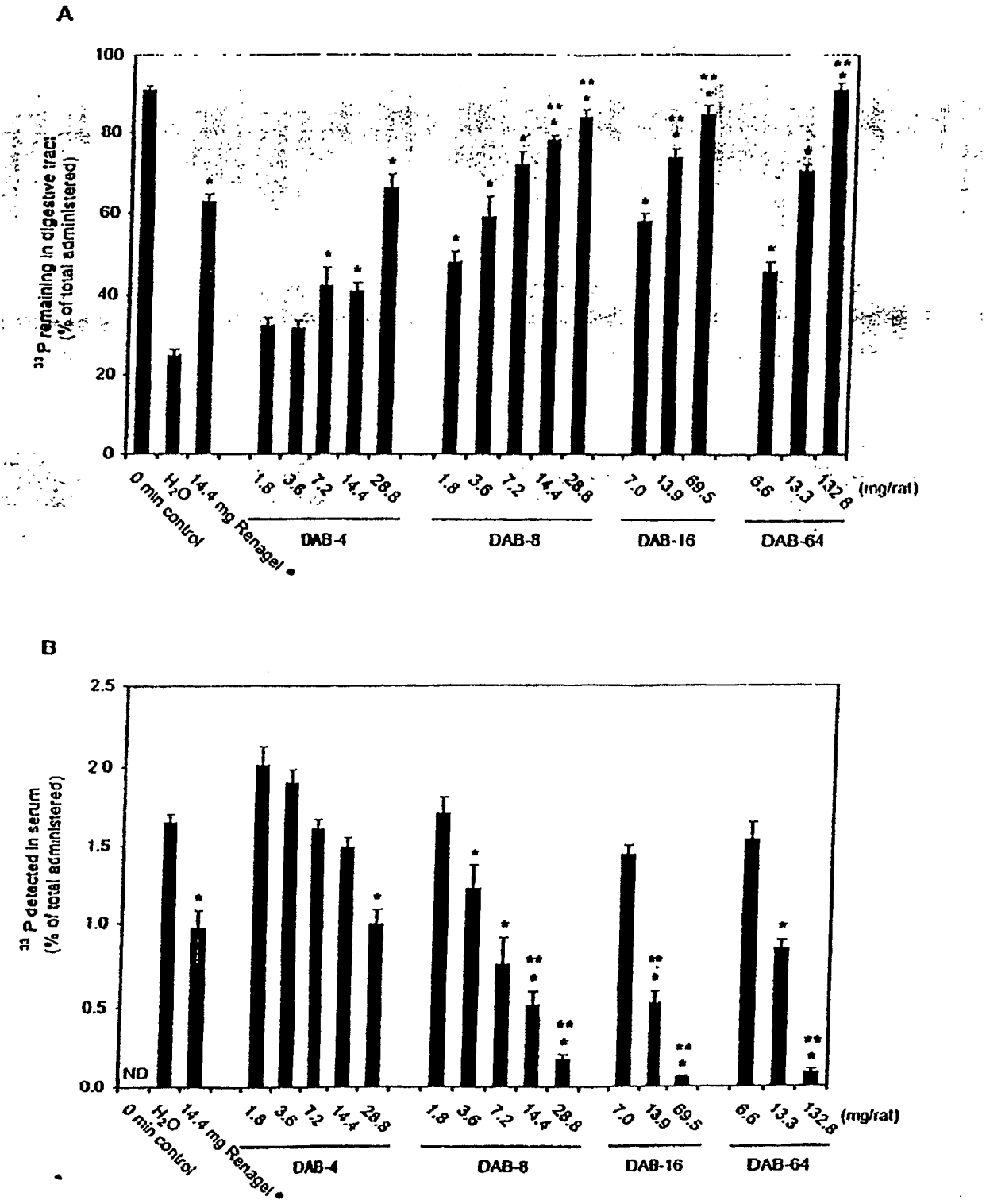

A
100
80
60
40
20
0
33P remaining in digestive tract (% of total administered)
0 min control
H2O
14.4 mg Renagel
1.8
3.6
7.2
14.4
28.8
1.8
3.6
7.2
14.4
28.8
7.0
13.9
69.5
6.6
13.3
132.8
(mg/rat)
DAB-4
DAB-8
DAB-16
DAB-64
B
2.5
2.0
1.5
1.0
0.5
0.0
33P detected in serum (% of total administered)
ND
0 min control
H2O
14.4 mg Renagel
1.8
3.6
7.2
14.4
28.8
1.8
3.6
7.2
14.4
28.8
7.0
13.9
69.5
6.6
13.3
132.8
(mg/rat)
DAB-4
DAB-8
DAB-16
DAB-64

