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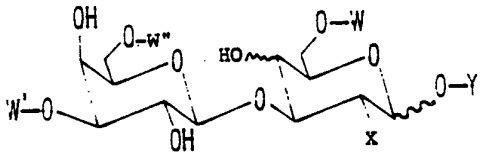

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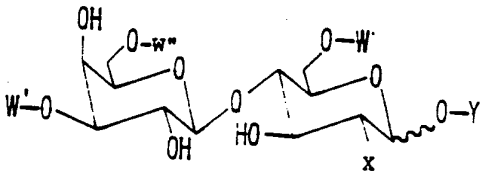

(II)

(57) Abstract

A composition for treating and/or inhibiting gastric and duodenal ulcers, comprising an oligosaccharide selected from Formula I, Formula II or mixtures thereof. Furthermore the compound of Formula II is not NAN alpha(2->3) Gal Beta 1-4 Glu or NAN alpha(2->6) Gal Beta 1-4 Glu. Compounds of the Formula I or Formula II detect the presence of *Helicobacter pylori* which is a microorganism that adheres to mammalian tissue. Also the compounds act to eliminate *Helicobacter pylori* from the stomach and duodenum.

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Description

Compositions For Treating and Inhibiting Gastric and Duodenal Ulcers

Related Applications:

This application is a Continuation-in-Part Application of U.S. Serial No. 07/922,519, filed on July 31, 1992, the entire contents of which are hereby incorporated by reference.

Technical Field

The present invention relates to compounds and compositions for treating and inhibiting gastric and duodenal ulcers, and to methods of treating and inhibiting gastric and duodenal ulcers.

Background Art

Infection by the gram-negative, spiral, microaerophilic bacterium *Helicobacter pylori* (*H. pylori*), formerly known as *Campylobactor pylori* (*C. pylori*), is a primary cause of non-autoimmune gastritis, is a factor in peptic ulcer disease and is more common in patients with gastric carcinoma. First isolated by Warren (Lancet (1983) 1:1273) and Marshal (Lancet (1983) 1:1273-5), *H. pylori* has been isolated in gastric tissue biopsies in patients throughout the world. While the precise mechanism of inflammation is not well understood, *H. pylori* is found in association with the apical surfaces of gastric mucous-secreting cells.

Due to the site specificity of attachment, it has been suggested that there are specific attachment sites for *H. pylori* which exist on gastric and duodenal mucous-secreting cells. Numerous studies have been undertaken to attempt to identify the specific binding site of *H. pylori*.

Evans et al (Infection and Immunity (1988) 56:2896-2906) reported that *H. pylori* binding to an erythrocyte receptor is preferentially inhibited by N-acetylneuraminyl-α(2→3)-Gal β1→4 Glc [NeuAc(2→3)-lactose] as compared with NeuAc(2→6)-lactose. Sialoproteins which contain the NeuAc(2→3)Gal isomer of NeuAc-lactose, i.e., human erythrocyte glycophorin A, fetuin, and human α_2-macroglobulin, also inhibited *H. pylori* binding, but at much higher concentrations (mg/ml) than that observed for NeuAc(2→3)-lactose, while no inhibition was observed for the corresponding asialoproteins.

These researchers further observed that NeuAc-lactose (also called sialyllactose) containing primarily the NeuAc(2→6)Gal isomer showed no inhibition of binding, leading the researchers to conclude that the receptor on the erythrocytes is a sialoprotein containing NeuAc(2→3)Gal. Although the NeuAc(2→3)Gal moiety, which Evans et al believed to be the specific site of binding for *H. pylori*, is a structure which occurs widely in nature, they rationalized the selective binding of *H. pylori* to be due to the unique gastrointestinal environment. This, in their view, accounts for the lack of further colonization by *H. pylori*.

Evans et al (Infection and Immunity (1989) 57:2272-2278) have also observed that *H. pylori* binds to monolayers of Y-1 mouse adrenal cells. But, this adherence can be prevented by pretreating the Y-1 cells with neuraminidase and is blocked by sialyllactose-containing fetuin.

Lingwood et al (Lancet (1989) 2:238-241) have reported the isolation of a gastric glycerolipid material which they observed to behave as a receptor for *H. pylori*. The material was isolated from red blood cells, and mucosal scrapings of pig stomach and human stomach. The investigators postulated that the material was a sulphated alkylacylglycero-lipid, but the actual structure of this material was not been reported. Subsequent investigations (Lingwood et al., Infection and

Immunity (1992) 60:2470-2474) showed that this receptor is phospatidylethanolamine.

Tzovelekis et al (Infection and Immunity (1991) 59:4252-4253) reported binding inhibition of *H. pylori* to HEp-2 cells by gastric mucin. The investigators observed that purified mucin showed the greatest inhibition of *H. pylori* binding while asialomucin exhibits somewhat diminished inhibition and periodate-oxidized mucin exhibited the lowest level of binding. On these observations, the researchers concluded that sialic acids are at least partially responsible for the binding interaction between *H. pylori* and human gastric mucin.

Thus the binding inhibition studies all point to a *H. pylori* binding specific receptor which possesses an N-acetylneuraminic acid (sialic acid) (Tzouvelekis et al and Evans et al) bound in a 2→3 manner to a lactose (Evans et al).

In addition to the numerous binding inhibition studies, methods have been pursued to treat gastric and duodenal ulcer patients. Colloidal bismuth subcitrate (CBS) has been used successfully in treating both gastric and duodenal ulcer diseases (for a review, see Lambert in Reviews of Infectious Diseases (1991) 13 (Suppl. 8):S691-5. CBS has proven effective as a histamine H2 antagonist and has been associated with lower relapse rates after cessation of therapy attributed to CBS's ability to eradicate *H. pylori*. Bismuth subsalicylate (BSS) has also been observed to inhibit *H. pylori*.

Additional studies in eliminating *H. pylori* have been conducted using the proton pump inhibitor, omeprazole.

Coleman et al (U.S. Patent No. 4,935,406) reported a method for relieving gastrointestinal disorder, resulting from *H. pylori* population, through the administration of bismuth (phosph/sulf)ated saccharide compositions. The saccharide

compositions according to this method are simple phosphates and sulfates of aldose and ketose monosaccharides.

Clinical trials have been reported (Evans et al, Ann. Internal Med. (1991) August 15, 115(4):266-9) in treating *H. pylori* using ranitidine in conjunction with a "triple therapy" of amoxicillin or tetracycline, metronidazole (an antiprotozoal), and BSS. The clinical studies suggested that ulcer healing was more rapid in patients receiving ranitidine plus the "triple therapy" than in patients receiving ranitidine alone.

