Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-145190- -00003-0000
Fecha: 2013-12-30 16:03:02 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 34

DIRECCIÓN DE NUEVAS CREACIONES
PRESENTACIÓN DE OPOSICIÓN

1. Oposición a solicitud de:

X Patente de invención
☐ Registro de Diseño Industrial
☐ Patente de Modelo de Utilidad
☐ Esquema de Trazado de Circuitos Integrados

Número de expediente: **13-145190**

Solicitante: **INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION**

Apoderado: **CARLOS ORLANDO MAYA RODRIGUEZ**

Título de la Nueva Creación: **"ANÁLOGOS DE GLUCAGON QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GLP"**

Gaceta: **676 DEL 1/OCT/2013 PUBLICACIÓN No. 676**

2. Datos del Opositor

☐ Persona Natural X Persona Jurídica

LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.

Nombre o denominación /Razón social completa

VÍCTOR JULIO CHUNCO
Nombre del representante legal

Documento de identificación: C.C. ☐ C.E ☐ NIT X Otr ☐ Cuál _____

Número **890.301.463-8**

Dirección: **Cra. 1 # 46-84** Ciudad: **Cali - Valle**

Teléfono: **052 6877700** Fax: Correo electrónico tparra@lafrancol.com

☐ **Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico**

3. Datos del representante o apoderado:

PARRA MURILLO TITO NOE	79.579.680	71168 C.S.J.
Apellidos y Nombre	Documento de identificación	Tarjeta profesional

Dirección del representante		
CALLE 108 # 51-45		
Dirección electrónica	No. Fax	Número telefónico
tparralfarncol.com	7424092	7422525

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo d-e poder general _____

TOMO 84 F.

4. Fundamentos de la oposición

Causal: **VER ANEXO**

Justificación: **VER ANEXO**

Prórroga: **SI** ☐ **NO** ☐

5. Anexos

- **X** Comprobante de pago de la tasa. No. **13-131186 de 18/DIC/2013**
- **X** Poder, si fuera el caso.
- ☐ Fundamentos de la oposición. Se anexan ___ folios (obligatorios indicar cuántos).
- ☐ Anexo de fundamentos de la oposición. Se anexan
- ☐ Folios (obligatorios indicar cuántos)
- **X** Copias para traslado.
- Copia de la solicitud y sus anexos en formato magnético.

6. Firma	
TITO NOE PARRA MURILLO	
Nombre del Firmante	Firma
79.579.680 DE BOGOTA	**71168 C.S.J.**
C.C.	Tarjeta Profesional

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 131186

Bogotá D.C., Diciembre 18 de 2013 - 16:22:28

RECIBIDO DE : LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. NI 890.301.463 RE

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
RECIBO DE CA	999999999	129961	16/12/2013		250.000.00
CONSIGNACION	BANCO DE BOGOTA	062754387	610280296	18/12/2013	3.000.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-04 PRESENTACION DE OBSERVACIONES A SOLICITUDES	12 OPOSICION POR CADA CLASE-MARCAS Y LEMAS COMERCIALES	325.000.00	325.000.00
				$325.000.00

SON: **TRESCIENTOS VEINTICINCO MIL PESOS MONEDA CORRIENTE***

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-145190- -00003-0000

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3

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF:	**EXPEDIENTE 13-145-190**
TÍTULO SOLICITADO:	**"ANÁLOGOS DE GLUCAGÓN QUE PRESENTAN ACTIVIDAD DE RECEPTOR DE GLP"**
PUBLICACIÓN:	**Gaceta 676 de 30 de septiembre de 2013, N° de publicación 676**
PRIORIDAD:	**US 61/514.609 03/08/2011** **US 61/426.285 22/12/2010**
SOLICITANTE:	**INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION**
APODERADO:	**CARLOS ORLANDO MAYA RODRÍGUEZ**
TRÁMITE:	**PRESENTACION DE OPOSICIÓN**

TITO NOÉ PARRA MURILLO, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 71168 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LAFRANCOL S.A.S.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente 13-145-190, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **LAFRANCOL S.A.S.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud colombiana en trámite 13-145-190, referida a análogos de glucagón que prestan actividad en el receptor de GLP, útiles en el tratamiento de diabetes y obesidad, presenta defectos en la solicitud y no cumple con los requisitos materiales de patentabilidad, tal como se demuestra a continuación:

1. Carencia de claridad y concisión

En primer lugar, las reivindicaciones no presentan claridad, por cuanto no se describen las características técnicas de los análogos de glucagón, reclamados por la solicitud. Así mismo, las reivindicaciones, en especial la reivindicación 1, revela diferentes grupos inventivos, dado que emplean diferentes generalidades para definir los distintos análogos, lo cual sin lugar a duda significa una amplitud tal que permite diferentes péptidos o proteínas de longitud de cadena indeterminada, sin tener una probabilidad razonable de cumplir con las ventajas técnicas divulgadas, veamos:

1-. Un análogo de glucagón (SEQ ID NO: 1) caracterizado porque comprende:

a. un aminoácido que comprende una cadena lateral de imidazola en posición 1,

b. un aminoácido protector de DPP-IV en posición 2, opcionalmente, AIB,

c. un aminoácido acilado o un aminoácido alquilado en cualquiera de las posiciones 9, 10, 12, 16 0 37-43, opcionalmente en donde el grupo acilo o alquilo se unen al aminoácido mediante un separador,

d. un aminoácido alfa-alfa-disustituido en posición 20, y

e. hasta 10 modificaciones de aminoácidos adicionales relacionadas con la SEQ ID NO: 1;

en donde, cuando el análogo de glucagón carece de una partícula hidrofílica, el análogo de glucagón presenta un porcentaje de potencia de GIP de, al menos, 0,1%, en donde el análogo de glucagón tiene una selectividad 100 veces menor para el receptor GLP-1 humano en comparación con el receptor GIP.

Las demás reivindicaciones carecen de igual forma de claridad, concisión y sustento al no mencionar las características estructurales de los análogos del glucagón, deberían incluir su secuencia específica y no hacer alusión a la secuencia del glucagón como tal para definirla de forma relativa con respecto a esta molécula.

2. Excepciones de patentabilidad, no invención, usos y métodos de tratamiento

La solicitud adicionalmente reclama usos, métodos de tratamiento y presenta materia no patentable según la D. 486 de la CAN.

3. Ausencia de Novedad y Nivel Inventivo de la solicitud.

La presente solicitud 13-145-190 en su primer grupo de invenciones se refiere a un análogo de glucagón que comprende un aminoácido que comprende una cadena lateral imidazol en posición 1, un aminoácido DPP-IV (dipeptidil peptidasa) en la posición 2, un aminoácido acilado o alquilado en las posiciones 9, 10, 12, 16, 20 o 37-43, un aminoácido alfa, alfa - disustituido en la posición 20, y mas de diez modificaciones de aminoácidos relativas a la molécula de glucagón no modificada.

Con relación a esta materia, el documento WO 2009/155257 A1 publicado el 23 de diciembre de 2009, describe análogos de glucagón que mantienen la actividad en el receptor de glucagón y exhiben una solubilidad relativa mejorada hacia el péptido de glucagón natural.

Las modificaciones al glucagón mencionadas por este documento incluyen acilación/ alquilación, adición de imidazol o aminoácidos alfa, alfa-disustituidos y modificaciones de aminoácidos, veamos el siguiente fragmento de la página 6:

In accordance with some embodiments, the glucagon peptides disclosed herein are modified to comprise an acyl group or alkyl group, e.g., an acyl or alkyl group which is non-native to a naturally-occurring amino acid. Acylation or alkylation can increase the half-life of the glucagon peptides in circulation, can advantageously delay the onset of action and/or extend the duration of action at the glucagon and/or GLP-1 receptors and/or improve resistance to proteases such as DPP-IV. Acylation or alkylation may also enhance solubility of the peptide at neutral pH. As shown herein, the activity at the glucagon receptor and GLP-1 receptor of the glucagon peptide is maintained if not enhanced after acylation.

Lo anterior demuestra la falta de novedad en sus características generales, así como la carencia del nivel inventivo de cualquier modalidad particular de lo reclamado, siendo la secuencia SEQ ID NO: 1 indicada de glucagón natural, una molécula conocida en el estado de la técnica sobre la que se realizan las modificaciones seleccionables de diversos grupos de aminoácidos como los ya citados en la anterioridad WO 2009/155257 A1.

Por otro lado, el documento de Pan Clark Q et al., *"Desing of a long acting peptide functioning as both a glucagon-like peptide-1 receptor agonist and a glucagón receptor antagonist"*, publicado el 1 de Mayo de 2006, describe a su vez péptidos híbridos modificados de glucagón, GLP-1 o exendina-4 que operan tanto como un receptor del péptido-1 tipo glucagón y como un antagonista del receptor de glucagón.

Este documento describe la forma de realizar análogos de glucagón y de GLP-1 tal como se observa en los resultados (ver a continuación):

RESULTS

To engineer a long acting dual GLP-1 agonist and glucagon antagonist peptide, a three-stage mutagenesis strategy was employed. In the first stage, glucagon, GLP-1, and the related GLP-1 agonist, exendin-4, were combined to generate a series of chimeric peptides with the goal of identifying peptides with high affinity toward GLP-1 and glucagon receptors. In stage two, mutations known to antagonize the glucagon receptor but not known to antagonize the GLP-1 receptor were selectively introduced into the GLP-1/glucagon binding-optimized chimeric peptide identified in stage one, leading to the identification of a peptide with potential to act as both a GLP-1 agonist and glucagon antagonist. The GLP-1 agonist and glucagon antagonist activities of the stage two selected peptide was then tested in secondary cell assays to examine its ability to promote pancreatic islet insulin secretion and inhibit glucagon-induced hepatocyte glucose output. Finally, in stage three, cysteine and lysine mutagenesis procedures were performed to identify a position suitable for attachment of a high molecular weight PEG to prolong duration of action *in vivo*.

En conclusión, los análogos de glucagón, los procedimientos, usos, métodos de tratamiento y las demás categorías reclamadas por la actual solicitud de patente, no son novedosos ni inventivos y no cumplen por tanto con los requisitos de patentabilidad.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

- La publicación internacional WO 2009/155257 A1 (Univ Indiana Res & Tech Cotrp [Us]; Dimarchi Richard D [Us]; Smiley Dav) 23 de diciembre de 2009.

- PAN CLARK Q ET AL: *"Desing of a long acting peptide functioning as both a glucagon-like peptide-1 receptor agonist and a glucagón receptor antagonist"*, Journal Of Biological Chemistry; The American Society Of Biological Chemists; Inc; Us; vol. 281, no. 18, 1 de Mayo de 2006.

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos:

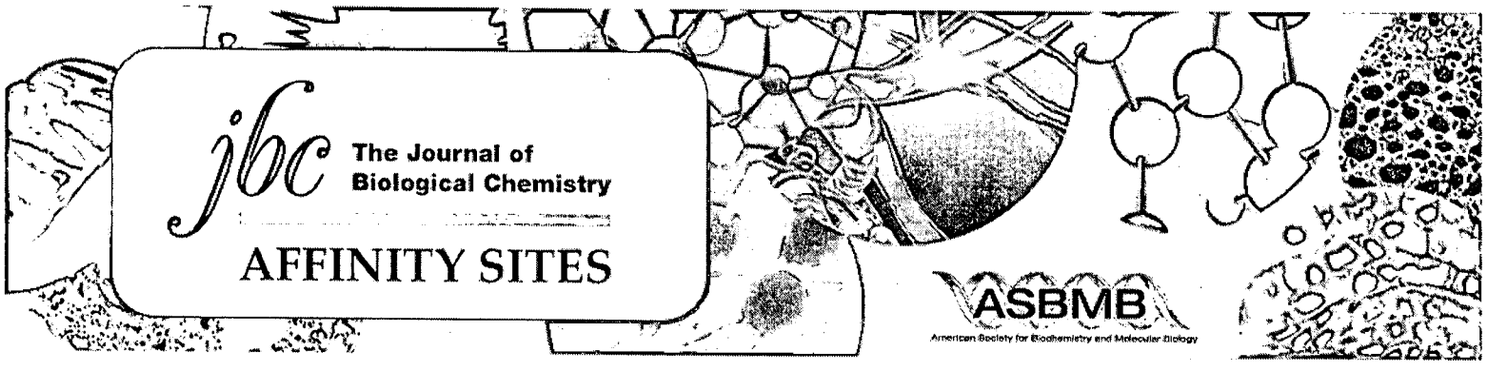
1. Certificado de existencia y representación legal de Lafrancol S.A.S.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Copia para correr traslado al solicitante.
5. Las mencionadas en el acápite de pruebas

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Calle 108 No. 51-45 de Bogotá, D.C.

Señor Superintendente,

TITO NOÉ PARRA MURILLO
C.C. 79.579.680 de Bogotá
T.P.A. 71.168 de C.S.J.