Figure 4

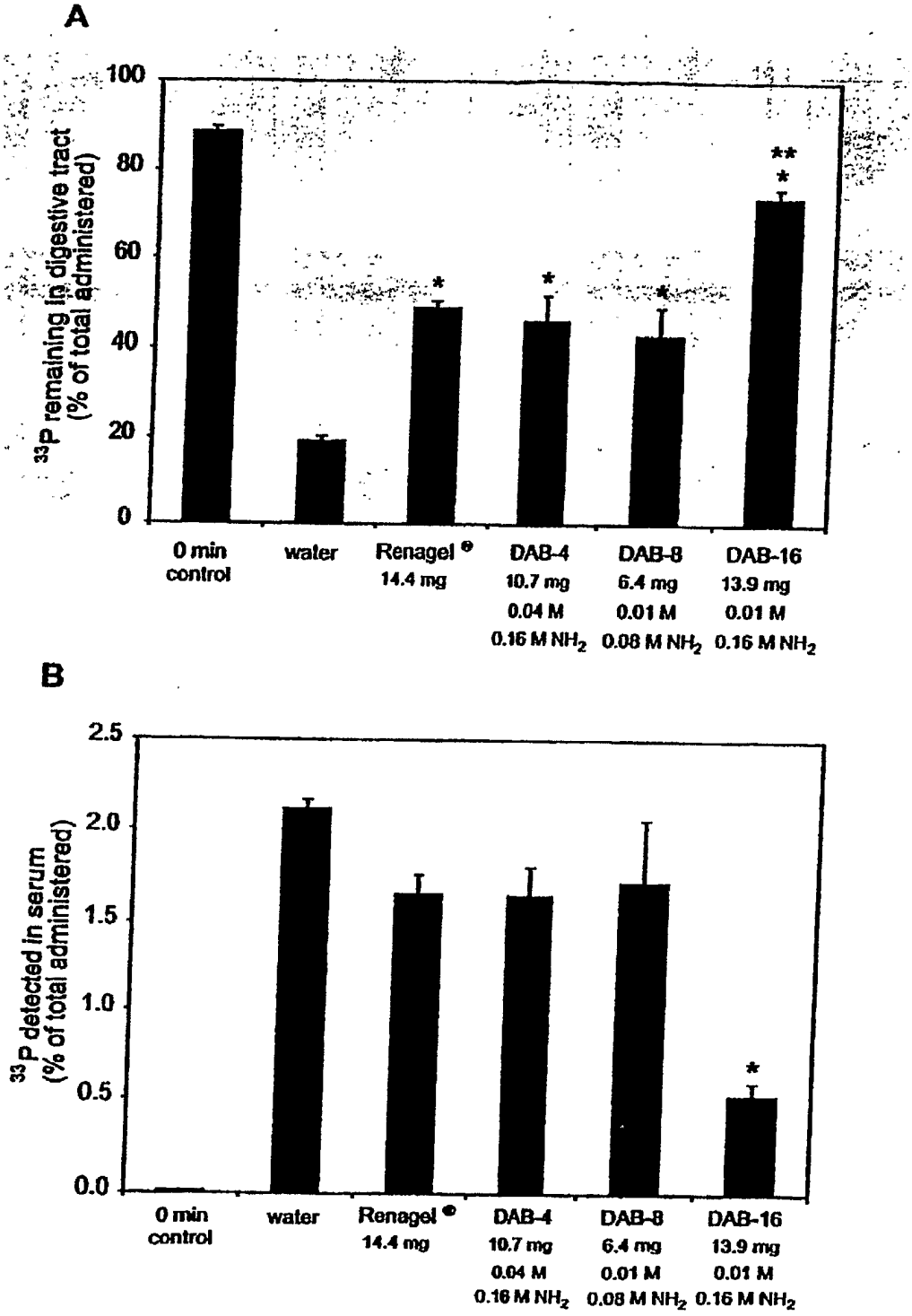

A
100
80
60
40
20
0
^{33}P remaining in digestive tract (% of total administered)
**
*
*
*
*
0 min control
water
Renagel ®
14.4 mg
DAB-4
10.7 mg
0.04 M
0.16 M NH2
DAB-8
6.4 mg
0.01 M
0.08 M NH2
DAB-16
13.9 mg
0.01 M
0.16 M NH2
B
2.5
2.0
1.5
1.0
0.5
0.0
^{33}P detected in serum (% of total administered)
*
0 min control