However, long-term eradication of this organism has been difficult with these therapies. The antibiotic approach runs the risk of the development of new antibiotic resistant strains. Thus, a method of treating *H. pylori* with good long-term eradication has not yet been developed.

Disclosure of the Invention

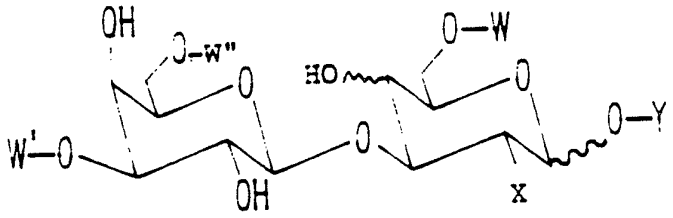
Accordingly, one object of the present invention is to provide novel compositions and methods for inhibiting and/or treating gastric and/or duodenal ulcers.

Another object of the present invention is to provide a method for inhibiting the adhesion of *Helicobacter pylori* to mammalian tissue, including eliminating *Helicobacter pylori* from the stomach and/or duodenum of a in need thereof patient.

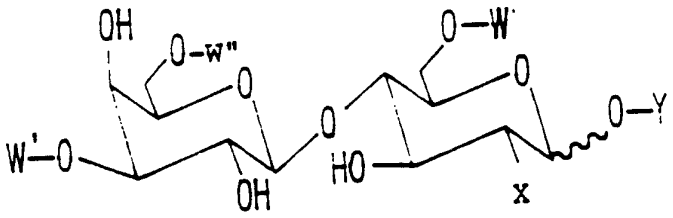
Another object of the present invention is to provide a kit for detecting the presence of *Helicobacter pylori* in a sample.

All of the above objects of the present invention and other objects which are apparent from the description of the invention given herein below have been discovered by the inventors to be satisfied by a composition comprising an

oligosaccharide selected from the group consisting of Formula I



; Formula II



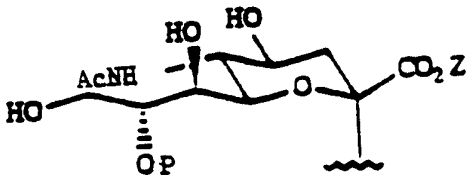
or a mixture thereof;

wherein:

X is independently OH or NHAc;

Y is independently H, or an amino acid or a peptide of 2-100, preferably 2-20, amino acids; and

W, W ′, and W" are each independently H or

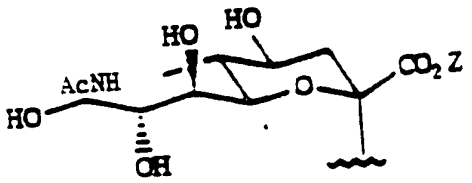


;

where Z is independently H or a pharmaceutically acceptable

cation; and

P is independently H or



where Z is defined as above;

wherein at least one of W, W′ or W" is an α-N-acetylneuraminic acid moiety, and

wherein W′ and W" are not simultaneously an α-N-acetylneuraminic acid moiety

with the proviso that the compound of Formula II is not NAN α(2→3)Gal β1-4 Glu or NAN α(2→6)Gal β1-4 Glu.

A composition containing at least one oligosaccharide of Formula I or Formula II alone, or in combination with an H2 blocker, an antibiotic and/or an antiulcerative compound, has been found by the inventors to be effective at inhibiting the binding of *Helicobacter pylori* to the gastric and duodenal mucosa and relieving the effects of gastric and duodenal ulcers.

Best Mode for Carrying Out the Invention

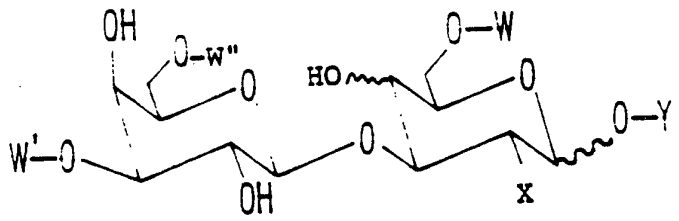
The following abbreviations are used throughout the text: "GalNAc" for N-Acetylgalactosamine; "Gal" for galactose; "Glc" for glucose; "GlcNAc" for N-Acetylglucosamine; "NAN" or "NeuAc" for N-Acetylneuraminic acid; and "ser" for serine.

In the oligosaccharide of Formula I, the OH group at the

4-position of the sugar on the right side of the molecule represents both the axial and equatorial epimeres. When the OH at the 4-position is in the axial position, then the sugar on the right side of the molecule is a Gal or GalNAc moiety. When the OH at the 4-position is in the equatorial position, then the sugar on the right side of the molecule is a Glc or GlcNAc moiety. Thus the core structure of Formula I represents either a Gal β1-3 Gal, Gal β1-3 GalNAc, Gal β1-3 Glc or Gal β1-3 GlcNAc.

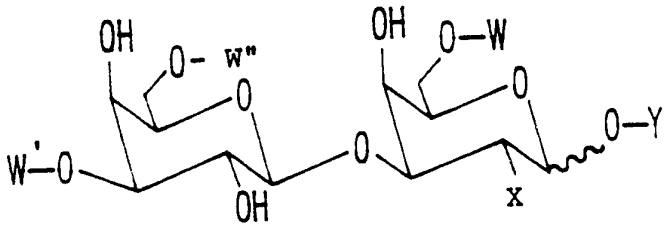
The oligosaccharides according to Formula I of the present invention comprise a core made up of an α-N-acetylneuraminic acid moiety bound *via* its 2-position to either the 3-position or 6-position of a β-galactose moiety, which, in turn, is bound *via* its 1-position to the 3-position of a galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety, which is bound *via* its 6-position to the 2-position of an α-N-acetylneuraminic acid. Alternatively, any of the α-N-acetylneuraminic acid groups, but not all simultaneously, may be replaced with H.

In a preferred embodiment, this core structure further comprises an amino acid bound to the oxygen at the 1-position of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety, to provide as shown below an oligosaccharide of Formula I:

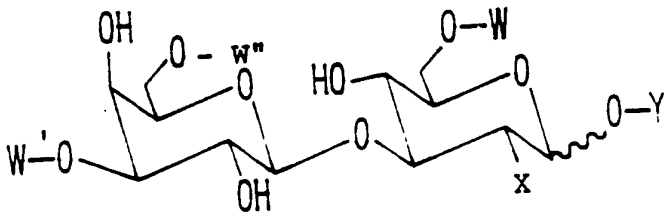


wherein X is OH or NHAc, Y is serine or threonine, W and W' are each an α-N-acetylneuraminic acid moiety, W" is H and Z is independently H or a known pharmaceutically acceptable cation.