Mechanisms of Signal Transduction:

Design of a Long Acting Peptide Functioning as Both a Glucagon-like Peptide-1 Receptor Agonist and a Glucagon Receptor Antagonist

Clark Q. Pan, Joanne M. Buxton, Stephanie L. Yung, Irene Tom, Ling Yang, Hongxing Chen, Margit MacDougall, Andrea Bell, Thomas H. Claus, Kevin B. Clairmont and James P. Whelan

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doi: 10.1074/jbc.M600127200 originally published online February 27, 2006

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 18, pp. 12506–12515, May 5, 2006

Design of a Long Acting Peptide Functioning as Both a Glucagon-like Peptide-1 Receptor Agonist and a Glucagon Receptor Antagonist*

Received for publication, January 5, 2006, and in revised form, February 15, 2006 Published, JBC Papers in Press, February 27, 2006, DOI 10.1074/jbc.M600127200

Clark Q. Pan[‡], Joanne M. Buxton[§], Stephanie L. Yung[‡], Irene Tom[‡], Ling Yang[§], Hongxing Chen[§], Margit MacDougall[§], Andrea Bell[§], Thomas H. Claus[§], Kevin B. Clairmont[§], and James P. Whelan[§1]

From the [‡]Department of Biotechnology, Bayer HealthCare, California 94701 and the [§]Department of Metabolic Disease Research, Bayer HealthCare, Pharmaceuticals, West Haven, Connecticut 06516

The closely related peptides glucagon-like peptide (GLP-1) and glucagon have opposing effects on blood glucose. GLP-1 induces glucose-dependent insulin secretion in the pancreas, whereas glucagon stimulates gluconeogenesis and glycogenolysis in the liver. The identification of a hybrid peptide acting as both a GLP-1 agonist and a glucagon antagonist would provide a novel approach for the treatment of type 2 diabetes. Toward this end a series of hybrid peptides made up of glucagon and either GLP-1 or exendin-4, a GLP-1 agonist, was engineered. Several peptides that bind to both the GLP-1 and glucagon receptors were identified. The presence of glucagon sequence at the N terminus removed the dipeptidylpeptidase IV cleavage site and increased plasma stability compared with GLP-1. Targeted mutations were incorporated into the optimal dual-receptor binding peptide to identify a peptide with the highly novel property of functioning as both a GLP-1 receptor agonist and a glucagon receptor antagonist. To overcome the short half-life of this mutant peptide *in vivo*, while retaining dual GLP-1 agonist and glucagon antagonist activities, site-specific attachment of long chained polyethylene glycol (PEGylation) was pursued. PEGylation at the C terminus retained the *in vitro* activities of the peptide while dramatically prolonging the duration of action *in vivo*. Thus, we have generated a novel dual-acting peptide with potential for development as a therapeutic for type 2 diabetes.

Glucagon-like peptide-1 (GLP-1)[2] and glucagon are members of a family of structurally related peptide hormones, the glucagon/secretin family. GLP-1 and glucagon originate from a common precursor, preproglucagon, which upon tissue-specific processing leads to production of GLP-1 in the intestine and glucagon in the pancreas (for review see Ref. 1). GLP-1 and glucagon constitute highly homologous peptides, displaying identity at 14 positions (45% identity) and similarity at 3 positions. The receptors for these two peptides are homologous (58% identity) and belong to the family of G-protein-coupled receptors (2, 3). However, despite the high degree of homology between the peptides and the homology between their receptors, GLP-1 and glucagon bind with a high degree of selectivity to their respective receptors (4). Exendin-4 is a structural homolog of GLP-1 (50% amino acid identity) isolated from saliva of the Gila monster that also acts as a potent agonist of the GLP-1 receptor (5).

Both GLP-1 and glucagon play major, but opposing, roles in overall glucose homeostasis. GLP-1 is synthesized in intestinal endocrine cells and induces glucose-dependent insulin secretion from the pancreas and thereby acts to lower plasma glucose concentrations without the risk of hypoglycemia (6, 7). This activity of GLP-1 has made it attractive as a therapeutic approach for the treatment of type 2 diabetes (for review see Ref. 8). Glucagon is secreted from α-cells in pancreatic islets in a glucose-dependent manner. Glucagon stimulates glycogenolysis and gluconeogenesis in the liver, resulting in elevation of plasma glucose. Glucagon has a critical role in maintaining serum glucose concentration, and glucagon receptor antagonists are being pursued as a potential therapeutic approach to inhibit hepatic glucose output (for review see Ref. 9). A molecule capable of both activation of the GLP-1 receptor and inhibition of the glucagon receptor has potential to be a highly effective and novel approach to control blood glucose in the treatment of type 2 diabetes.

Structure-activity studies have been performed to determine the role of individual amino acids within both GLP-1 and glucagon sequences. GLP-1 and glucagon have no defined structure in aqueous solution, but in the presence of micelles, adopt an α-helical structure in the midsection, with flexible N- and C-terminal regions (10, 11). This suggests that the helical structure is required for binding to their respective receptors. Mutations in the N-terminal region of both peptides result in receptor antagonists, suggesting the importance of the N terminus for receptor activation by both GLP-1 (12, 13) and glucagon (14–18).

In addition to the N-terminal region, many amino acids within the glucagon sequence have been shown to contribute to receptor binding and activation (for review see Ref. 19). For example, mutations at positions 11, 12, 16, 17, and 18 of glucagon appear to negatively affect receptor activation more than binding (20–23). Some possible increase in glucagon antagonism may also be provided by changes in amino acids 5, 7, 9, 11, 13, 21, and 29 (24).

For GLP-1, extensive mutational analysis has been reported. For example, alanine-scanning mutagenesis on GLP-1-(7–36)-amide implicates positions 1, 4, 6, 7, 9, 13, 15, 22, 23, and 26 to be critical for receptor binding (25, 26). On the other hand, combinatorial alanine substitutions at positions 8, 11, 12, and 16 of GLP-1 have a minimal effect on GLP-1 receptor binding and activation (27). *In vivo*, GLP-1 is rapidly degraded by dipeptidylpeptidase IV (DPP-IV), a protease responsible for cleaving peptides containing proline or alanine residues in the penultimate N-terminal position, resulting in the inactive GLP-1-(9–36)-amide metabolite (28). Removal of the DPP-IV site by mutagenesis greatly

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[1] To whom correspondence should be addressed: Bayer HealthCare, Pharmaceuticals, Dept. of Metabolic Disease Research, 400 Morgan Lane, West Haven, CT 06516. Tel.: 203-812-2131; Fax: 203-812-2686; E-mail: james.whelan.b@bayer.com.

[2] The abbreviations used are: GLP-1, glucagon-like peptide-1; IPGTT, intraperitoneal glucose tolerance test; DPP, dipeptidylpeptidase; PEG, polyethylene glycol; PEGylation, PEGylated, covalent attachment of long chained PEG to a target molecule; PBS, phosphate-buffered saline; TES, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid; AUC, area under the curve; PEG-DAPD, PEGylated dual-acting peptide for diabetes; FA, fatty acid.

improves plasma stability (29). Exendin-4 has a prolonged half-life *in vivo* relative to GLP-1 likely due to the lack of the DPP-IV site (5).

The similarity between GLP-1 and glucagon, and between their respective receptors, raises the possibility of producing a hybrid peptide that can bind to both receptors. Indeed, Hjorth *et al.* (4) generated a chimeric peptide consisting of the N-terminal part of the glucagon molecule joined to the C-terminal part of the GLP-1 molecule that displayed high affinity for both receptors. However, the design of a peptide that binds both receptors and exhibits agonist activity on the GLP-1 receptor but antagonist activity on the glucagon receptor is more challenging.

A therapeutic drug based upon a hybrid peptide with dual GLP-1 agonist and glucagon antagonist action would still have to overcome the very short half-life (<5 min) inherent to its parent peptides, which makes them unsuitable for the treatment of type 2 diabetes. PEGylation, the covalent attachment of long chained polyethylene glycol (PEG) to a target molecule, has been successfully employed to increase the circulation half-life of proteins, allowing once-a-week dosing (for review see Ref. 30). PEGylation protects the protein from protease digestion and keeps the protein out of the kidney filtrate. However, the high molecular weight PEGs required for significant improvement of plasma half-life tend to have a negative effect on protein activity, due to steric hindrance. Given the small size of GLP-1 and glucagon peptides, and the fact that functionally important amino acids are spread throughout the peptide sequences, it is a further challenge to generate a PEGylated peptide that retains dual GLP-1 agonist and glucagon antagonist activities.

In the current study, we have engineered hybrid glucagon/GLP-1 peptides with targeted mutations that result in the identification of a peptide with both GLP-1 agonist and glucagon antagonist activity. We believe this is the first example of a single chimeric peptide that possesses opposing agonist and antagonist effects on related receptors. To enhance the duration of action, we have site-specifically modified the hybrid peptides with high molecular weight PEGs and succeeded in identifying a PEGylated peptide retaining both GLP-1 agonist and glucagon antagonist activities.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and PEGylation—Peptides were supplied by Sigma Genosys (The Woodlands, TX) or SynPep (Dublin, CA). The peptides were characterized by high-performance liquid chromatography and mass spectrometry and were >90% pure (data not shown). Peptides were site-specifically PEGylated with 22- or 43-kDa PEG-maleimide purchased from Nektar Therapeutics (San Carlos, CA) and purified to >95% purity (data not shown).

RINm5F Cell Culture—RINm5F cells were maintained in RPMI 1640 medium containing 5% fetal bovine serum (JRH Biosciences) and 1% antibiotic-antimycotic solution (Invitrogen) at 37 °C in a humidified 5% CO_2 incubator.

Preparation of RINm5F Cell Membranes—RINm5F cells were washed with PBS, scraped in ice-cold HES buffer (20 mM Hepes, 1 mM EDTA, 250 mM sucrose buffer containing protease inhibitors), and homogenized. Unbroken cells and nuclei were removed by centrifugation at 500 × *g* for 5 min at 4 °C. The supernatant was centrifuged at 40,000 × *g* for 20 min. Membranes were resuspended in HES.

Preparation of Plasma Membranes from Rat Liver—Male Sprague-Dawley rats were sacrificed, and livers were removed and placed into ice-cold TES buffer (20 mM Tris, pH 7.5, 1 mM EDTA, 255 mM sucrose containing protease inhibitors). The tissue was minced in 5 volumes of ice-cold TES, and a slurry was prepared using a Polytron. The slurry was further homogenized using a handheld Dounce homogenizer. The homogenate was passed through several layers of cheesecloth and spun at 25,000 × *g* for 10 min. The pellets were resuspended in TES, and plasma membranes were isolated from the interface of 1.2 M sucrose and TES cushion centrifugation at 180,000 × *g*. The resulting membranes were washed with TES using a 15-min centrifugation at 200,000 × *g*, resuspended in TES again, and purified on a second sucrose cushion at 200,000 × *g*. The final pellet was resuspended in TES.

Competitive Binding of Peptide to the GLP-1 and Glucagon Receptors—GF/C filtration plates (Millipore, Bedford, MA) were blocked with 0.3% or 0.1% polyethyleneimine for RINm5F membranes and rat liver plasma membranes, respectively. Filter plates were washed twice with binding buffer consisting of 20 mM Tris, 2 mM EDTA, pH 7.5, 1 mg/ml bovine serum albumin, and 1 mg/ml bacitracin. 5 μg of RINm5F cell plasma membranes or 3–5 μg of rat liver plasma membranes diluted in binding buffer were combined with 0.05 μCi of ^{125}I-labeled GLP-1 or ^{125}I-labeled glucagon and unlabeled test peptide at the indicated concentrations. Following a 1-h incubation at room temperature, the plates were washed three times with ice-cold PBS containing 1 mg/ml bovine serum albumin. The plates were dried, scintillant was added to each well, and plates were counted using a Wallac Microbeta counter.

Measurement of Peptide Signaling through GLP-1 Receptor—1.5 × 10^5 RINm5F cells per well were seeded in 96-well plates and grown overnight. The cells were then washed twice with PBS and then incubated with peptide in Hepes-PBS containing 1% bovine serum albumin and 100 μM isobutylmethylxanthine for 15 min at 37 °C. The cells were lysed, and intracellular cAMP was determined using the cAMP Scintillation Proximity Assay direct screening assay system (Amersham Biosciences).

Measurement of Peptide Signaling through Glucagon Receptor—Hepatocytes were isolated according to a modified procedure of Berry (31). Freshly isolated rat hepatocytes were plated in 96-well plates at 7.5 × 10^4 cells/well in Hepes-bicarbonate-PBS containing 1% bovine serum albumin and 100 μM isobutylmethylxanthine. Following equilibration at 37 °C in a 5% CO_2/95% O_2 environment for 10 min, peptide was added for an additional 15 min. The cells were lysed, and intracellular cAMP was determined as described above. The ability of the hybrid peptide to inhibit glucagon activity was measured as follows: Following equilibration at 37 °C in a 5% CO_2/95%O_2 environment for 10 min, 10 μM peptide was added to the cells followed immediately by either 2 or 10 nM glucagon for 15 min. The cells were lysed, and cAMP was determined.