water
Renagel ®
14.4 mg
DAB-4
10.7 mg
0.04 M
0.16 M NH2
DAB-8
6.4 mg
0.01 M
0.08 M NH2
DAB-16
13.9 mg
0.01 M
0.16 M NH2

Figure 5

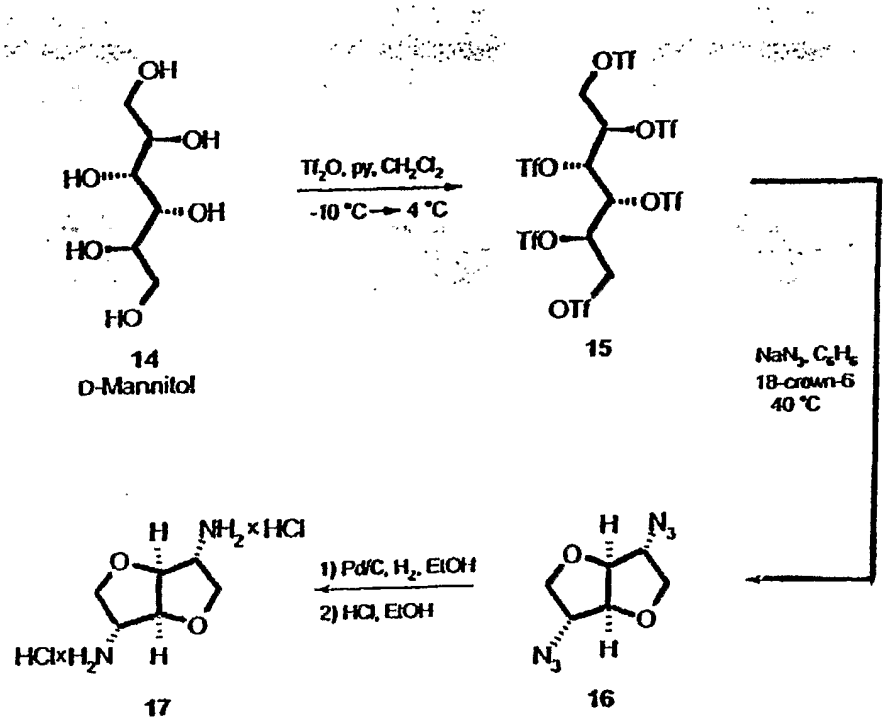

OH
OH
HO
OH
HO
HO
14
D-Mannitol
Tf2O, py, CH2Cl2
-10 °C → 4 °C
OTf
OTf
TfO
OTf
TfO
OTf
15
NaN3, C6H5
18-crown-6
40 °C
H
NH2×HCl
O
O
HClxH2N
H
17
1) Pd/C, H2, EtOH
2) HCl, EtOH
H
N3
O
O
N3
H
16