Specifically, the oligosaccharide of Formula I may be Formula Ia



or Formula Ib



where X, Y, W, W', W", Z and P are as defined above for Formula I.

In other preferred embodiments, the oligosaccharide of Formula I is selected from the group NANα2-3Galβ1-3(NANα2-6)GalNAc, NANα2-3Galβ1-3(NANα2-6)GalNAcα1-ser, NANα2-3Galβ1-3GalNAc,

NANα2-6Galβ1-3(NANα2-6)GalNAc, NANα2-6Galβ1-3(NANα2-6)GalNAcα1-ser, NANα2-6Galβ1-3GalNAc, Galβ1-3(NANα2-6)GalNAc,

NANα2-3Galβ1-3(NANα2-6)Gal, NANα2-3Galβ1-3(NANα2-6)Galα1-ser, NANα2-3Galβ1-3Gal,

NANα2-6Galβ1-3(NANα2-6)Gal, NANα2-6Galβ1-3(NANα2-6)Galα1-ser, NANα2-6Galβ1-3Gal, Galβ1-3(NANα2-6)Gal,

NANα2-3Galβ1-3(NANα2-6)GlcNAc, NANα2-3Galβ1-3(NANα2-6)GlcNAcα1-ser, NANα2-3Galβ1-3GlcNAc,

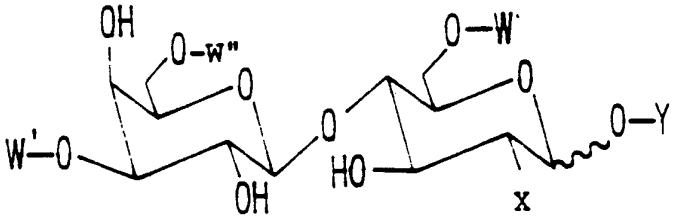
NANα2-6Galβ1-3(NANα2-6)GlcNAc, NANα2-6Galβ1-3(NANα2-6)GlcNAcα1-ser, NANα2-6Galβ1-3GlcNAc, Galβ1-3(NANα2-6)GlcNAc,

NANα2-3Galβ1-3(NANα2-6)Glc, NANα2-3Galβ1-3(NANα2-6)Glcα1-ser, NANα2-3Galβ1-3Glc,

NANα2-6Galβ1-3(NANα2-6)Glc, NANα2-6Galβ1-3(NANα2-6)Glcα1-ser, NANα2-6Galβ1-3Glc, Galβ1-3(NANα2-6)Glc and a mixture thereof.

The oligosaccharides according to Formula II of the present invention comprise a core made up of an α-N-acetylneuraminic acid moiety bound *via* its 2-position to either the 3-position or 6-position of a β-galactose moiety, which, in turn, is bound *via* its 1-position to the 4-position of a glucose or N-acetylglucosamine moiety, which is bound *via* its 6-position to the 2-position of an α-N-acetylneuraminic acid. Alternatively, any of the α-N-acetylneuraminic acid groups, but not all simultaneously, may be replaced with H. However, the compound of Formula II does not include NAN α(2→3)Gal β1-4 Glu or NAN α(2→6)Gal β1-4 Glu.

In a preferred embodiment, this core structure further comprises an amino acid bound to the oxygen at the 1-position of the glucose or N-acetylglucosamine moiety, to provide as shown below an oligosaccharide of Formula II:



wherein X is OH or NHAc, Y is serine or threonine, W and W' are each an α-N-acetylneuraminic acid moiety and Z is independently H or a known pharmaceutically acceptable cation.

In other preferred embodiments, the oligosaccharide of Formula I is selected from the group NANα2-3Galβ1-4(NANα2-6)GlcNAc, NANα2-3Galβ1-4(NANα2-6)GlcNAcα1-ser, NANα2-3Galβ1-4GlcNAc,

NANα2-6Galβ1-4(NANα2-6)GlcNAc, NANα2-6Galβ1-4(NANα2-6)GlcNAcα1-ser, NANα2-6Galβ1-4GlcNAc, Galβ1-4(NANα2-6)GlcNAc,

NANα2-3Galβ1-4(NANα2-6)Glc, NANα2-3Galβ1-4(NANα2-6)Glcα1-ser,

NANα2-6Galβ1-4(NANα2-6)Glc, NANα2-6Galβ1-4(NANα2-6)Glcα1-ser, Galβ1-4(NANα2-6)Glc and a mixture thereof.

In addition, for the oligosaccharide of Formula I or Formula II the group Y may represent an amino acid or peptide of from 2-100 amino acids, preferably 2-20 amino acids. It is noted that the glycoprotein fetuin, contains a peptide of at least 500 amino acids.

In addition, any one of or all of the free hydroxyl groups on the oligosaccharide of Formula I or Formula II may be acylated with a C_{1-6} acyl group by treatment with a suitable acylating agent such as acetyl chloride, propionyl chloride, butyryl chloride or acetic anhydride.

The α-N-acetylneuraminic acid moieties as they appear in Formula I or II may further be substituted at the 8-position with an α-N-acetylneuraminic acid moiety. Accordingly for all of the above-identified compounds of Formula I and Formula II any of the α-N-acetylneuraminic acid moieties may be replaced by NANα2-8NANα2-. This may be done by treating a mono α-N-acetylneuraminic acid compound of Formula I or II with CMP-NAN

and an α-N-acetylneuraminic acid transferase specific for transfer to an α-N-acetylneuraminic acid.

Of the bis sialylated oligosaccharides of the present invention, are for example the bis sialylated compounds of Formula II, NANα2-8NANα2-3Galβ1-4Glc and NANα2-8NANα2-6Galβ1-4Glc.

Any known suitable pharmaceutically acceptable cations may be used with the oligosaccharides of Formula I or Formula II, including the cations of conventional non-toxic salts including a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) or an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N, N'-dibenzylethylenediamine salt, etc.), an organic acid salt (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.), and the like.