Measurement of Glucose Release from Rat Hepatocytes—Hepatocytes were added into a flat bottom 96-well plate (2 × 10^5/100 μl/well) and preincubated in a 37 °C incubator with constant shaking and under 95% O_2/5% CO_2 flow for 10 min. Hepatocytes were incubated for another 30 min after addition of glucagon with or without peptide. Cells were then lysed with 15% perchloric acid and plates were spun. The supernatant was neutralized with 1 M Tris-HCl (pH 8.0):2.5 N KOH (45:55) and spun again. The resulting supernatant was analyzed for glucose with hexokinase and glucose-6-phosphate dehydrogenase and the A340 read on a fMAX plate reader (Molecular Devices, Sunnyvale, CA). Glucose output was calculated in the following manner: after subtracting the amount of glucose produced in the unstimulated hepatocytes from each data point, the percent inhibition was calculated.

Insulin Release from Perifused Rat Islets—The bi-phasic response of insulin release stimulated by peptides was tested in perifused rat islets. Fifty islets were loaded in a perifusion chamber and perifused with HEPES-Krebs-Ringer bicarbonate buffer containing 3 mM glucose at 37 °C. After 60 min, islets were exposed to buffer containing 8 mM glucose with or without peptide (50 nM) and perifused for another 30 min. Fractions of perifusate were collected at 1- or 5-min intervals for insulin determination. Insulin was measured using an enzyme-linked immunosorbent assay kit (Alpco Diagnostics, Windham, NH).

Measurement of Peptide Stability in Rat Plasma—Fresh rat plasma was collected from male Wistar rats. The peptides were diluted in 100% rat plasma to 10-fold desired final concentration and incubated at 37 °C for 0, 3, 6, 18, or 24 h. Samples were then added to RINm5F cells, and cAMP production was measured after a 15-min exposure as outlined above. Percent peptide remaining was determined by comparing cAMP production at a given time point to cAMP production in a freshly diluted sample (zero time).

Intraperitoneal Glucose Tolerance Test—Male Wistar rats (220–250 g) were purchased from Harlan (Indianapolis, IN). All procedures were approved by the Bayer Animal Care and Use Committee, and all experiments were performed in accordance with relevant guidelines and regulations. Male Wistar rats were either fasted overnight and then given peptide or vehicle by subcutaneous injection or they were given peptide or vehicle first and then fasted overnight, depending on the time interval between dosing and when the IPGTT was to be performed. At the appropriate time after dosing, the fasting blood glucose level was measured from tail-tip blood using a Glucometer (Bayer HealthCare, Mishawaka, IN), and the animals were given 2 g/kg glucose by intraperitoneal injection. Blood glucose was measured again after 15, 30, and 60 min. The area under the glucose curve (AUC) was calculated using the trapezoidal method, and the effect of the peptide on the AUC was expressed as a percentage of the AUC for the vehicle-treated group.

Statistical Analysis—*In vitro* results are means ± S.E. for the number of experiments (*n*) indicated in the figure legends. *In vitro* assay statistics were calculated by *t* test using Graphpad Prizm (GraphPad Software, Inc., San Diego, CA). *In vivo* data are expressed ± S.E. Statistical analyses were performed using InStat (GraphPad). Treatment effects were analyzed by analysis of variance with post hoc analysis using Tukey-Kramer Multiple Comparisons Test (parametric methods) or the Kruskal-Wallis Test (non-parametric methods) when necessary. Differences are considered significant at p values of <0.05.

RESULTS

To engineer a long acting dual GLP-1 agonist and glucagon antagonist peptide, a three-stage mutagenesis strategy was employed. In the first stage, glucagon, GLP-1, and the related GLP-1 agonist, exendin-4, were combined to generate a series of chimeric peptides with the goal of identifying peptides with high affinity toward GLP-1 and glucagon receptors. In stage two, mutations known to antagonize the glucagon receptor but not known to antagonize the GLP-1 receptor were selectively introduced into the GLP-1/glucagon binding-optimized chimeric peptide identified in stage one, leading to the identification of a peptide with potential to act as both a GLP-1 agonist and glucagon antagonist. The GLP-1 agonist and glucagon antagonist activities of the stage two selected peptide was then tested in secondary cell assays to examine its ability to promote pancreatic islet insulin secretion and inhibit glucagon-induced hepatocyte glucose output. Finally, in stage three, cysteine and lysine mutagenesis procedures were performed to identify a position suitable for attachment of a high molecular weight PEG to prolong duration of action *in vivo*.

Stage 1: Optimization of Dual Binding Affinities to GLP-1 and Glucagon Receptors—As previously reported, both glucagon and GLP-1 displayed high affinity (IC_{50}) and potency (EC_{50}) for their own receptors but no significant cross-reactivity for the other receptor (Table 2). To identify novel peptides capable of binding to both receptors, 15 peptides (A1–A15) were synthesized (Table 1). Peptides A1 to A6 were designed to progressively introduce GLP-1-specific amino acids into the glucagon sequence from the C terminus. The N-terminal 18 amino acids of glucagon are critical for high affinity binding to its receptor, because

TABLE 1
Hybrid peptide sequences

Peptide sequences are represented as follows: Normal font = glucagon amino acids, shaded normal font = GLP-1 amino acids, shaded italic font = exendin-4 amino acids, bold font = mutated amino acids. The non-natural amino acid norleucine is denoted as X. C-terminal amide is symbolized by asterisk.

Peptide	Sequence
Glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR*
Exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPP
A1	HSQGTFTSDYSKYLDSRRAQDFVQWLVKGR*
A2	HSQGTFTSDYSKYLDSRRAQDFIAWLVKGR*
A3	HSQGTFTSDYSKYLDSRRAKEFIAWLVKGR*
A4	HSQGTFTSDYSKYLDSRAAKEFIAWLVKGR*
A5	HSQGTFTSDYSKYLDSQAAKEFIAWLVKGR*
A6	HSQGTFTSDYSKYLEGQAAKEFIAWLVKGR*
A7	HSQGTFTSDYSKYLDSRRAQDFVQWLKNGGPSSGAPPP
A8	HSQGTFTSDYSKYLDSRRAQDFIEWLKNGGPSSGAPPP
A9	HSQGTFTSDYSKYLDSRAVRLFIEWLKNGGPSSGAPPP
A10	HSQGTFTSDYSKYLEEEAVRLFIEWLKNGGPSSGAPPP
A11	HSQGTFTSDYSKQMEEEAVRLFIEWLKNGGPSSGAPPP
A12	HSQGTFTSDYSKYLDSRRAQDFIAWLVKGGPSSGAPPP
A13	HSQGTFTSDYSKYLEGQAAKEFIAWLVKGGPSSGAPPP
A14	HSQGTFTSDYSKYLEEEAVRLFIEWLKNGR*
A15	HSQGTFTSDYSKYLEEEAVRLFIEWLVKGR*
AN1	YSQGTFTSEYSKYLDSRRAKEFIAWLVKGR*
AN2	SQGTFTSDYSKYLDSRRAKEFIAWLVKGR*
AN3	YSQGTFTSDYSKYLDSRRAKEFIAWLVKGR*
AN4	HSQGTFTSEYSKYLDSRRAKEFIAWLVKGR*
AN5	HSQGTFTSDYAKYLDSRRAKEFIAWLVKGR*
AN6	HSQGTFTSDYSKYLDARRAKEFIAWLVKGR*
AN7	HSQGTFTSDYSKYLDGRRAKEFIAWLVKGR*
AN8	HSQGTFTSDYSKYLDERRAKEFIAWLVKGR*
AN9	HSQGTFTSXYSKYLDARRAKEFIAWLVKGR*
AN10	HSQGTFTSDYAKYLDARRAKEFIAWLVKGR*
AN11	HSQGTFTSXYAKYLDARRAKEFIAWLVKGR*
AN12	HSQGTFTSXYAKYLDQRRAKEFIAWLVKGR*
AN13	HSQFTFTSDYSKYLDSRRAKEFIAWLVKGR*
AN14	HSQGYFTSDYSKYLDSRRAKEFIAWLVKGR*
AN15	HSQGTFTSDYSRYLDSRRAKEFIAWLVKGR*
AN16	HSQFYFTSDYSRYLDSRRAKEFIAWLVKGR*
AN17	HSDFSFTSDYSKYLDSKKAKEFIAWLVKGR*
AN18	HSDFSFTSDYSRYLDSRRAKEFIAWLVKGR*

C-terminal replacements with GLP-1 sequence beyond this point greatly reduce glucagon binding affinity (Table 2). The IC_{50} of A4–A6 at the glucagon receptor were at least 10-fold higher than that of peptides A1–A3. Exchange of only the three C-terminal amino acids of glucagon with the corresponding C-terminal GLP-1 amino acids, while only causing <3-fold decrease in glucagon receptor affinity, reduced glucagon agonist activity by >100-fold, as exhibited by peptide A1. Therefore, the precise amino acid sequence of the C-terminal portion of glucagon appears to be required for activation but not binding to the glucagon receptor. In contrast, binding to the GLP-1 receptor is much more tolerant to insertion of glucagon amino acids into the GLP-1 sequence. Even peptide A1, with 12 amino acid differences from GLP-1 in the N-terminal 26 positions, displayed relatively high affinity (IC_{50} = 6 nM) and potency (EC_{50} = 21.7 nM) for the GLP-1 receptor. Successive introduction of more GLP-1 amino acids into glucagon further enhanced activity at the GLP-1 receptor culminating in peptide A6, which has four amino acid differences from GLP-1 but almost an identical IC_{50} and EC_{50} as GLP-1. Peptide A3, a chimera sequence of glucagon-(1–19) and GLP-1-(20–30) possesses the most desired *in vitro* profile of a potent GLP-1 agonist (EC_{50} = 4.9 nM) and a strong glucagon receptor binder (IC_{50} = 7.7 nM). Moreover, peptide A3 is a weak glucagon agonist (EC_{50} = 361 nM) and could potentially act as a glucagon antagonist. However, consistent glucagon antago-

TABLE 2

Hybrid peptide GLP-1/glucagon receptor binding and activity

GLP-1 and glucagon receptor binding (IC_{50}) and activation (EC_{50}) to receptors on RINm5F cells or rat liver membranes, respectively. Data are the mean ± S.E. of at least two experiments.

Peptide	GLP-1 receptor		Glucagon receptor		
	IC_{50}	EC_{50}	IC_{50}	EC_{50}	Maximum
	nM	nM	nM	nM	%
Glucagon	>1 μM		2.3 ± 0.3	4.1 ± 1.2	100
GLP-1	0.4 ± 0.1	1.9 ± 0.5	>1 μM		
Exendin-4	0.4	0.8 ± 0.2			
A1	6.0 ± 1.7	21.7 ± 7.1	6.6 ± 1.2	505 ± 142	103.1 ± 6.3
A2	3.5 ± 0.4	14.4 ± 4.0	8.4 ± 1.3	567 ± 129	96.6 ± 7.4
A3	0.9 ± 0.2	4.9 ± 1.0	7.7 ± 1.6	361 ± 124	92.9 ± 4.0
A4	0.18 ± 0.07	10.5 ± 6.8	84 ± 14	365	100
A5	2.1 ± 1.2	15.1 ± 0.7	193 ± 96	3021 ± 954	99.7 ± 9.5
A6	0.5 ± 0.1	2.0 ± 0.6	243 ± 71	938 ± 200	106.5 ± 8.6
A7	12.3 ± 3.2	39 ± 15	98 ± 7	75 ± 23	80.8 ± 8.4
A8	1.9 ± 0.7	5.6 ± 1.8	9.7 ± 0.3	12.8 ± 5.3	80.2 ± 3.8
A9	0.2 ± 0.1	9.2 ± 4.6	53	108	80.3
A10	1.4	0.8	>1 μM		
A11	0.4 ± 0.1	6.6 ± 3.3	>1 μM	>1 μM	49.6 ± 16.1
A12	0.8	8.8 ± 6.9	21 ± 13	13	89.4
A13	0.6 ± 0.4	6.1 ± 5.5	105 ± 8	93	92.3
A14	0.2 ± 0.02	1.6 ± 0.4	452 ± 238	1458 ± 340	97.9 ± 16.7
A15	0.27 ± 0.04	1.9 ± 0.3	216 ± 48	543 ± 177	78.4 ± 9.2
AN1	41 ± 12	>1 μM	101 ± 9	>10 μM	10.5 ± 10.5
AN2	99 ± 4.7	>1 μM	264 ± 101	>10 μM	0.6
AN3	28 ± 11	91 ± 19	13.1 ± 6.3	143 ± 25	81.3 ± 6.2
AN4	64 ± 25	132 ± 19	372 ± 180	>10 μM	6.8
AN5	5.9 ± 3.6	40 ± 17	116 ± 25	321 ± 102	46.8 ± 17.7
AN6	1.5 ± 0.7	12.9 ± 3.3	28.8 ± 6.3	468 ± 182	72.1 ± 11.1
AN7	4.5 ± 2.3	11.6 ± 0.6	31 ± 10	241	94.7
AN8	0.6 ± 0.1	2.5 ± 0.5	16.4 ± 7.8	166 ± 80	103.1 ± 18.0
AN9	10.2 ± 2.6	53 ± 14	42 ± 34	>10 μM	0.0
AN10	3.0 ± 0.7	13.3 ± 4.9	69 ± 16	713 ± 140	43.6 ± 4.3
AN11	9.7 ± 0.2	>1 μM	43 ± 26	>10 μM	0.0
AN12	11.4 ± 4.3	>1 μM	26 ± 20	>10 μM	0.0
AN13	>10 μM		>1 μM	NT[a]	NT
AN14	61 ± 29	463 ± 197	591 ± 176	NT	NT
AN15	2.7 ± 1.6	31 ± 22	136 ± 100	705 ± 440	75.5 ± 16.0
AN16	17.5 ± 6.3	>1 μM	163 ± 47	837 ± 656	58.2 ± 18.3
AN17	40 ± 19	171	799 ± 245	>10 μM	2.7 ± 3.9
AN18		>1 μM	1429 ± 364	NT	NT

[a] NT, not tested.

nism could not be demonstrated with peptide A3 (data not shown) perhaps because it can act as a full agonist of the glucagon receptor at sufficiently high concentrations.