Figure 6

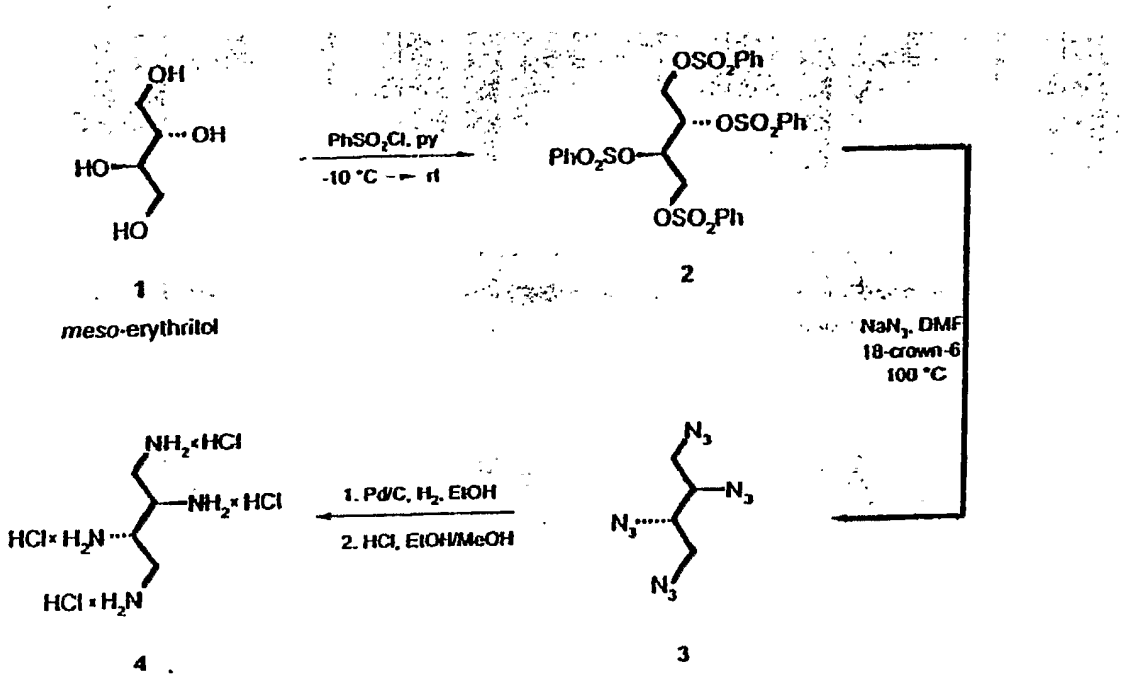


Figure 7

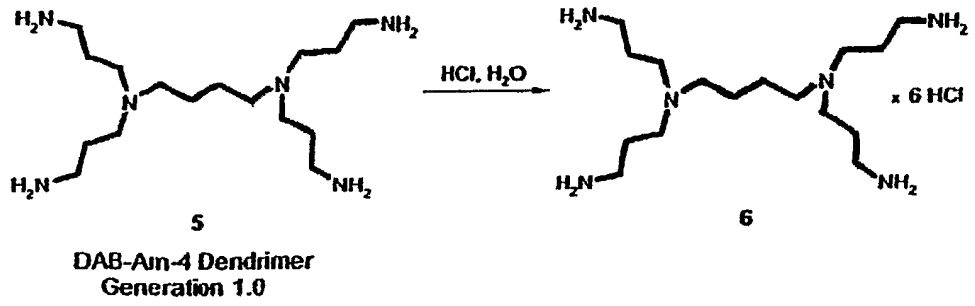
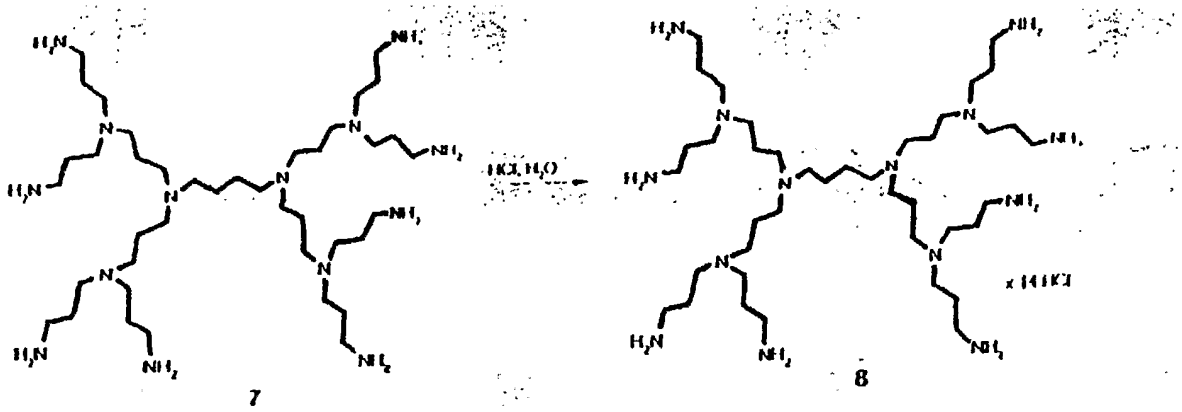