The structure NANα2-3Galβ1-3(NANα2-6)GalNAc has been identified as an O-linked carbohydrate found in bovine Factor X (Mizvochi et al, J. Biol. Chem. (1977) 255:3526), bovine high-molecular weight ininogen (Endo et al, J. Biochem. Tokyo (1977) 82:545), human plasminogen, (Hayes et al, J. Biol. Chem. (1979) 254:8777), immunoglobulin (Chandraskaren et al, J. Biol. Chem., (1981) 256:1549), the β-subunit of human chorionic gonadotropin, (M. Kessler et al, J. Biol. Chem. (1979) 254:7909), bovine fetuin (R. Spiro et al, J. Biol. Chem. (1974) 249:5704) and human apolipoprotein C-III (P. Yaith, Biochem. Biophys. Acta (1978) 541:234).

The oligosaccharide of the present invention may be

obtained using any known method, including (1) enzymatically, using one of the inventor's method described in published international application WO 91/16449, (2) synthetically, using classical organic chemistry, or (3) by degradation of a natural occurring oligosaccharide, glycolipid, or glycopeptide.

The compound NANα2-3Galβ1-3(NANα2-6)GalNAc-itol, wherein the acetal of the terminal GalNAc has been reduced to the primary alcohol, may be obtained by alkaline hydrolysis and $NaBH_4$ reduction of fetuin. Illustrative hydrolysis conditions consist of reacting fetuin in 0.05 M NaOH and 1M $NaBH_4$ at 50°C for 16h in water.

The compound NANα2-3Galβ1-3(NANα2-6)GalNAc-peptide may be obtained by protease mediated degradation of fetuin in aqueous solution at about 50°C.

However, as a pharmaceutically acceptable source of this compound, the hydrolysis product of fetuin may be disfavored due to the possible presence of mad cow's disease. While purification and treatment techniques can rule out transfer of this virus to the composition, enzymatic synthesis is still preferred.

The present oligosaccharide may be administered in conjunction with a known proton pump inhibitor or a known H2 receptor antagonist. A representative proton pump inhibitor is omeprazole, and representative H_2 antagonists include cimetidine, ranitidine, nizatidine and famotidine. The amount of proton pump inhibitor and H_2 antagonist administered in conjunction with the present oligosaccharide is about the same amount administered for their known therapy. Accordingly, effective dosages of the proton pump inhibitor and H_2 can be determined by routine experimentation.

Alternatively a known antiulcerative compound may be used

in conjunction with or as a replacement for the H2 receptor antagonist. Suitable antiulceratives include aceglutamide aluminum complex, ϵ-acetamidocaproic acid zinc salt, acetoxolone, arbaprostil, benexate hydrochloride, bismuth subcitrate sol, carbenoxolone, cetraxate, cimetidine, enprostil, esaprazole, famotidine, ftaxidide, gefarnate, guaiazulene, irsogladine, misoprostol, nazatidine, ornoprostil, γ-oryzanol, pifarnine, pirenzepine, plaunotol, ranitidine, rioprostil, rosaprostol, rotraxate, roxatidine acetate, sofalcone, spizofurone, sucralfate, teprenone, trimoprostil, trithiozine, troxipide, and zolimidine. The amount of antiulcerative administered in conjunction with the present oligosaccharide is about the same amount administered for its known therapy. Accordingly, effective dosage of the antiulcerative can be determined by routine experimentation.

Alternatively, the present oligosaccharide may be administered in conjunction with an antibiotic with activity against *H. pylori*. Suitable antibiotics include metronidazole, tetracycline, bismuth, erythromycin, macrolide, quinoline and amoxicillin. The amount of antibiotic administered in conjunction with the present oligosaccharide is about the same amount administered for its known therapy. Accordingly, effective dosage of the antibiotic can be determined by routine experimentation.

The anti-*H. pylori* compositions of the present invention contains one or a plurality of oligosaccharides of Formula I or Formula II in association with any suitable liquid or solid, pharmaceutically acceptable carrier or excipient, preferable in a form suitable for oral or enteral administration. In addition, the anti-*H. pylori* compositions of the present invention are preferably pyrogen free.

The anti-*H. pylori* compositions are usually administered as a mixture with a carrier suitably selected depending upon the route for administration using standard formulations. For

example, the compound of the present invention may be administered in the form of tablets which may be prepared using known techniques by adding to a powder of the active ingredient of the present invention an excipient such as starch, lactose, sucrose, glucose, crystalline cellulose, calcium carbonate or kaolin, a hydroxypropylcellulose, a glucose solution, a sucrose solution, water or ethanol, a disintegrator such as starch, agar, gelatin powder, carboxymethylcellulose calcium (CMC-Ca), carboxymethylcellulose sodium (CMC-Na), crystalline cellulose, calcium carbonate or sodium hydrogencarbonate, or a lubricant such as magnesium stearate, calcium stearate, talc, macrogoal 4,000, macrogoal 6,000 or stearic acid.

The mixture is then subjected to compression molding by a conventional tableting method, and if necessary, applying a sugar coating by means of a concentrated sugar solution containing e.g. gum arabic, talc, polyvinylpyrrolidone, polyethyleneglycol and/or titanium oxide, applying a film coating by means of a film-forming agent composed of e.g. polyvinyl acetal diethylaminoacetate, hydroxypropylmethylcellulose, hydroxypropylcellulose, ethylcellulose or polyvinylpyrrolidone or applying an enteric coating by means of a film-forming agent composed of e.g. ethylcellulose phthalate, cellulose acetate phthalate or hydroxypropylmethylcellulose phthalate.

These pharmaceutical compositions may be in the form of granules or fine granules which may be prepared by adding to the active ingredient of the present invention a binder such as starch, gelatin, gum arabic, methylcellulose, sodium carboxymethylcellulose, heavy silicic anhydride or light silicic anhydride, followed by kneading and granulation by usual methods; or as a powder of the active ingredient of the present invention by itself; or as capsules which may be prepared by adding to the active ingredient of the present invention an excipient such as lactose, starch or crystalline

cellulose and/or a lubricant such as magnesium stearate, calcium stearate or talc, and filling the mixture into capsules.

A solution or suspension may be prepared by adding any diluent customarily, used in the art. For example, suitable diluents include water, ethyl alcohol, propylene glycol, polyoxyethylene sorbitol, and sorbitan esters. Sodium chloride, glucose or glycerol may be incorporated into such a liquid preparation in an amount sufficient to prepare an isotonic solution. The therapeutic composition may also further contain ordinary dissolving aids, buffers, pain-alleviating agents, art preservatives, and optionally coloring agents, fragrances, flavors, sweeteners and other pharmacologically active agents such are well known in the art.

Suitable compositions may take the form of a solution, suspension, tablet, coated tablet or any pharmaceutically acceptable form suitable for delivery to the stomach or duodenum.