Similar substitution of glucagon with exendin-4 amino acids (A7–A11) showed comparable results at the GLP-1 receptor, suggesting GLP-1- or exendin-4-specific amino acids in the N-terminal portion are not required for potent activities at the GLP-1 receptor. Unlike the GLP-1 amino acids, substitution of exendin-4 amino acids into glucagon had a more profound effect on glucagon receptor binding. Only peptide A8 displayed high affinity and potency at the glucagon receptor. Introduction of a combination of GLP-1 and exendin-4 amino acids into peptides (A12–A15) showed a similar trend and again did not produce any peptide with a better profile with respect to GLP-1 agonist activity and lack of glucagon agonist activity when compared with peptide A3. Thus, peptide A3 was selected as the lead peptide from this stage and the template sequence for the next stage of mutagenesis.

Stage 2: Optimization of GLP-1 Agonism and Glucagon Antagonism—To generate a peptide mutant that retains GLP-1 agonism but blocks glucagon from activating its receptor (*i.e.* glucagon antagonism) 18 peptides were designed that introduced known glucagon antagonist mutations into the template peptide A3 (Table 1). Mutations at positions 11, 12, and 16, which have been shown to affect glucagon receptor activation much more than glucagon receptor binding (21, 32), had a modest effect on GLP-1 receptor binding and activation as demonstrated by peptides AN5–AN10 and AN15 (Table 2). On the other hand, mutations in the N-terminal region, including residues 1, 3–5, and 9, which lead to strong glucagon antagonism (14–18), also greatly affected interactions at the GLP-1 receptor as illustrated by peptides AN1–AN4, AN11–AN14, and AN16–AN18. Furthermore, with the exception of peptides AN3, AN11, and AN12, these N-terminal mutations reduced glucagon receptor binding affinity by at least 10-fold as compared with the template peptide A3. Therefore, even though these mutations had little effect on glucagon receptor binding in the context of the glucagon sequence, they had a greater effect when combined with the C-terminal GLP-1 sequence. Unlike the parent peptide A3, the S11A/S16A double mutant, AN10, is only a partial agonist at the glucagon receptor, making it more likely to act as a glucagon antagonist.

Peptide AN10: A GLP-1 Agonist and Glucagon Antagonist—The hybrid peptide AN10 was selected from the panel of 33 peptides analyzed as having the optimal *in vitro* characteristics with respect to GLP-1 and glucagon receptor binding and activity. Peptide AN10 binds to the GLP-1 and glucagon receptors with IC_{50} = 3 nM and 69 nM, respectively (Table 2 and Fig. 1, *a* and *c*). Peptide AN10 activates the GLP-1 receptor (EC_{50} = 13 nM) and activates the receptor to 98% the maximal level of receptor activation achieved by GLP-1 (Table 2 and Fig. 1*b*). Peptide AN10 is a weak agonist of the glucagon receptor (EC_{50} = 713 nM) reaching only 44% the maximum activity of glucagon (Table 2 and Fig. 1*d*).

Peptide AN10 (10 μM) antagonizes glucagon (2 nM)-mediated cAMP production 22% in rat hepatocytes. The benchmark glucagon antagonist peptide DesHis(Glu^9) glucagon (10 μM) antagonizes glucagon (2 nM)-

12

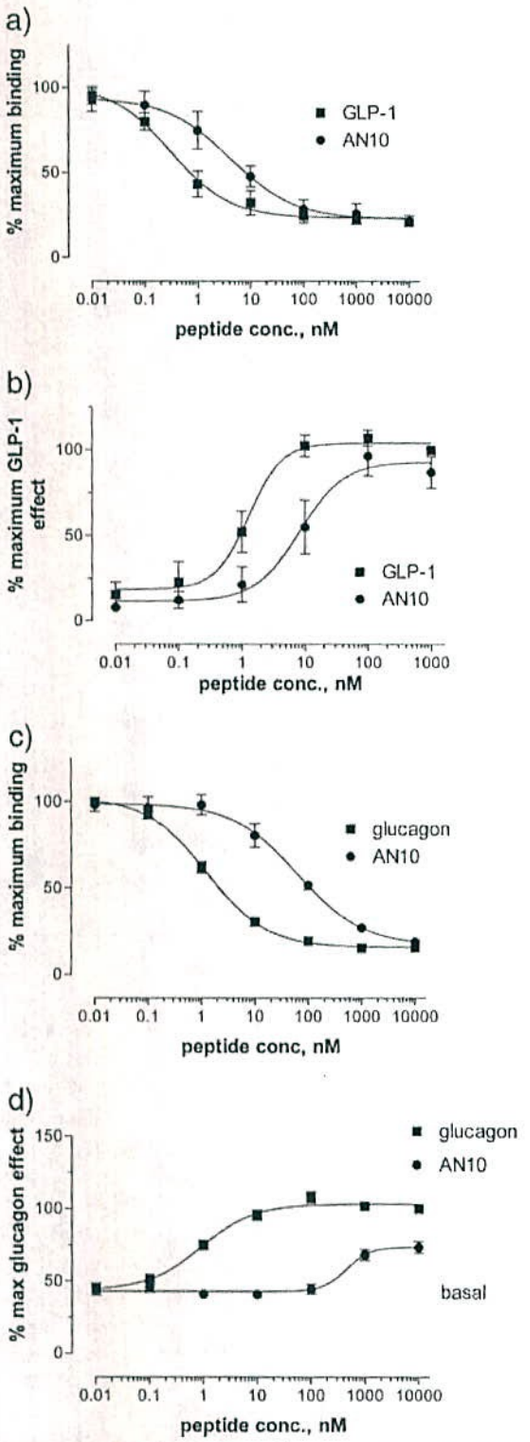

FIGURE 1. **Binding to and activation of GLP-1 and glucagon receptors by AN10.** *a* and *c*, binding to GLP-1 and glucagon receptors on RINm5F cell and rat liver membranes, respectively. Maximum binding is the cpm bound in the absence of competing unlabeled peptide. Data shown are the mean ± S.E. of 5 or 6 separate experiments. *b* and *d*, GLP-1 and glucagon receptor activation in RINm5F and rat liver cells, respectively. Data were normalized to percent maximum GLP-1 (1 μM) or glucagon (10 μM) effect. Values shown are the mean ± S.E. from 10 or 7 trials for GLP-1 and glucagon receptor activation, respectively.

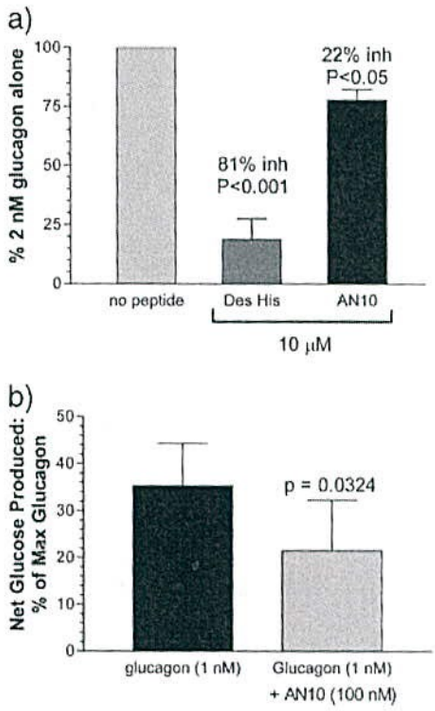

FIGURE 2. **Inhibition of glucagon-mediated cAMP and glucose production in rat hepatocytes.** *a*, inhibition of glucagon-mediated cAMP production in rat hepatocytes. The *bars* are the mean ± S.E. of six trials. *b*, inhibition of glucagon-mediated glucose production in rat hepatocytes. The *bars* are the mean ± S.E. of three trials. *p* value was determined using a paired *t* test.

mediated cAMP production 81% in rat hepatocytes (Fig. 2*a*). In addition, peptide AN10 (100 nM) antagonizes glucagon (1 nM)-mediated glucose output from rat hepatocytes 39% (Fig. 2*b*).

GLP-1 agonism was further assessed by examining the ability of peptide AN10 to increase insulin secretion from perfused rat islets. Islets were perifused with 8 mM glucose, 8 mM glucose plus AN10 (50 nM), or 8 mM glucose plus GLP-1 (50 nM), and samples were collected over 25 min. Insulin secretion increased over time in all conditions, but incubation with GLP-1 or AN10 increased the area under the curve 1.8-fold ($p < 0.05$) as compared with 8 mM glucose alone. Therefore, AN10 increases insulin secretion from perifused rat islets in the presence of 8 mM glucose equivalent to that achieved by GLP-1 (Fig. 3).

The short *in vivo* duration of action of GLP-1 is accounted for in part by DPP-IV. Because AN10 does not contain the N-terminal sequence of GLP-1 (*i.e.* it does not contain an alanine in the second position), we tested whether it would be more stable than GLP-1 in plasma. Exendin-4, which has a glycine at position 2 instead of the alanine found in GLP-1, is resistant to DPP-IV cleavage and has prolonged activity *in vivo* (33). GLP-1, exendin-4, and AN10 were incubated in rat plasma at 37 °C for time periods ranging from 3 to 24 h. At various time points during this period the percentage of active peptide remaining was assessed in the GLP-1 receptor activation assay. For GLP-1, after 6-h incubation in rat plasma only 14% of active peptide remained. At this 6-h time point 40 and 95% of active exendin-4 and AN10 remained, respectively. Furthermore, after 18-h incubation 27 and 52% of active exendin-4 and AN10 remained, respectively (Fig. 4). Thus, AN10 has greatly improved stability in plasma compared with GLP-1.

Stage 3: Optimization of Site-specific PEGylation—Protein PEGylation has often been limited by the loss of potency due to blockage of the protein active site by the large unstructured PEG polymer, which may explain the very few examples of successful peptide, as opposed to protein, PEGylation reported to date. In an effort to maintain both GLP-1 agonism and glucagon antagonism of the hybrid peptide, it was neces-

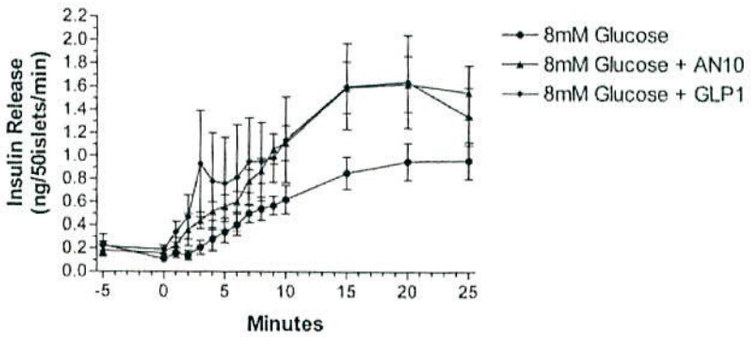

FIGURE 3. **Insulin secretion from perifused rat islets.** Rat islets were perifused with a solution containing 8 mM glucose with or without 50 nM GLP-1 or AN10 as indicated. Insulin in the perifusate was measured using enzyme-linked immunosorbent assay. The results are the mean ± S.E. of 10 trials.

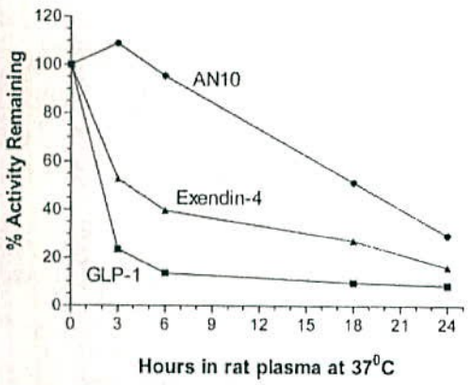

FIGURE 4. **Activity of peptides following incubation in rat plasma.** Peptides were incubated in 100% rat plasma at the indicated concentrations, for the indicated time periods. The samples were then added to RINm5F cells, and cAMP production was measured after a 15-min exposure. Percent peptide remaining was determined by comparing cAMP production at a given time point to cAMP production in a freshly diluted sample (zero time).