Figure 8

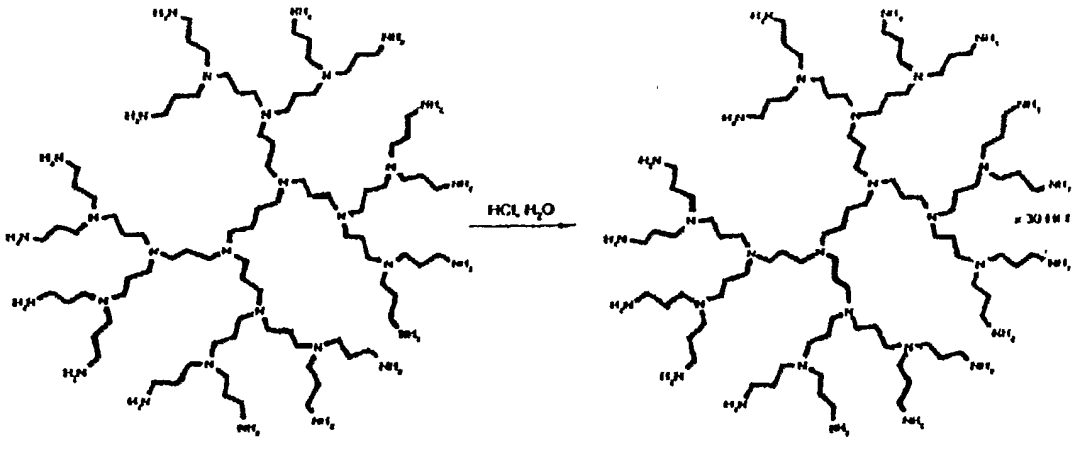


7

DAB-Am-8 Dendrimer
Generation 2.0

8

Figure 9



9

DAB Am-16 Dendrimer
Generation 3.0

10

Figure 10

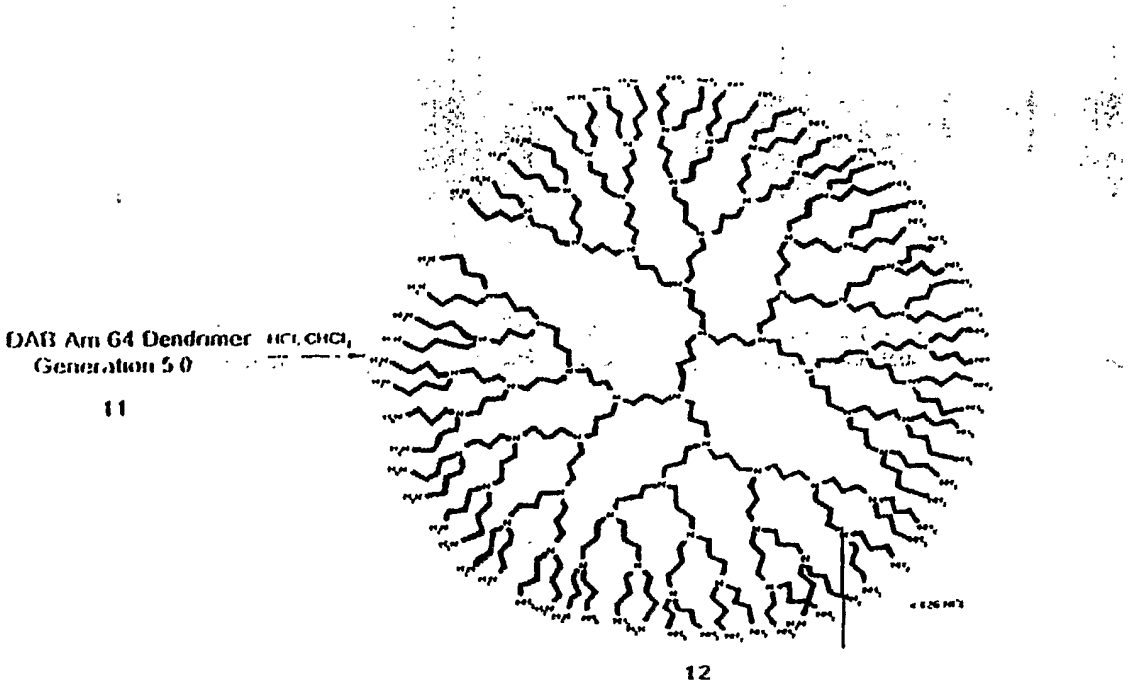


Figure 11

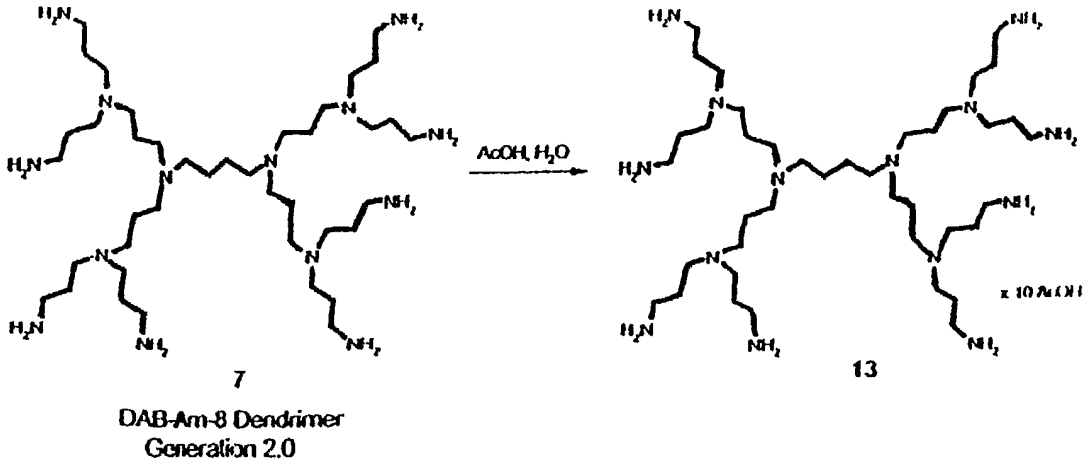


Figure 12