According to a preferred embodiment of the present invention, the oligosaccharide or anti-*H. pylori* compositions are administered enterally to a patient in need thereof to inhibit *H. pylori* binding or eliminate *H. pylori* colonies from the patient's stomach and/or duodenum.

Typically, suitable patients are humans. However the present method is also applicable to treatment of animals, including but not limited to mammals such as cows, horses, sheep, goats, dogs, cats, rodents and non-human primates, fowl such a chickens, turkeys and ducks, and fish.

Suitable amounts of the composition to be administered include those which produce an effective stomach concentration of oligosaccharide of from 1 μg to 10,000 mg/ml per dose,

preferably 100 to 1000 μg/ml. When a proton pump inhibitor, H2 antagonist, or antiulcerative is coadministered, the composition is formulated to provide between 10-500 mg, preferably 100-300 mg of the proton pump inhibitor, H2 antagonist, or antiulcerative daily. Dosage forms include such unit dosage forms such as tablets, capsules, solutions or suspensions.

In another embodiment of this invention the oligosaccharide of Formula I or Formula II is provided as a multivalent molecule. In this embodiment, at least one type of the oligosaccharide of Formula I or Formula II is bound to a polymer using known techniques so as to produce a polymer to which more than one individual molecules of the oligosaccharide of Formula I or Formula II are covalently attached. The polymer backbone is sufficiently long to provide a multivalent molecule leaving from between 2-1,000, preferably 10-100, more preferably 20-30 molecules of the compound of Formula I or Formula II bound to the polymer.

The oligosaccharide of Formula I or Formula II is preferably bound to the polymer *via* the free anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II when Y = H, or the peptide when Y = peptide. Suitable polymers are any polymer material which may be reacted with the anomeric carbon of a saccharide, such as a polylysine, a polyacrylamide or a cyclodextrin.

For example, the oligosaccharide of Formula I or Formula II may be bound to a support to form a bead wherein the surface of the bead is bound with the compound of Formula I or Formula II.

Another embodiment of this invention, which relates to one of the inventor's application serial no. 07/241,012, filed September 2, 1988, provides a kit for detecting *H. pylori*. The kit is analogous to a kit for performing ELISAs, but uses a compound of Formula I or Formula II which is bound to a solid support, instead of the antigens or antibodies bound to solid supports normally found with ELISA kits. The kit comprises a container, and contained therein, the compound of Formula I or Formula II bound to a solid support. The compound of Formula I or Formula II is bound to a polymer support through the anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II in the case where Y = H and through the amino acid or the peptide when Y = amino acid or peptide. A sample to be tested for *H. pylori* is contacted with the bound compound of Formula I or Formula II. Bound *H. pylori* may be detected by standard means such as labeled antibodies.

* * *

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

* * *

Example 1. Synthesis of tetrasaccharide NANα2-3 Gal β1-3(NANα2-6)GalNAc

An aqueous solution of equimolar equivalents of galNAc and UDP-gal is stirred at 40°C in the presence of a β 1-3 galactosyltransferase for a sufficient time to effect coupling of the two moieties. The β 1-3 galactosyltransferase is isolated from porcine submaxillary glands. Next, three molar equivalents of CMP-NAN are introduced along with an α 2-6 sialyltransferase, to form the monosialo compound. Finally an

α 2-3 sialyltransferase is introduced in the presence of three more equivalents of CMP-NAN to form the tetrasaccharide.

Example 2. Synthesis of glycopeptidesaccharide NANα2-3 Gal β1-3(NANα2-6) GalNAcβ1-Serine

An aqueous solution of equimolar equivalents of galNAc O-linked to serine and UDP-gal is stirred at 40°C in the presence of a β 1-3 galactosyltransferase for a sufficient time to effect coupling of the two moieties. The β 1-3 galactosyltransferase is isolated from porcine submaxillary glands. Next three molar equivalents of CMP-NAN are introduced along with an α 2-6 sialyltransferase to form the monosialo compound. Finally an α 2-3 sialyltransferase is introduced in the presence of three more equivalents of CMP-NAN to form the glycopeptidesaccharide.

Example 3

An anti-*Helicobacter* composition is prepared by mixing 100 mg of the tetrasaccharide of Example 1 with 250 mg of the H_2 receptor antagonist ranitidine. The mixture is then suspended in a mixture of water and propylene glycol.

Example 4

An anti-*Helicobacter* composition is prepared by mixing 100 mg of the tetrasaccharide of Example 1 with 250 mg of the proton pump inhibitor omeprazole. The mixture is then suspended in a mixture of water and propylene glycol.

Example 5

An anti-*Helicobacter* composition is prepared by mixing 100 mg of the tetrasaccharide of Example 1 with 250 mg of a combination of metronidazole, tetracycline, and amoxicillin. The mixture is then suspended in a mixture of water and

propylene glycol.

Example 6

As a therapeutic treatment, a patient infected with *H. pylori* is treated with the composition of Example 3. The patient is treated orally four times daily with each dosage providing an effective stomach concentration of 100 μg/ml. Therapy is continued for two weeks, after which examination showed eradication of the *H. pylori* bacteria. After eradication, maintenance therapy with the composition of the present invention is continued to prevent recurrence.

Example 7. Synthesis of tetrasaccharide NANα2-3 Gal β1-4(NANα2-6)GlcNAc

An aqueous solution of equimolar equivalents of glcNAc and UDP-gal is stirred at 40°C in the presence of a β 1-4 galactosyltransferase for a sufficient time to effect coupling of the two moieties. The β 1-4 galactosyltransferase is isolated from porcine submaxillary glands. Next three molar equivalents of CMP-NAN are introduced along with an α 2-6 sialyltransferase, to form the monosialo compound. Finally an α 2-3 sialyltransferase is introduced in the presence of three more equivalents of CMP-NAN to form the tetrasaccharide.

Example 8. Synthesis of tetrasaccharide NANα2-3 Gal β1-3(NANα2-6)GlcNAc

An aqueous solution of equimolar equivalents of glcNAc and UDP-gal is stirred at 40°C in the presence of a β 1-3 galactosyltransferase for a sufficient time to effect coupling of the two moieties. The β 1-3 galactosyltransferase is isolated from porcine submaxillary glands. Next three molar equivalents of CMP-NAN are introduced along with an α 2-6 sialyltransferase, to form the monosialo compound. Finally an α 2-3 sialyltransferase is introduced in the presence of three

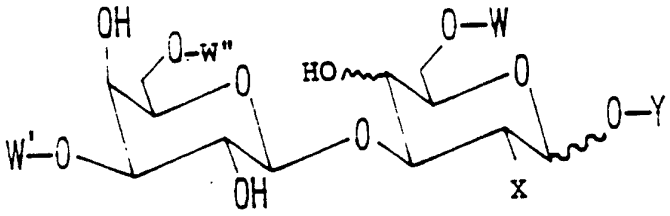
more equivalents of CMP-NAN to form the tetrasaccharide.