TABLE 3
Cysteine and lysine mutant peptides
Bold font indicates mutated amino acids.

Peptide	Sequence
AN10G	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGRG
ANC1	HSQGTFTSDY**A**KYLD**A**RRA**C**EEIIAWLVKGRG
ANC2	HSQGTFTSDY**A**KYLD**A**RRAK**C**FIAWLVKGRG
ANC3	HSQGTFTSDY**A**KYLD**A**RRAKEFI**C**WLVKGRG
ANC4	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLV**C**GRG
ANC5	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVK**C**RG
ANC6	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKG**C**G
ANC7	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGR**C**
ANC8	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGRG**C**
ANC9	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGRG**GSC**
ANC10	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGRG**PPPSC**
ANC11	HSQGTFTSDY**A**KYLD**A**RRAKEFIAWLVKGRG**GSGGSC**
ANK1	HSQGTFTSDY**A**RYLD**A**RRAKEFIAWLV**R**GRG
ANK2	HSQGTFTSDY**A**RYLD**A**RRA**R**EFI**K**WLV**R**GRG
ANK3	HSQGTFTSDY**A**RYLD**A**RRA**R**EFIAWLVKGRG
ANK4	HSQGTFTSDY**A**RYLD**A**RRA**R**EFIAWLV**R**GRG**K**
ANC7K2	HSQGTFTSDY**A**RYLD**A**RRA**R**EFI**K**WLV**R**GR**C**

FIGURE 5. **Model of AN10G.** The "twisted helix" model of AN10G when in contact with the cell surface. The helical backbone of AN10G is represented by a *thin ribbon*. Receptor binding side of the peptide is predicted to be at the *top*, surrounded by the nine hydrophobic side chains (depicted as *thick sticks*) Phe^{6}, Tyr^{10}, Tyr^{13}, Leu^{14}, Phe^{22}, Leu^{23}, Trp^{25}, Leu^{26}, and Val^{27}. Side chains for the remaining residues (all hydrophilic) are shown as *thin sticks*. The flexible N-terminal region is predicted to insert into the receptor during receptor activation. The C-terminal three residues are also expected to be flexible. Each C_α atom of the seven positions within GLP-1 sequence chosen for PEGylation is represented by a *ball*.

sary to target sites of PEGylation distal to the active site. Thus, a site-specific PEGylation strategy was pursued.

Potential sites of PEGylation in the AN10 peptide were selected in an effort not to hinder its ability to interact with either the glucagon or the GLP-1 receptors. Based on the crystal structure of glucagon and NMR structure of GLP-1 (34, 35), a "twisted helix" model was generated for the hybrid peptide AN10G (Fig. 5), which is identical to peptide AN10 except for the replacement of the C-terminal amide with a glycine found in some of the native GLP-1 species. Changes within the highly flexible N-terminal region (residues 1–5) and residue 15 are not well tolerated by either GLP-1 or glucagon (22, 26). Mutations at position 8 reduce glucagon binding, whereas those at positions 7 and 9 reduce GLP-1 activation (32). Residues 6, 10, 13, 14, 22, 23, and 25–27 are all hydrophobic and thus may be involved in receptor binding, consistent with the "twisted helix" model. Mutations at many of these positions reduced GLP-1 and glucagon binding and activity (36). Position 19 is also directed toward the receptor and thus would not serve as an ideal PEGylation site. As outlined above, our results demonstrate the importance of positions 11, 12, and 16–18 for activity. This detailed structure-activity analysis leaves only positions 20, 21, 24, and 28–31 as potential sites for PEGylation. All seven positions are predicted to be directed away from the receptor, and there are no known deleterious effects associated with mutations at these positions.

Two site-specific PEGylation strategies were pursued. Because peptide AN10G contains no cysteine, introduction of a free cysteine into positions 20, 21, 24, and 28–31 would allow highly specific attachment of a single PEG molecule. Peptides ANC1–ANC7 were designed with a cysteine inserted individually in each of these seven positions (Table 3). To further reduce the potential of PEG interfering with receptor binding, the 31-amino acid peptide was extended by a cysteine to allow PEGylation at position 32 (ANC8) or a rigid spacer such as GS, PPPS, or GSGGS (ANC9–ANC11). Alternatively, single unique lysines were created at positions 20, 24, 28, and 32 to allow site-specific amine PEGylation of N-terminally blocked peptides (ANK1–ANK4). The two existing lysines (Lys-20 and Lys-28) were mutated to arginines to allow single-site PEGylation.

Peptides ANC1–ANC11 and ANK1–ANK4 were analyzed for GLP-1 receptor binding and activation as well as glucagon receptor binding and activation, and glucagon receptor antagonism. With the exception of ANC2, all peptides displayed reasonable GLP-1 receptor potency (EC_{50} < 50 nM) (Table 4). Likewise, with the exception of ANC2, ANC10, and ANC11, these peptides exhibited reasonable glucagon

TABLE 4

Receptor binding and activity of the cysteine and lysine mutant peptides

GLP-1 and glucagon receptor binding (IC_{50}) and activation (EC_{50}) to receptors on RINm5F cells or rat liver membranes, respectively. Inhibition of glucagon activity is determined by assaying rat hepatocyte response to 10 nM glucagon in the presence or absence of 10 μM peptide. Data are the mean ± S.E. of at least two experiments.

	GLP-1 receptor		Glucagon receptor		
	RIN IC_{50}	RIN EC_{50}	Liver IC_{50}	Maximum glucagon activity	Inhibition of glucagon activity
	nM	nM	nM	%	%
ANC1	5.7 ± 1.4	35.0 ± 3.5	25.6 ± 7.9	67.4 ± 12.5	11.3 ± 30.3
ANC2	NT[a]	110 ± 15.3	166 ± 44	47.0 ± 7.1	NI[b]
ANC3	NT	41.5 ± 5.0	74.5 ± 2.4	57.4 ± 13.8	4.4 ± 27.7
ANC4	4.4 ± 0.2	36.4 ± 6.9	21.7 ± 7.8	70.4 ± 19.4	23.7 ± 20.3
ANC5	7.7 ± 2.2	44.2 ± 8.1	54.0 ± 6.3	55.4 ± 15.9	17.1 ± 21.8
ANC6	10.2 ± 1.7	23.6 ± 2.1	59.1 ± 15.9	49.9 ± 15.7	23.0 ± 25.4
ANC7	8.8 ± 4.2	25.2 ± 1.9	65.5 ± 6.6	52.4 ± 14.7	18.6 ± 19.3
ANC8	NT	17.4 ± 2.2	63.2 ± 1.9	66.5 ± 18.3	NI
ANC9	NT	16.1 ± 1.7	87.6 ± 7.5	66.4 ± 9.9	NI
ANC10	NT	21.4 ± 3.9	243 ± 43	64.3 ± 7.6	NI
ANC11	NT	18.1 ± 2.2	209.2	63.2 ± 15.9	NI
ANK1	8.1 ± 4.5	14.8 ± 7.8	33.6 ± 7.5	50.9 ± 11.6	30.2 ± 4.5
ANK2	3.2 ± 1.6	7.0 ± 0.4	32.3 ± 4.6	58.2 ± 14.3	24.7 ± 5.3
ANK3	1.4 ± 0.9	4.1 ± 0.9	11.7 ± 2.5	44.1 ± 2.2	25.2 ± 9.9
ANK4	1.5 ± 1.0	3.3 ± 0.5	16.1 ± 2.6	47.6 ± 8.7	27.9 ± 4.6
ANC7K2	3.5 ± 0.5	12.7 ± 1.7	117 ± 17	46.8 ± 5.7	35.6 ± 10

[a] NT, not tested.
[b] NI, no inhibition.

TABLE 5

GLP-1 receptor activity of PEGylated peptides

Potency (EC_{50}, nM) of PEGylated and non-PEGylated peptides toward the GLP-1 receptor was evaluated in the RINm5F cell cAMP assay as described under "Experimental Procedures." Data are mean ± S.E. of at least two trials.

Peptide	No PEG	22-kDa PEG	43-kDa PEG
ANC1	35.0 ± 3.5	346.1 ± 79.0	358.6 ± 21.7
ANC2	110 ± 15.3	476 ± 137.7	411.3 ± 16.1
ANC3	41.5 ± 5.0	144.5 ± 9.5	294.7 ± 60.3
ANC4	36.4 ± 6.9	114.7 ± 10.1	337.4 ± 22.3
ANC5	44.2 ± 8.1	275.8 ± 37.8	388.4 ± 2.4
ANC6	23.6 ± 2.1	106.6 ± 19.3	383.0 ± 7.0
ANC7	25.2 ± 1.9	49.7 ± 17.6	236.1 ± 16.6
ANC8	17.4 ± 2.2	65.9 ± 15.4	91.6 ± 17.4
ANC9	16.1 ± 1.7	60.2 ± 16.7	66.9 ± 14.5
ANC10	21.4 ± 3.9	98.1 ± 31.8	
ANC11	18.1 ± 2.2	92.8 ± 1.7	49.6 ± 11.5
ANC7K2	12.7 ± 1.7	16.5 ± 4.3	227 ± 19

receptor affinity (IC_{50} < 100 nM). In the glucagon receptor activation assay, none of the peptides reached the maximum of glucagon-induced activity. Peptides ANC4–ANC7 and ANK1–ANK4 inhibited glucagon-induced cAMP activation (>15%). The lysine mutant peptides ANK1–ANK4 appear to possess a better *in vitro* profile compared with the cysteine mutant peptides ANC1–ANC11 in that the former displays more consistent glucagon receptor antagonism.

The eleven single cysteine mutants were PEGylated with a 22-kDa linear PEG or a 43-kDa branched PEG and tested in the GLP-1 receptor activation assay. PEG had a greater negative effect on activity when introduced on internal positions of the peptide (ANC1–ANC6) than at the C terminus (ANC7–ANC11) (Table 5). Therefore, the optimal site for PEGylation was determined to be at the C-terminal position 31 for 22-kDa PEG (*i.e.* ANC7). The branched 43-kDa PEG led to even greater activity loss, which can be recovered by inserting spacers between the PEG and the body of the peptide. For example, the 5-amino acid spacer allowed the 43-kDa PEGylated ANC11 to have the same *in vitro* activity as 22-kDa PEGylated ANC7.

To assess whether PEGylation improves *in vivo* duration of action ANC7-PEG, which has a cysteine added to the C terminus of AN10G followed by modification with a 22-kDa PEG, and the benchmark peptide, fatty acid-modified GLP-1 (FA-GLP-1), were examined for their ability to lower the blood glucose in an intraperitoneal glucose tolerance

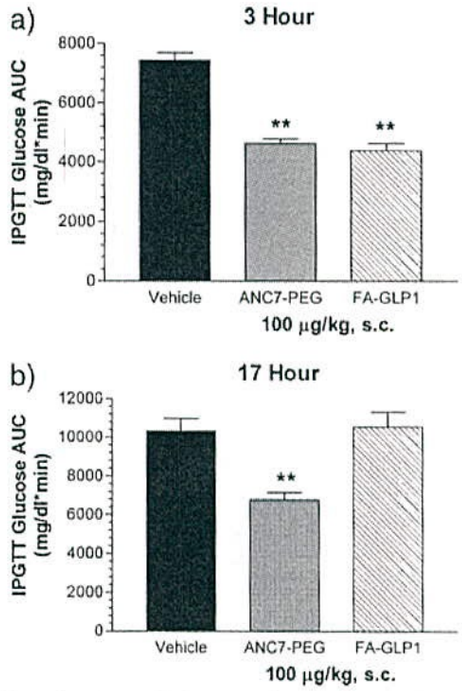

FIGURE 6. **Glucose lowering activity in an IPGTT in mice.** Overnight fasted BALB/c mice were injected subcutaneously with vehicle or 100 μg/kg of ANC7-PEG or FA-GLP-1, and either 3 or 17 h later an IPGTT was performed. The glucose AUC was determined, and the percent reduction due to each treatment was calculated. Data are the mean ± S.E. for 8–16 mice/group. **, $p < 0.05$.

test (IPGTT) performed 3 or 17 h after subcutaneous administration of the peptides (100 μg/kg) in mice. FA-GLP-1 is equivalent to the long acting GLP-1 analogue, NN2211/liraglutide (37). When the IPGTT is performed 3 h after peptide administration, both ANC7-PEG (PEGylated with 22-kDa PEG) and FA-GLP-1 significantly lower the blood glucose area under the curve (AUC) by 38% ($p < 0.01$) and 41% ($p < 0.01$), respectively. When the IPGTT is performed 17 h after peptide administration FA-GLP-1 fails to significantly lower the glucose AUC, whereas ANC7-PEG decreases the glucose AUC 34% ($p < 0.01$) (Fig. 6).

We conclude that the PEGylated ANC7 peptide has prolonged activity *in vivo*.