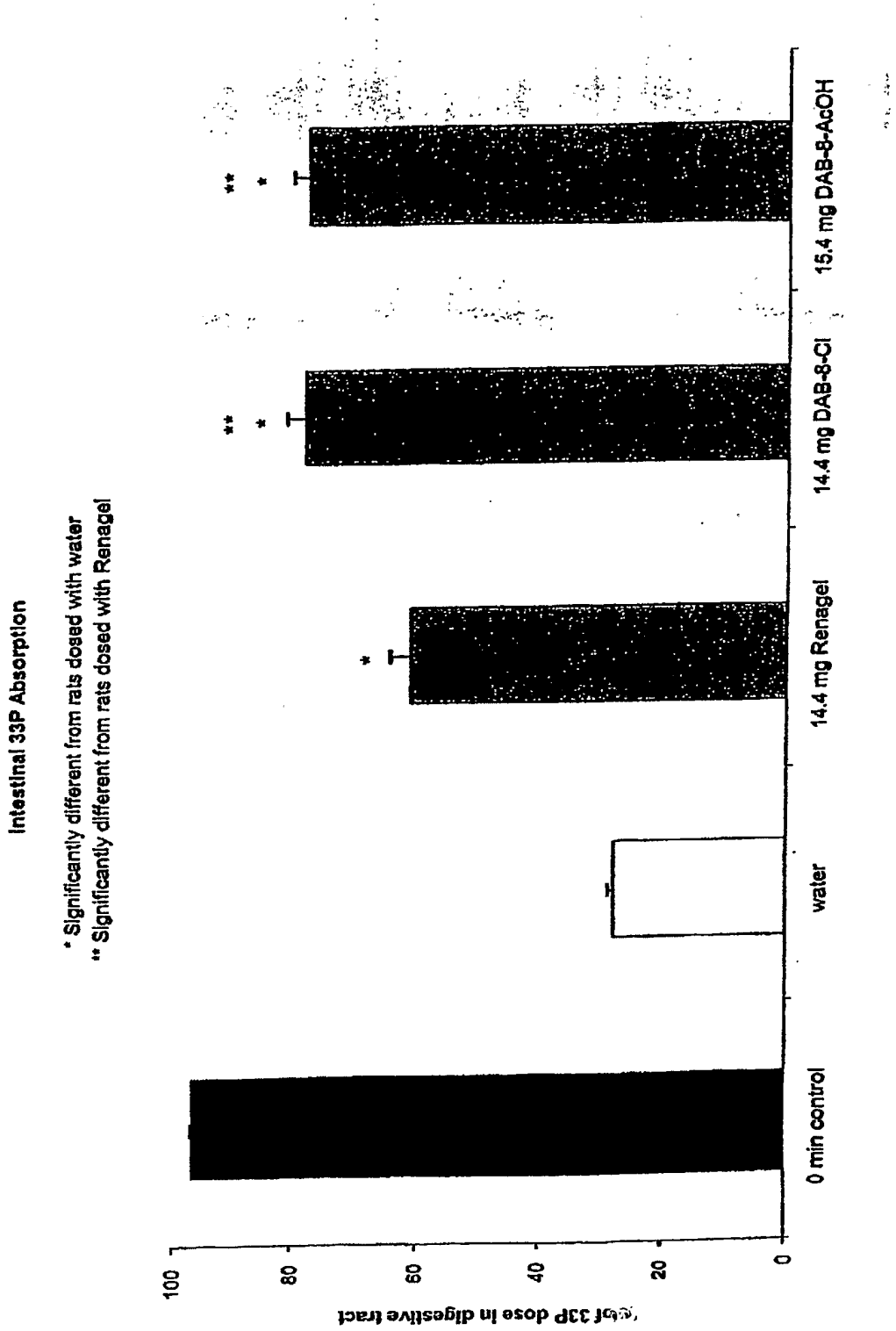

Intestinal 33P Absorption
* Significantly different from rats dosed with water
** Significantly different from rats dosed with Renagel
% of 33P dose in digestive tract
100
80
60
40
20
0
0 min control
water
14.4 mg Renagel
14.4 mg DAB-8-Cl
15.4 mg DAB-8-AcOH