* * * *

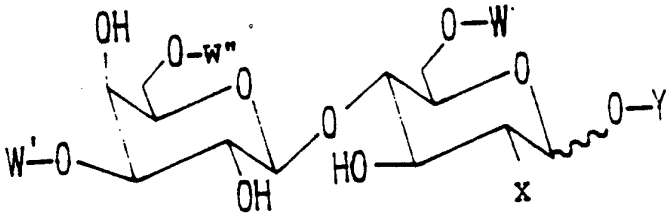
Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

Claims

1. A method of treating or inhibiting an ulcer in the stomach or duodenum of a patient in need thereof, comprising administering to the stomach or duodenum of said patient, an effective amount of a composition comprising an oligosaccharide selected from the group consisting of Formula I



; Formula II



or a mixture thereof;

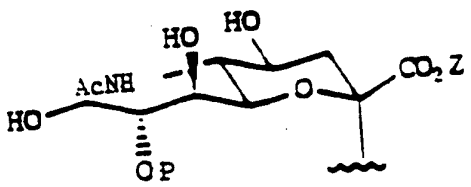
wherein:

X is independently OH or NHAc;

Y is independently H, or an amino acid or a peptide of 2-100, preferably 2-20, amino acids; and

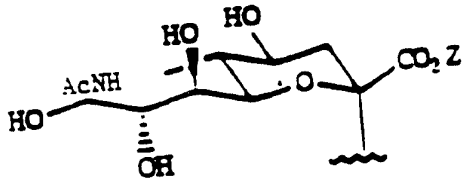
W, W ', and W" are each independently H or

;



where Z is independently H or a pharmaceutically acceptable cation; and

P is independently H or



where Z is defined as above;

wherein at least one of W, W′ or W" is an α-N-acetylneuraminic acid moiety, and

wherein W′ and W" are not simultaneously an α-N-acetylneuraminic acid moiety

with the proviso that the compound of Formula II is not NAN α(2→3)Gal β1-4 Glu or NAN α(2→6)Gal β1-4 Glu.

2. The method of Claim 1, wherein X is OH.

3. The method of Claim 1, wherein X is NHAc.

4. The method of Claim 1, wherein Y is H.

5. The method of Claim 1, wherein Y is serine or threonine.

6. The method of Claim 1, wherein Y is an amino acid or 2 to 20 amino acids long peptide.

7. The method of Claim 1, wherein Y is a peptide of from 2 to 20 amino acids.

8. The method of Claim 1, wherein at least one of Z is H.

9. The method of Claim 1, wherein at least one of Z is a pharmaceutically acceptable cation.

10. The method of Claim 1, comprising inhibiting a gastric ulcer in said patient.

11. The method of Claim 1, comprising inhibiting a duodenal ulcer in said patient.

12. The method of Claim 1, comprising treating a gastric ulcer in said patient.

13. The method of Claim 1, comprising treating a duodenal ulcer in said patient.

14. The method of Claim 1, comprising coadministering an H2 blocker to said patient.

15. The method of Claim 1, comprising coadministering an antiulcerative compound to said patient.

16. The method of Claim 1, comprising coadministering a proton pump inhibitor to said patient.

17. The method of Claim 1, comprising coadministering an antibiotic compound to said patient.

18. The method of Claim 1, wherein a plurality of molecules of said oligosaccharide are covalently bonded to a pharmaceutically acceptable polymer *via* the free anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II when Y is H and *via* the amino acid or the 2 to 100 amino acids-long peptide moiety

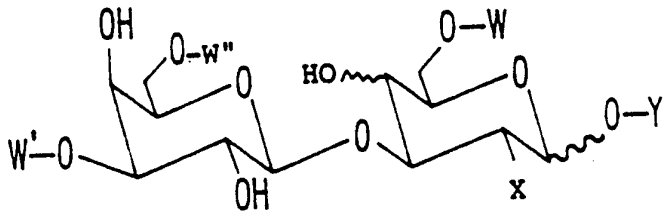
when Y is an amino acid or a 2 to 100 amino acids-long peptide.

19. The method of Claim 18, wherein said pharmaceutically acceptable polymer is a polylysine, a polyacrylamide or a cyclodextrin.

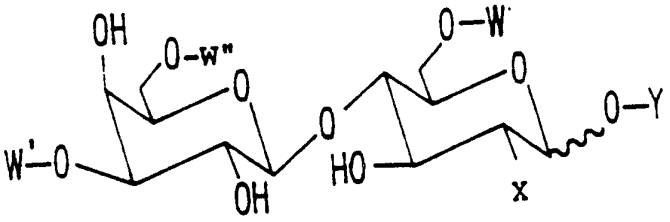
20. The method of Claim 10, wherein a plurality of molecules of said oligosaccharide are covalently bonded to a pharmaceutically acceptable polymer, *via* the free anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II when Y is H and *via* the amino acid or the 2 to 100 amino acids-long peptide moiety when Y is an amino acid or a 2 to 100 amino acids-long peptide.

21. The method of Claim 20, wherein said pharmaceutically acceptable polymer is a polylysine, a polyacrylamide or a cyclodextrin.

22. A composition comprising, in association with a carrier or excipient suitable for enteral administration, an amount effective for *Helicobacter pylori* binding inhibition, of a composition comprising an oligosaccharide selected from the group consisting of Formula I



; Formula II



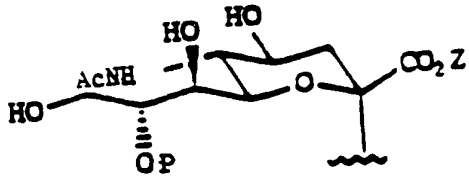
or a mixture thereof;

wherein:

X is independently OH or NHAc;

Y is independently H, or an amino acid or a peptide of 2-100, preferably 2-20, amino acids; and

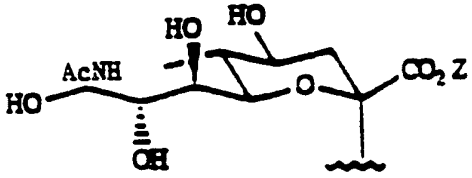
W, W ′, and W" are each independently H or



;

where Z is independently H or a pharmaceutically acceptable cation; and

P is independently H or



where Z is defined as above;

wherein at least one of W, W′ or W" is an α-N-acetylneuraminic acid moiety, and

wherein W′ and W" are not simultaneously an α-N-

acetylneuraminic acid moiety

with the proviso that the compound of Formula II is not NAN $\alpha(2\rightarrow3)$Gal β1-4 Glu or NAN $\alpha(2\rightarrow6)$Gal β1-4 Glu.