Based on the overall *in vitro* profile of unPEGylated peptides (Table 4) and the GLP-1 receptor activity of PEGylated peptides (Table 5), the C-terminal position 31 (peptide ANC7) was selected as the optimal position for cysteine PEGylation. Because the lysine mutants ANK1–ANK4 showed more consistent glucagon receptor antagonism, compared with ANC7, cysteine substitution at position 31 was performed on one of the ANK series of peptides (ANK2) to generate the peptide ANC7K2 (Table 3). ANC7K2 is a cross between ANC7 and ANK2 and was selected as the optimal peptide for PEGylation. ANC7K2 is a potent GLP-1 receptor agonist (12.7 nM EC_{50}) and consistently inhibited the glucagon receptor (Table 4). Modification of ANC7K2 with a 22-kDa PEG had minimal effects on GLP-1 receptor activation (Table 5).

DISCUSSION

In this report we describe the design of hybrid peptides with the unique property of activating the GLP-1 receptor but inhibiting the related glucagon receptor. This was achieved by first identifying peptides that bind to both receptors, followed by introduction of mutations that selectively reduce glucagon receptor activation without affecting glucagon receptor binding, while retaining GLP-1 receptor activation. Through detailed structure-activity and mutational analysis we then identified several potential sites for PEGylation. PEGylation at the C-terminal amino acid of the hybrid peptide was shown to retain *in vitro* activity and displayed prolonged duration of action *in vivo*.

In a previous study by Hjorth *et al.* (4), two hybrid peptides, identical to peptides A1 and A6, that bind to both the GLP-1 and glucagon receptors were described. However, the functional activity of these peptides was not described. We determined that peptide A1 is 11-fold less potent compared with GLP-1 in activating the GLP-1 receptor. Peptide A6, while binding to both the GLP-1 and glucagon receptors, has relatively low affinity for the glucagon receptor compared with glucagon itself. Thus these hybrid peptide backbones were not suitable as template sequences for optimization of GLP-1 agonism and glucagon antagonism. To identify a peptide that retains potent GLP-1 receptor activation and glucagon receptor affinity, we designed hybrid peptides A2 to A5 where the number of C-terminal GLP-1 amino acids are intermediate between that of peptides A1 and A6. Receptor binding and activation analysis of these novel peptides revealed A3 as the optimal peptide for GLP-1 receptor activation, with only 2.6-fold reduction in potency, yet retaining glucagon receptor binding affinity. Peptide A3 displays ~3-fold greater potency at the GLP-1 receptor compared with peptide A2, presumably because peptide A3 contains GLP-1 amino acids at positions 20 and 21, adjacent to positions 22 and 23, which are reported to be critical for GLP-1 receptor binding (25, 26). On the other hand, peptides A2 and A3 show comparable potency at the glucagon receptor, consistent with no significant effect on glucagon receptor interaction being detected upon replacement of the amino acid at position 21 of glucagon with that of GLP-1 (22). Likewise, peptide A2 shows ~2-fold greater potency at the GLP-1 receptor but comparable potency at the glucagon receptor compared with peptide A1, consistent with GLP-1 alanine-scanning mutagenesis data and the fact that positions 23 and 24 are not reported to be important for glucagon receptor interaction. Peptide A3 displays ~11-fold greater affinity for the glucagon receptor compared with peptide A4, because peptide A3 contains a glucagon amino acid at position 18, whereas peptide A4 contains a GLP-1 amino acid at this position. When this amino acid is mutated to that of GLP-1 in the context of glucagon, it results in an ~8-fold reduction in glucagon receptor affinity (20). Thus, our results lend further support to previous structure activity studies of GLP-1 and glucagon.

To engineer a selective glucagon antagonist peptide based on the A3 peptide backbone sequence, we introduced mutations known to antagonize the glucagon receptor. The literature was reviewed for glucagon peptide mutations that are reported to reduce receptor activation more than binding. [Tyr^1Glu^9]-, des-His^1-, and [Glu^9]-glucagon bind to the glucagon receptor with comparable affinity to glucagon, but have at least 100-fold lower activity (17, 18). This is similar to what we observed for peptides AN1, AN2, and AN4, where we introduced the same mutations into the A3 peptide backbone. However, these mutations also greatly affected GLP-1 receptor activation, consistent with alanine-scanning mutagenesis of GLP-1 that suggest the importance of positions 1 and 9 for GLP-1 receptor activation (25, 26). The more conservative H1Y mutation of glucagon slightly improved affinity and reduced activity by 3-fold (17), but in the context of the A3 peptide (*i.e.* peptide AN3) this mutation did not reduce glucagon receptor activation. More importantly, AN3 is almost 20-fold less potent compared with the A3 peptide in activating the GLP-1 receptor. This indicates that GLP-1 receptor activation is more sensitive to mutations at position 1 compared with glucagon receptor activation.

In addition to positions 1 and 9 of peptide A3, we evaluated positions 3, 4, 5, 12, 17, and 18 based on glucagon antagonists [Asp^3,D-Phe^4,Ser^5,$Lys^{17,18}$,Glu^{21}]-glucagon and [D-Phe^4,Tyr^5,3,5-I_2-Tyr^{10},Arg^{12},$Lys^{17,18}$,Glu^{21}]-glucagon, which have 20-fold and 2-fold, respectively, lower affinity for the glucagon receptor compared with glucagon (38). In the context of the peptide A3 sequence, we detected a significant loss of glucagon receptor affinity due to the Q3D mutation, as demonstrated by the comparison of AN18 *versus* AN16. However, even AN16, with the G4F/T5Y/K12R triple mutation lost 21-fold affinity for glucagon receptor, compared with peptide A3. This suggests that the R17K/R18K mutations in [D-Phe^4,Tyr^5,3,5-I_2-Tyr^{10},Arg^{12},$Lys^{17,18}$,Glu^{21}]-glucagon may contribute to improved glucagon receptor affinity. This is supported by the triple mutant R17K/R18K/D21E of glucagon that is five times more potent compared with glucagon in the glucagon receptor binding assay (39). Finally, single mutations at positions 4, 5, and 12 of peptide A3 as found in AN13, AN14, and AN15, respectively, greatly reduced glucagon receptor binding. Although we were able to confirm and further extend the understanding of glucagon receptor binding, mutations at positions 3, 4, 5, 12, 17, and 18 did not lead to a selective glucagon antagonist.

Positions 11 and 16 have not been implicated as being critical for GLP-1 activity by alanine scanning mutagenesis (25, 26). Moreover, S11A/S16A and S11A/S16N mutations of glucagon have minimal effect on glucagon receptor binding, but reduced receptor activation by at least 10-fold (21). Although these mutations did not result in glucagon antagonists in the context of glucagon backbone, double mutation S11A/S16A of peptide A3 resulted in the peptide AN10 that is a potent GLP-1 receptor agonist while at the same time inhibits glucagon receptor activation. Presumably this is because of its ability to bind to the glucagon receptor with relatively high affinity, yet it only partially activates the glucagon receptor with relatively weak potency. This large drop in glucagon, but not GLP-1, receptor activation may be caused by greater structure rigidity in this region when the native serine amino acids at positions 11 and 16 are replaced with alanine, which possesses a greater helix-forming propensity (40). Glucagon receptor activation perhaps requires a more flexible midsection than GLP-1 receptor activation, as suggested by GLP-1 having a more defined structure than glucagon in this region. The midsection of GLP-1 covering positions 9 to 15 forms an α-helix (35), whereas positions 11 and 16 bracket the

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glucagon "hinge region" that connects and orients the N-terminal activation and C-terminal binding regions (41). Alternatively, these serines may act as part of an active site triad together with His^1 and Asp^9 that has been suggested for glucagon receptor activation but not GLP-1 receptor activation (32). In conclusion, the combination of two sets of mutations, hybrid N-terminal glucagon and C-terminal GLP-1 sequence of the A3 peptide and double S11A/S16A mutations, that individually have some tendency for glucagon antagonism and minimal effect on GLP-1 activation, is sufficient to produce a selective glucagon antagonist.

GLP-1 has the potential to be a very effective therapeutic drug for the treatment of type 2 diabetes. Multiple approaches are being pursued in an effort to attain a duration of action that would allow for once daily, or more preferably, once weekly administration. Exendin-4 (Exenatide/Byetta®), which lacks the DPP-IV cleavage site, is the only GLP-1 analogue approved for treatment of type 2 diabetes, but it requires twice daily dosing. We eliminated the DPP-IV cleavage site by using glucagon amino acids at the N-terminus of the hybrid peptides. Lipidation, which is the attachment of a fatty acid moiety to a protein or peptide, has been used as an approach to improve plasma half-life. Fatty acid-modified GLP-1 (NN2211/liraglutide) is currently in Phase II clinical development and has demonstrated efficacious glycemic control in clinical studies (42, 43). To identify the optimal site for lipidation of GLP-1 the activity of GLP-1 lipidated at various positions throughout the peptide was examined. GLP-1 was lipidated at positions 2, 12, 17, 20, 21, 28, and 30 by introducing lysine mutations while mutating the existing lysines to arginines. Lipidation at all of these positions, except position 2, had no significant effect on peptide activity *in vitro* (44). In contrast, we observed that PEGylation with high molecular weight PEGs has a more significant effect on peptide activity *in vitro* compared with lipidation, especially when the PEG is attached to internal amino acids of the peptide, such as positions 20 and 21. The reduction in receptor activation appears to be mediated by a corresponding loss of receptor affinity, as PEGylated peptides also display weaker affinity for the receptor compared with the unPEGylated peptides.[3] Thus, it is unlikely that the PEG makes any specific interaction with a particular region of the receptor, consistent with the nonspecific nature of PEG-protein interactions. Because PEG effects on binding and activation are similar, the high molecular weight PEGs conjugated to the C terminus of the peptide may simply interfere with receptor binding through steric interference of the C-terminal region of the peptide, responsible for the initial contact with the receptor. This interference with the C-terminal interaction would result in a subsequent reduction of activation potency mediated by the N-terminal region of the peptide, consistent with the two-site model of activation of family B GPCRs (41). Nevertheless, in many cases the reduced activity of PEGylated proteins *in vitro* due to reduced receptor affinity is more than compensated for by the significantly increased stability and extended half-life of the protein *in vivo*, which results in prolonged exposure and receptor occupancy (45).

From the studies described, ANC7K2 was identified as the optimal GLP-1 agonist/glucagon antagonist peptide for PEGylation. This peptide has been PEGylated with 43-kDa PEG, and its *in vivo* activity has been studied. This PEGylated peptide, referred to as PEG-DAPD (PEGylated dual-acting peptide for diabetes), has prolonged glucose lowering activity following glucose tolerance tests in mice, rats, and dogs. This is evidence of insulin secretion through GLP-1 receptor agonism *in vivo*. In rats glucose-lowering efficacy is evident when a glucose tolerance test is performed up to 65 h after a single subcutaneous injection of the peptide, demonstrating the dramatically prolonged *in vivo* activity of the peptide compared with native GLP-1. Furthermore, PEG-DAPD lowers blood glucose following a glucagon challenge in mice, evidence of *in vivo* glucagon antagonism. PEG-DAPD also lowers fasting blood glucose in a diabetic animal model, *db/db* mice.[4] In conclusion, the mutagenesis data described herein provide further mechanistic insights into GLP-1 and glucagon receptor activation and inhibition, respectively, and have led to the identification of novel dual-acting GLP-1 agonist/glucagon antagonist peptides, which could have potential for development as a therapy for the treatment of type 2 diabetes.

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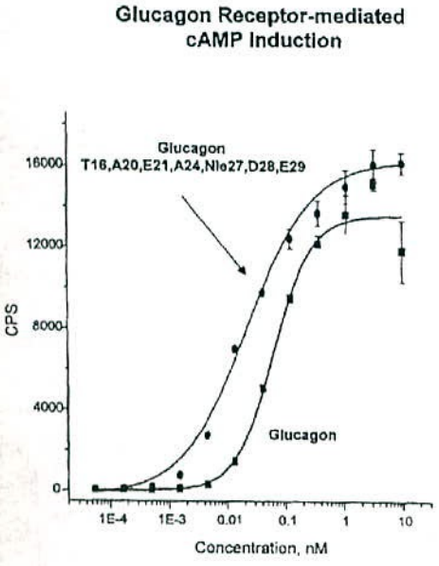
Figures 11A



(57) Abstract: Modified glucagon peptides are disclosed having improved solubility and/or half-life while retaining glucagon agonist activity. The glycogen peptides have been modified by substitution of native amino acids with, and/or addition of, charged amino acids to the carboxy terminus of the peptide. The modified glucagon agonists can be further modified by pegylation, or the addition of a carboxy terminal peptide selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 23, or both to further enhance the solubility of the glucagon agonist analogs.