Figure 13

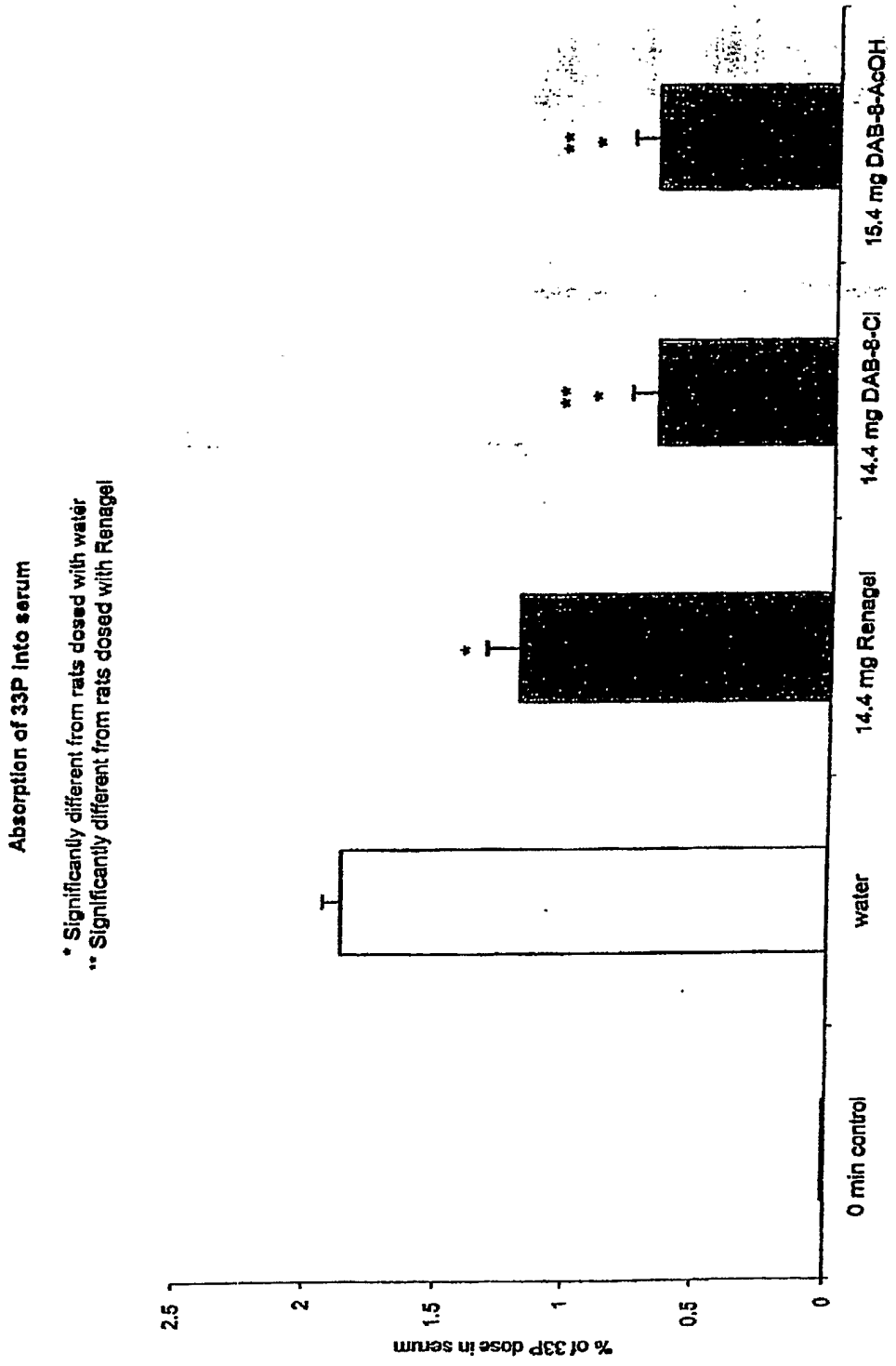

Absorption of 33P into serum
* Significantly different from rats dosed with water
** Significantly different from rats dosed with Renagel
% of 33P dose in serum
2.5
2
1.5
1
0.5
0
0 min control
water
14.4 mg Renagel
14.4 mg DAB-8-Cl
15.4 mg DAB-8-AcOH
*
**
*
**
*

Figure 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/035717

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/34 A61K31/132 A61P5/18

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZIMMER A ET AL: "Complex formation of Ni(II), Cu(II), Pd(II), and Co(III) with 1,2,3,4-tetraaminobutane." CHEMISTRY (WEINHEIM AN DER BERGSTRASSE, GERMANY) 2001, vol. 7, no. 4, 2001, pages 917-931, XP009076062 ISSN: 0947-6539 page 929, left-hand column, last paragraph	9,10
X	BACHMANN, FRANK ET AL: "Synthesis of novel polyurethanes and polyureas by polyaddition reactions of dianhydrohexitol configurated diisocyanates" MACROMOLECULAR CHEMISTRY AND PHYSICS , 202(17), 3410-3419 CODEN: MCHPES; ISSN: 1022-1352, 2001, XP009076061 Scheme 1	9

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2006	27/12/2006

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/035717