23. The composition of Claim 22, further comprising an amount effective for H2 blocking of an H2 blocker.

24. The composition of Claim 23, wherein said H2 blocker is selected from the group consisting of cimetidine, octreotide, enterogastrone, ranitidine, nizatidine and famotidine.

25. The composition of Claim 22, further comprising an antiulcerative amount of an antiulcerative compound.

26. The composition of Claim 22, further comprising a proton pump inhibiting amount of a proton pump inhibitor.

27. The composition of Claim 26, wherein said proton pump inhibitor is omeprazole.

28. The composition of Claim 22, further comprising an antibiotic effective amount of an antibiotic effective against *H. pylori*.

29. The composition of Claim 28, wherein said antibiotic is selected from the group consisting of metronidazole, tetracycline, bismuth, erythromycin, macrolide, quinoline, amoxicillin and a mixture thereof.

30. A solid composition according to Claim 22.

31. A liquid composition according to Claim 22.

32. The composition of Claim 22, wherein X is OH.

33. The composition of Claim 22, wherein X is NHAc.

34. The composition of Claim 22, wherein Y is H.

35. The composition of Claim 22, wherein Y is an amino acid or 2 to 100 amino acids-long peptide.

36. The composition of Claim 35 wherein Y is a peptide of from 2 to 20 amino acids.

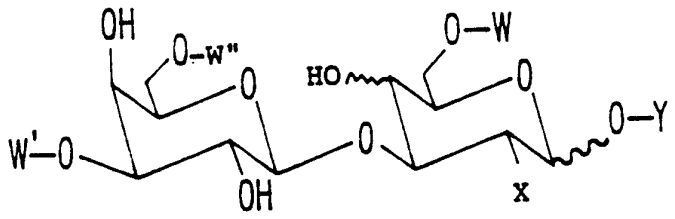
37. The composition of Claim 22, wherein at least one of Z is H.

38. The composition of Claim 22, wherein at least one of Z is a pharmaceutically acceptable cation.

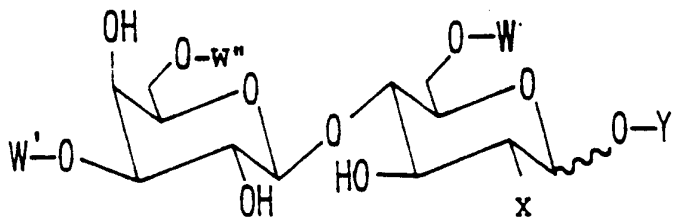
39. A kit for determining the presence of *Helicobacter pylori* in a sample, comprising:

(i) a container and

(ii) an oligosaccharide selected from the group consisting of Formula I



; Formula II



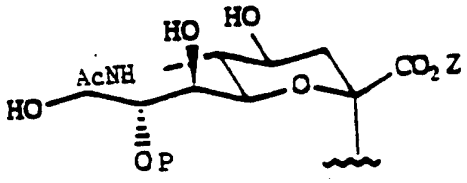
or a mixture thereof;

wherein:

X is independently OH or NHAc;

Y is independently H, or an amino acid or a peptide of 2-100, preferably 2-20, amino acids; and

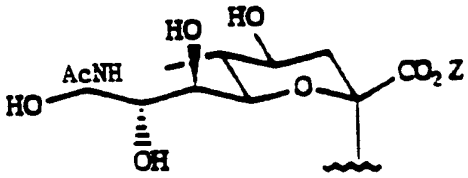
W, W ′, and W" are each independently H or



;

where Z is independently H or a pharmaceutically acceptable cation; and

P is independently H or



where Z is defined as above;

wherein at least one of W, W′ or W" is an α-N-acetylneuraminic acid moiety, and

wherein W′ and W" are not simultaneously an α-N-acetylneuraminic acid moiety

with the proviso that the compound of Formula II is not NAN α(2→3)Gal β1-4 Glu or NAN α(2→6)Gal β1-4 Glu.

40. The kit of claim 39 wherein X is OH.

41. The kit of claim 39 wherein X is NHAc.

42. The kit of claim 39 wherein Y is H.

43. The kit of claim 39 wherein Y is an amino acid or a 2 to 100 amino acids-long peptide.

44. The kit of claim 43, wherein Y is a peptide of from 2 to 20 amino acids.

45. The kit of claim 39 wherein at least one of Z is H.

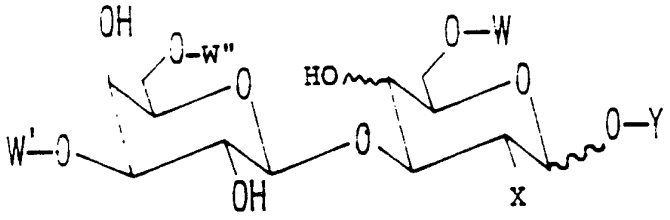
46. The kit of claim 39 wherein at least one of Z is a pharmaceutically acceptable cation.

47. The kit of Claim 39, wherein a plurality of said oligosaccharide, covalently bonded to a pharmaceutically acceptable polymer *via* the free anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II when Y is H and *via* the amino acid or the 2 to 100 amino acids-long peptide moiety when Y is an amino acid or a 2 to 100 amino acids-long peptide, are bound to said solid support.

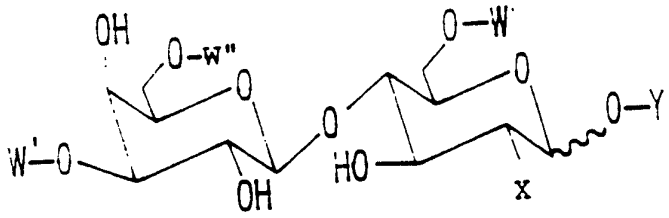
48. The kit of Claim 47, wherein said pharmaceutically acceptable polymer is a polylysine, a polyacrylamide or a cyclodextrin.