WO 2009/155257 A1

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

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

GLUCAGON ANALOGS EXHIBITING ENHANCED SOLUBILITY AND STABILITY IN PHYSIOLOGICAL PH BUFFERS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to the following: U.S. Provisional Patent Application No. 61/073,193 filed on June 17, 2008, U.S. Provisional Patent Application No. 61/078,165 filed July 3, 2008, and U.S. Provisional Patent Application No. 61/090,415 filed on August 20, 2008. The disclosure of each application is hereby expressly incorporated by reference in its entirety.

BACKGROUND

Pre-proglucagon is a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides, including glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin (OXM), that are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. Glucagon is a 29-amino acid peptide that corresponds to amino acids 33 through 61 of pre-proglucagon, while GLP-1 is produced as a 37-amino acid peptide that corresponds to amino acids 72 through 108 of pre-proglucagon.

Hypoglycemia occurs when blood glucose levels drops too low to provide enough energy for the body's activities. In adults or children older than 10 years, hypoglycemia is uncommon except as a side effect of diabetes treatment, but it can result from other medications or diseases, hormone or enzyme deficiencies, or tumors. When blood glucose begins to fall, glucagon, a hormone produced by the pancreas, signals the liver to break down glycogen and release glucose, causing blood glucose levels to rise toward a normal level. Thus, glucagon's most recognized role in glucose regulation is to counteract the action of insulin and maintain blood glucose levels. However for diabetics, this glucagon response to hypoglycemia may be impaired, making it harder for glucose levels to return to the normal range.

Hypoglycemia is a life threatening event that requires immediate medical attention. The administration of glucagon is an established medication for treating acute hypoglycemia and it can restore normal levels of glucose within minutes of

administration. When glucagon is used in the acute medical treatment of hypoglycemia, a crystalline form of glucagon is solubilized with a dilute acid buffer and the solution is injected intramuscularly. While this treatment is effective, the methodology is cumbersome and dangerous for someone that is semi-conscious. Accordingly, there is a need for a glucagon analog that maintains the biological performance of the parent molecule but is sufficiently soluble and stable, under relevant physiological conditions, that it can be pre-formulated as a solution, ready for injection.

Additionally, diabetics are encouraged to maintain near normal blood glucose levels to delay or prevent microvascular complications. Achievement of this goal usually requires intensive insulin therapy. In striving to achieve this goal, physicians have encountered a substantial increase in the frequency and severity of hypoglycemia in their diabetic patients. Accordingly, improved pharmaceuticals and methodologies are needed for treating diabetes that are less likely to induce hypoglycemia than current insulin therapies.

As described herein, high potency glucagon agonists are provided that exhibit enhanced biophysical stability and aqueous solubility at physiological pH in pharmaceutical compositions suitable for commercial use. Native glucagon is neither soluble, nor stable in the physiological pH range and thus must be manufactured as a dry product that requires reconstitution and immediate use. The glucagon analogs described herein have enhanced physical properties that render them superior for use in current medicinal settings where the native hormone is currently employed. These compounds can be used in accordance with one embodiment to prepare pre-formulated solutions ready for injection to treat hypoglycemia. Alternatively, the glucagon agonists can be co-administered with insulin to buffer the effects of insulin to allow for a more stable maintenance of blood glucose levels. In addition, other beneficial uses of compositions comprising the modified glucagon peptides disclosed herein are described in detail below.

SUMMARY

One embodiment of the invention provides glucagon peptides that retain glucagon receptor activity and exhibit improved solubility relative to the native glucagon peptide (SEQ ID NO: 1). Native glucagon exhibits poor solubility in

aqueous solution, particularly at physiological pH, with a tendency to aggregate and precipitate over time. In contrast, the glucagon peptides of one embodiment of the invention exhibit at least 2-fold, 5-fold, or even higher solubility compared to native glucagon at a pH between 6 and 8, or between 6 and 9, for example, at pH 7 after 24 hours at 25◦C.

In one embodiment the glucagon peptides retain at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75% activity, 80% activity, 85% activity, or 90% of the activity of native glucagon (calculated as the inverse ratio of EC50s for the glucagon peptide vs. glucagon, e.g., as measured by cAMP production using the assay generally described in Example 13). In one embodiment, the glucagon peptides of the present invention have the same or greater activity (used synonymously with the term "potency" herein) than glucagon. In some embodiments, the glucagon peptides retain up to about 100%, 1000%, 10,000%, 100,000%, or 1,000,000% of the activity of native glucagon.

Glucagon normally has about 1% of the activity of native GLP-1 at the GLP-1 receptor. GLP-1(7-36) amide (SEQ ID NO: 57) or GLP-1(7-37)acid (SEQ ID NO: 58) are biologically potent forms of GLP-1, that demonstrate essentially equivalent activity at the GLP-1 receptor. Glucagon is also 10- to 20- fold more selective for the glucagon receptor compared to GLP-1 receptor (selectivity calculated as the inverse ratio of EC50 of glucagon for the glucagon receptor vs. for the GLP-1 receptor). For example, for an assay in which glucagon's EC50 at the glucagon receptor is 0.22 nM and glucagon's EC50 at the GLP-1 receptor is 3.85 nM, the calculated selectivity is 17.5-fold. Activity can be measured, e.g., by cAMP production using the assay generally described in Example 13. In some embodiments, the glucagon peptides of the present invention exhibit less than about 5%, 4%, 3%, 2% or 1% of the activity of native GLP-1 at the GLP-1 receptor and/or a greater than about 5-fold, 10-fold, or 15-fold selectivity for glucagon receptor compared to GLP-1 receptor. For example, in some embodiments, the glucagon peptides of the present invention exhibit less than 5% of the activity of native GLP-1 at the GLP-1 receptor and exhibit a greater than 5-fold selectivity for glucagon receptor compared to GLP-1 receptor.

Any of the glucagon peptides of the invention may additionally exhibit improved stability and/or reduced degradation, for example, retaining at least 95% of the original peptide after 24 hours at 25 ℃. In some embodiments, the glucagon

peptides of the invention exhibit improved stability, such that at least 75% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, more than 95%, up to 100%) of a concentration of the peptide or less than about 25% (e.g., less than 20%, less than 15%, less than 10%, less than 5%, 4%, 3%, 2%, 1%, down to 0%) of degraded peptide is detectable at 280 nm by an ultraviolet (UV) detector after 1 or more weeks (e.g., 2 weeks, 4 weeks, 1 month, two months, four months, six months, eight months, ten months, twelve months) in solution at a temperature of at least 20 °C (e.g., 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, 26 °C, at least 27.5 °C, at least 30 °C, at least 35 °C, at least 40 °C, at least 50 °C) and less than 100 °C, less than 85 °C, less than 75 °C, or less than 70 °C.

In accordance with one embodiment a glucagon peptide is provided with improved solubility, wherein the peptide is modified by amino acid substitutions and/or additions that introduce a charged amino acid into the C-terminal portion of the peptide, and in one embodiment at a position C-terminal to position 27 of SEQ ID NO: 1. Optionally, one, two or three charged amino acids may be introduced within the C-terminal portion, and in one embodiment C-terminal to position 27. In accordance with one embodiment the native amino acid(s) at positions 28 and/or 29 are substituted with a charged amino acid, and/or one to three charged amino acids are added to the C-terminus of the peptide, after position 29. In exemplary embodiments, one, two or all of the charged amino acids are negatively charged. Additional modifications, e.g. conservative substitutions, may be made to the glucagon peptide that still allow it to retain glucagon activity.

In accordance with one exemplary embodiment the glucagon peptide comprises an amino acid sequence of SEQ ID NO: 11, or an analog thereof that contains 1 to 3 further amino acid modifications relative to native glucagon, or a glucagon agonist analog thereof. SEQ ID NO: 11 represents a modified glucagon peptide wherein the asparagine residue at position 28 of the native protein has been substituted with an aspartic acid. In another exemplary embodiment the glucagon peptide comprises an amino acid sequence of SEQ ID NO: 38, wherein the asparagine residue at position 28 of the native protein has been substituted with glutamic acid. Other exemplary embodiments include glucagon peptides of SEQ ID NOS: 24, 25, 26, 33, 35, 36 and 37.

In accordance with another embodiment, glucagon peptides are provided that have enhanced potency at the glucagon receptor, wherein the peptides comprise an amino acid modification at position 16 of native glucagon (SEQ ID NO: 1). By way of nonlimiting example, such enhanced potency can be provided by substituting the naturally occurring serine at position 16 with glutamic acid or with another negatively charged amino acid having a side chain with a length of 4 atoms, or alternatively with any one of glutamine, homoglutamic acid, or homocysteic acid, or a charged amino acid having a side chain containing at least one heteroatom, (e.g. N, O, S, P) and with a side chain length of about 4 (or 3-5) atoms. Substitution of serine at position 16 with glutamic acid enhances glucagon activity at least 2-fold, 4-fold, 5-fold and up to 10-fold greater at the glucagon receptor. In some embodiments, glucagon peptide retains selectivity for the glucagon receptor relative to the GLP-1 receptors, e.g., at least 5-fold, 10-fold, or 15-fold selectivity.

The solubility of any of the foregoing compounds can be further improved by attaching a hydrophilic moiety to the peptide. Introduction of such groups also increases duration of action, e.g. as measured by a prolonged half-life in circulation. In one embodiment the hydrophilic moiety is a polyethylene glycol (PEG) chain or other water soluble polymer that is covalently linked to the side chain of an amino acid residue at one or more of positions 16, 17, 21, 24, 29, 40 of said glucagon peptide, within a C-terminal extension, or at the C-terminal amino acid. In some embodiments, the native amino acid at that position is substituted with an amino acid having a side chain suitable for crosslinking with hydrophilic moieties, to facilitate linkage of the hydrophilic moiety to the peptide. Exemplary amino acids include Cys, Lys, Orn, homo-Cys, or acetyl phenylalanine (Ac-Phe). In other embodiments, an amino acid modified to comprise a hydrophilic group is added to the peptide at the C-terminus. The polyethylene glycol chain in accordance with one embodiment has a molecular weight selected from the range of about 500 to about 40,000 Daltons. In one embodiment the polyethylene glycol chain has a molecular weight selected from the range of about 500 to about 5,000 Daltons. In another embodiment the polyethylene glycol chain has a molecular weight of about 10,000 to about 20,000 Daltons. In yet other exemplary embodiments the polyethylene glycol chain has a molecular weight of about 20,000 to about 40,000 Daltons.

NOTARÍA 75 - BOGOTÁ D. C.
REPUBLICA DE COLOMBIA

ESCRITURA: 75 ----------

SETENTA Y CINCO ----------

Fecha: ENERO – 23 – 2013 ----------

ACTO: PODER GENERAL ----------

DE: Laboratorio Franco Colombiano LAFRANCOL S.A.S. Sigla: LAFRANCOL S.A.S., Nit. 890.301.463-8. ----------

Representante legal: Juan Camilo Palacio Ramírez, cc. 19.455.622 de Bogotá. ------

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A FAVOR DE: Tito Noe Parra Murillo, cc. 79.579.680 de Bogotá ----------

T.P. 71.168 C. S. de la J. ----------

VIGENCIA: TÉRMINO INDEFINIDO. ----------

En Bogotá, Distrito Capital, República de Colombia, a los veintitrés (23) días del mes de enero de dos mil trece (2013), ante mí, **MIGUEL ÁNGEL LOZADA URREGO, NOTARIO SETENTA Y CINCO (75) DEL CÍRCULO DE BOGOTÁ - ENCARGADO,** se otorgó escritura en los siguientes términos: ----------

---

OTORGANTE COMPARECIENTE CON MINUTA: ----------

JUAN CAMILO PALACIO RAMÍREZ, varón, colombiano, mayor de edad, domiciliado y residente en esta ciudad e identificado con cédula de ciudadanía 19.455.622 de Bogotá y manifestó: ----------

---

PRIMERO.- Que obra en su carácter de representante legal de **LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.** Sigla: **LAFRANCOL S.A.S.**, Nit. 890.301.463-8, sociedad comercial organizada y existente conforme a las leyes colombianas, con domicilio y sede principal en Cali (Valle), constituida mediante escritura mil cuatrocientos treinta y cuatro (1.434) de la Notaría Cuarta (4ª) del

Círculo de Cali del veintinueve (29) de septiembre de mil novecientos cincuenta y ocho (1958), reformada en distintas oportunidades, según consta en el certificado expedido por la Cámara de Comercio de Cali.----------