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COVASSIN L ET AL: "Synthesis of spermidine and norspermidine dimers as high affinity polyamine transport inhibitors" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 9, no. 12, 21 June 1999 (1999-06-21), pages 1709-1714, XP004167745 ISSN: 0960-894X page 1710 - page 1711	9
X	US 5 530 092 A (MEIJER EGBERT W [NL] ET AL) 25 June 1996 (1996-06-25) the whole document in particular column 5 - column 12	9-13
A	WO 2005/041902 A2 (ILYPSA INC [US]; CONNOR ERIC [US]; CHARMOT DOMINIQUE [US]; CHANG HAN T) 12 May 2005 (2005-05-12) paragraphs [0002], [0009], [0011], [0012], [0014], [0016], [0018], [0028], [0039], [0101], [0102] Claims	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/035717

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-8 and 14-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/035717

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5530092 A	25-06-1996	NONE	
WO 2005041902 A2	12-05-2005	AU 2004285609 A1	12-05-2005
		CA 2542730 A1	12-05-2005
		EP 1687349 A2	09-08-2006
		EP 1682606 A2	26-07-2006
		GB 2426248 A	22-11-2006
		US 2005239901 A1	27-10-2005
		US 2005147580 A1	07-07-2005
		WO 2005041900 A2	12-05-2005