49. A method of eliminating *Helicobacter pylori* from the stomach or duodenum of a patient in need thereof, comprising administering to said patient an *Helicobacter pylori*

eliminating effective amount of composition comprising an oligosaccharide selected from the group consisting of Formula I



; Formula II



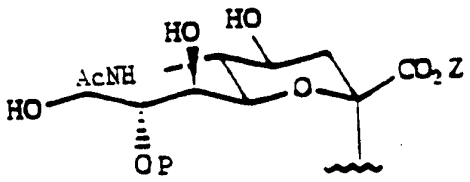
or a mixture thereof;

wherein:

X is independently OH or NHAc;

Y is independently H, or an amino acid or a peptide of 2-100, preferably 2-20, amino acids; and

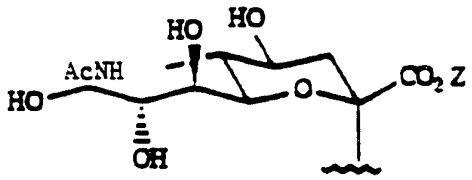
W, W ′, and W" are each independently H or



;

where Z is independently H or a pharmaceutically acceptable cation; and

P is independently H or



where Z is defined as above;

wherein at least one of W, W′ or W″ is an α-N-acetylneuraminic acid moiety, and

wherein W′ and W″ are not simultaneously an α-N-acetylneuraminic acid moiety

with the proviso that the compound of Formula II is not NAN α(2→3)Gal β1-4 Glu or NAN α(2→6)Gal β1-4 Glu.

50. The method of Claim 49, wherein X is OH.

51. The method of Claim 49, wherein X is NHAc.

52. The method of Claim 49, wherein Y is serine or threonine.

53. The method of Claim 49, wherein Y is an amino acid or a 2 to 20 amino acids long peptide.

54. The method of Claim 49, wherein at least one of Z is H.

55. The method of Claim 49, wherein at least one of Z is a pharmaceutically acceptable cation.

56. The method of Claim 49, wherein said patient has been diagnosed as having a duodenal ulcer.

57. The method of Claim 49, wherein said patient has been diagnosed as having a gastric ulcer.

58. The method of Claim 49, wherein said administration is oral.

59. The method of Claim 49, wherein said administration is enteral.

60. The method of Claim 49, wherein a plurality of said oligosaccharide are covalently bonded to a pharmaceutically acceptable polymer, *via* the free anomeric carbon of the galactose, N-acetylgalactosamine, glucose or N-acetylglucosamine moiety of Formula I or the glucose or N-acetylglucosamine moiety of Formula II when Y is H and *via* the amino acid or the 2 to 100 amino acids-long peptide moiety when Y is an amino acid or a 2 to 100 amino acids-long peptide.

61. The method of Claim 60, wherein said pharmaceutically acceptable polymer is a polylysine, a polyacrylamide or a cyclodextrin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07010

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70, 31/715
US CL :514/42, 53, 58, 61
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)

U.S. : 514/42, 53, 58, 61

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 practicable, search terms used)

CAS ONLINE, MEDLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,935,406 (COLEMAN ET AL.) 19 JUNE 1990, SEE ENTIRE DOCUMENT.	1-21, 49-61
Y	US, A, 4,938,967 (NEWTON ET AL.) 03 JULY 1990, SEE ENTIRE DOCUMENT.	22-48
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, VOLUME 260, NO. 14, ISSUED 15 JULY 1985, D. K. PODOLSKY, "OLIGOSACCHARIDE STRUCTURES OF HUMAN COLONIC MUCIN", PAGES 8262-8271, ESPECIALLY SEE TABLE XIV.	1-61

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

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 01 November 1993	Date of mailing of the international search report NOV 15 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DEBORAH K. WARE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07010

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	INFECTION AND IMMUNITY, VOLUME 56, NO. 11, ISSUED NOVEMBER 1988, D. G. EVANS ET AL., "N-ACETYLNEURAMINYLLACTOSE-BINDING FIBRILLAR HEMAGGLUTININ OF CAMPYLOBACTER PYLORI: A PUTATIVE COLONIZATION FACTOR ANTIGEN", PAGES 2896-2906, SEE ENTIRE REFERENCE.	1-61
Y	INFECTION AND IMMUNITY, VOLUME 57, NO. 8, ISSUED AUGUST 1989, D. G. EVANS ET AL., "RECEPTOR-MEDIATED ADHERENCE OF CAMPYLOBACTER PYLORI TO MOUSE Y-1 ADRENAL CELL MONOLAYTERS", PAGES 2272-2278, SEE ENITRE REFERENCE.	1-61
Y	THE LANCET, ISSUED 29 JULY 1989, LINGWOOD ET AL., "GASTRIC GLYCEROLIPID AS RECEPTOR FOR CAMPYLOBACTER PYLORI", PAGES 238-241, ENTIRE DOCUMENT.	1-61
Y	INFECTION AND IMMUNITY, VOLUME 59, NO. 11, ISSUED NOVEMBER 1991, L. S. TZOUVELEKIS ET AL., "IN VITRO BINDING OF HELICOBACTER PYLORI TO HUMAN GASTRIC MUCIN", PAGES 4252-4254, ENTIRE DOCUMENT.	1-61
Y	REVIEWS OF INFECTIOUS DISEASES, VOLUME 13 (SUPPL. 8), ISSUED 1991, LAMBERT, "PHARMACOLOGY OF BISMUTH-CONTAINING COMPOUNDS", PAGES S691-S695, ENTIRE DOCUMENT.	1-61
Y	REVIEWS OF INFECTIOUS DISEASES, VOLUME 13 (SUPPL. 8), ISSUED 1991, DUNN ET AL., "ADHERENCE OF HELICOBACTER PYLORI TO GASTRIC CARCINOMA CELLS: ANALYSIS BY FLOW CYTOMETRY", PAGES S657-S664, ENTIRE DOCUMENT.	1-61

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07010

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-21 and 30-38, drawn to a first method of treating or inhibiting an ulcer in the stomach or duodenum of a patient and first product used thereby, classified 514/62.

II. Claims 23-24, drawn to second product comprising a H2 blocker, classified 514/646.

III. Claim 25, drawn to third product comprising an antiulcerative compound, classified 514/54.

IV. Claims 26-27, drawn to fourth product comprising a proton pump inhibitor, classified 514/438.

V. Claims 28-29, drawn to fifth product comprising an antibiotic, classified 514/412.

VI. Claims 39-48, drawn to sixth product comprising Formula I and II, classified 435/174.

VII. Claims 49-61, drawn to second method of using first product, classfied 424/93R.

Note that Claim 22 will be examined with the elected Group II, III, IV or V.