---

SEGUNDO.- Que en tal carácter, por este público instrumento concede **PODER GENERAL, AMPLIO Y SUFICIENTE** a **TITO NOE PARRA MURILLO**, varón, colombiano, mayor de edad, domiciliado y residente en esta ciudad e identificado con cédula de ciudadanía 79.579.680 de Bogotá, abogado en ejercicio, portador de la Tarjeta Profesional 71.168 del Consejo Superior de la Judicatura, domiciliado en Bogotá, D.C., República de Colombia con dirección en la Diagonal 108 número 51-45 de la misma ciudad, como su representante con poder general amplio y suficiente, para obtener la protección de los derechos de propiedad industrial, protección a la competencia y asuntos jurisdiccionales en materia de competencia desleal y protección al consumidor de LAFRANCOL S.A.S, a cuyo efecto lo autorizo, para que nos represente ante cualesquiera entidad o persona, ejerza o no jurisdicción, especialmente ante las del orden administrativo, contencioso administrativo y judicial, en todo lo concerniente a los siguientes asuntos:----------

a) En cuanto a marcas, lemas, enseñas comerciales, depósito de nombres comerciales, diseños industriales y modelos de utilidad, patentes de invención y derechos de autor para que solicite su registro o depósito, lleve los procesos respectivos hasta su culminación, haga solicitudes, solicite traspasos, cesiones, modificaciones o correcciones, solicite prórrogas, preste caución, solicite renovaciones, inscriba afectaciones, acuerdo comercialización y licencias de uso, desista de solicitudes, presente derechos de petición, responda cualquier requerimiento o auto, presente recursos, presente demandas, intervenga en procesos administrativos y jurisdiccionales, asista audiencias, presente y solicite pruebas, conteste demandas, proteja en general los derechos y prioridades, formule observaciones, presente acciones de oposición, de cancelación, de nulidad, de medidas cautelares y en general intervenga e procesos civiles, penales, administrativos, o contenciosos – administrativos relacionados con estos derechos u otros de similar naturaleza, que promueva LAFRANCOL S.A.S, o se promuevan contra ésta. ----------

b) En cuanto a procesos jurisdiccionales, en materia de competencia desleal y en materia de protección al consumidor, lleve los mismos hasta su culminación,

presentando demandas y respondiendo a las mismas, actuando en todo lo atinente con la naturaleza de dichos procesos. Así mismo, represente a la sociedad poderdante en todos los trámites relativos a protección de la competencia que la entidad encargada pueda adelantar. En general, faculto a mi apoderado a realizar todos aquellos trámites en materia de protección al consumidor, protección de la competencia, reglamentos técnicos y metrología legal, incluso aquellas investigaciones administrativas por reporte de precios, protección de datos personales, asuntos relacionados con propiedad industrial y asuntos jurisdiccionales en materia de protección al consumidor y competencia desleal. ------

c) Para que en todos los asuntos arriba determinados, tanto en los términos señalados por los artículos 42 y complementarios de la Decisión 486 de la Comunidad Andina de Naciones como del artículo 70 del C.P.C., y normas pertinentes del Código Contencioso Administrativo, pueda sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistir, cancelar, recibir, renunciar y ratificar los actos de agentes oficiosos, de todos los asuntos antes mencionados que se deban tramitar ante la Superintendencia de Industria y Comercio, los Tribunales Contencioso Administrativos, Consejo de Estado y jurisdicción penal, civil o comercial. ----------

NOTA.- Manifiesto que de la veracidad de las declaraciones y autenticidad de los documentos anexos en este instrumento público solo responde la parte compareciente. ----------

---

DECLARACIÓN DEL REPRESENTANTE LEGAL DE LA PODERDANTE: ----------

El representante legal declara bajo juramento: ----------

a) Que la sociedad a la cual representa, no ha sido disuelta a la fecha. ----------

b) Que las facultades que sirven de fundamento para el otorgamiento de la presente escritura se encuentran vigentes y se hace responsable conforme a la Ley. ----------

c) Que no existe impedimento legal alguno para ejercer el presente mandato. -------

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Presente **TITO NOE PARRA MURILLO,** de condiciones civiles indicadas, declaró que acepta el poder que por esta escritura se le otorga.----------

---

HASTA AQUÍ EL CONTENIDO DE LA MINUTA PRESENTADA PREVIAMENTE ELABORADA, REVISADA, APROBADA Y ACEPTADA//----------

---

LOS COMPARECIENTES HACEN CONSTAR QUE: ----------

1. Han verificado cuidadosamente sus nombres y apellidos, su real estado civil, números correcto de sus documentos de identificación y aprueban este instrumento sin reserva alguna, en la forma como quedó redactado. ----------
Por consiguiente, sólo solicitarán correcciones, aclaraciones o modificaciones al texto de la presente escritura en la forma y en los casos previstos por la Ley. --------

---

2. Las declaraciones consignadas en este instrumento corresponden a la verdad y los otorgantes las aprueban totalmente, sin reserva alguna.----------

---

3. Conocen la ley y saben que el Notario Encargado responde de la regularidad formal de los instrumentos que autoriza, pero no de la veracidad de las declaraciones de los otorgantes, ni de la autenticidad de los documentos que forman parte de este instrumento.----------

---

Política de privacidad: Los otorgantes, expresamente declaran que **NO** autorizan la divulgación, ni comercialización, ni publicación por ningún medio, sin excepción alguna, de su imagen personal y/o fotografía tomada en la Notaría 75 - Bogotá, ni su huella digital, ni de sus documentos de identidad, ni su dirección electrónica ni física, ni teléfonos, salvo lo relacionado con el presente instrumento y demás actos notariales que personalmente o por intermedio de apoderado soliciten por escrito, conforme a la Ley. ----------

---

OTORGAMIENTO Y AUTORIZACIÓN

LEIDO, APROBADO TOTALMENTE SIN OBJECION ALGUNA Y FIRMADO por los otorgantes este instrumento, que se elaboró conforme a su voluntad, declaraciones e instrucciones, se les hicieron las advertencias de Ley. El Notario Encargado lo autoriza y da fe de ello. ----------

Instrumento elaborado papel notarial números: Aa001312464, Aa001312473, Aa001312498.-

Enmendado: "Aa001312473". SI VALE.-

ESTA HOJA CORRESPONDE A LA ESCRITURA: **75**

DE FECHA: **ENERO – 23 – 2013**

OTORGADA EN LA NOTARIA SETENTA Y CINCO (75) DEL CÍRCULO DE BOGOTÁ

ESCRITURACIÓN

RECIBIÓ Marcela — RADICÓ Marcela

DIGITÓ — Vo.Bo. Marcela

IDENTIFICÓ — HUELLAS/FOTO P.C. — o —

LIQUIDÓ 1 Alejandro — LIQUIDÓ 2 Giovanni

REV./LEGAL Giovanni — CERRÓ

ORGANIZÓ

J. ROJAS R. 52445602

Derechos notariales:	**$45.320**
Total gastos de escrituración	**$58.842**
IVA:	**$16.666**
Superintendencia de Notariado y registro:	**$4.250**
Cuenta especial para el Notariado:	**$4.250**

JUAN CAMILO PALACIO RAMÍREZ

C.C. 1947822

DIRECCION.

TELEFONO.

CELULAR

E-MAIL:

Representante legal de LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S., sigla: LAFRANCOL S.A.S.

REPÚBLICA
RAMÓN ALBERTO
NOTARIA 75

J. ROJAS R.
52445802

TITO NOE PARRA MURILLO
CC 79174680
T.P. 71168
DIRECCION:
TELEFONO:
CELULAR:
E-MAIL:
FECHA:

REPÚBLICA DE COLOMBIA
MIGUEL ANGEL LOZADA URREGO
NOTARIO ENCARGADO
NOTARÍA 75 -BOGOTÁ D.C.

MIGUEL ÁNGEL LOZADA URREGO
NOTARIO SETENTA Y CINCO (75) DEL CÍRCULO DE BOGOTÁ - ENCARGADO

0075/13

Avenida Suba No. 106-52
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E-mail: notaria75@gmail.com
Preparó: Johanna Rojas – 878/13.-

Ramón Alberto Lozada de la Cruz
Notaria75@gmail.com

COLOMBIA
ADA DE LA CRUZ
GOTÁ D.C.

REPÚBLICA DE COLOMBIA
RAMÓN ALBERTO LOZADA DE LA CRUZ
75 NOTARIA 75 -BOGOTÁ D.C.

ES FIEL Y DOSCIENTOS NOVENTA (290) COPIA, DE LA ESCRITURA 75 DE ENERO 23 DE 2013, TOMADA DE SU ORIGINAL EN CUATRO (4) HOJAS (INCLUIDA ESTA). DEC. 960/70 ART. 80 - MODIFICADO ART. 42 DEC. 2163/70 – ART. 41 DEC. 2148/83 -

HOY 28/10/2013, SIENDO LAS 11:30 A.M.; LA PRESENTE ESCRITURA NO TIENE NOTA DE REVOCACIÓN O SUSTITUCIÓN DE PODER.

NOTA: Según lo dispuesto por el artículo 2189 del Código Civil el poder termina, entre otras causas, por:

1.- La muerte o interdicción del poderdante,

2.- La quiebra o insolvencia del poderdante o apoderado.

SE EXPIDE CON DESTINO A:

NUESTRO USUARIO

BOGOTÁ D.C., 28/10/2013

REPÚBLICA DE COLOMBIA
RAMÓN ALBERTO LOZADA DE LA CRUZ
75 NOTARIA 75 -BOGOTÁ D.C.

RAMÓN ALBERTO LOZADA DE LA CRUZ
NOTARIO 75 - BOGOTÁ

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1368326881

Cámara de Comercio de Bogotá

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2013/12/13 HORA: 08:22:54

OPERACION: 02LJS1213021 PAGINA: 1

****

REPUBLICA DE COLOMBIA

CERTIFICADO DE EXISTENCIA Y REPRESENTACION DE SOCIEDADES

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE CALI

CERTIFICA:

NOMBRE:LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.

SIGLA : LAFRANCOL S.A.S.

DOMICILIO: CALI VALLE

DIRECCION DOMICILIO PRINCIPAL:CRA. 1 NRO. 46 84

DIRECCION NOTIFICACION JUDICIAL:CRA. 1 NRO. 46 84

CIUDAD:CALI

MATRICULA MERCANTIL NRO. 5233-16 FECHA MATRICULA : 02 DE OCTUBRE DE 1958

DIRECCION ELECTRONICA : lafrancolsa@lafrancol.co

DIRECCION WEB : www.lafrancol.com

AFILIADO

CERTIFICA:

NIT : 890301463-8

CERTIFICA:

QUE POR ESCRITURA NRO. 1434 DEL 29 DE SEPTIEMBRE DE 1958 NOTARIA CUARTA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 02 DE OCTUBRE DE 1958 BAJO EL NRO. 18319 DEL LIBRO IX ,SE CONSTITUYO LABORATORIO FRANCO COLOMBIANO LAFRANCOL S. A.

CERTIFICA:

QUE POR ESCRITURA NRO. 3672 DEL 24 DE AGOSTO DE 2009 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 27 DE AGOSTO DE 2009 BAJO EL NRO. 9876 DEL LIBRO IX ,CAMBIO SU NOMBRE DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. . POR EL DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. SIGLA: LAFRANCOL S. A. .

CERTIFICA:

QUE POR ACTA NRO. 124 DEL 30 DE MARZO DE 2012 ASAMBLEA GENERAL ,INSCRITA EN LA CAMARA DE COMERCIO EL 31 DE MAYO DE 2012 BAJO EL NRO. 6645 DEL LIBRO IX ,SE TRANSFORMO DE SOCIEDAD ANÓNIMA EN SOCIEDAD POR ACCIONES SIMPLIFICADA BAJO EL NOMBRE DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. SIGLA: LAFRANCOL S.A. S. .

CERTIFICA:

QUE POR ACTA NRO. 124 DEL 30 DE MARZO DE 2012 ASAMBLEA DE ACCIONISTAS ,

*** CONTINUA ***

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2013/12/13 HORA: 08:22:54

OPERACION: 02LJS1213021 PAGINA: 2

****

INSCRITA EN LA CAMARA DE COMERCIO EL 03 DE DICIEMBRE DE 2012 BAJO EL NRO. 14136 DEL LIBRO IX ,SE APROBO LA ESCISION ENTRE (ESCINDENTE) AMERICAN GENERICS S.A.S. Y (BENEFICIARIA(S)) LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. .

CERTIFICA:

REFORMAS

DOCUMENTO	FECHA.DOC	ORIGEN	FECHA.INS	NRO. INS	LIBRO
E.P. 1104	30/06/1959	NOTARIA CUARTA DE CALI	07/07/1959	19467	
E.P. 2309	26/12/1959	NOTARIA CUARTA DE CALI	29/12/1959	20089	
E.P. 1725	24/07/1962	NOTARIA CUARTA DE CALI	26/07/1962	24528	
E.P. 1824	31/07/1963	NOTARIA CUARTA DE CALI	14/08/1963	26360	
E.P. 2422	25/10/1963	NOTARIA CUARTA DE CALI	28/10/1963	26717	
E.P. 1720	16/09/1964	NOTARIA CUARTA DE CALI	22/09/1964	28406	
E.P. 101	31/01/1966	NOTARIA CUARTA DE CALI	01/02/1966	31039	
E.P. 552	21/03/1968	NOTARIA CUARTA DE CALI	01/04/1968	35711	
E.P. 4964	12/12/1972	NOTARIA CUARTA DE CALI	16/01/1973	2975	IX
E.P. 1956	30/05/1973	NOTARIA CUARTA DE CALI	13/06/1973	4337	IX
E.P. 4964	12/12/1972	NOTARIA CUARTA DE CALI	28/11/1973	5994	IX
E.P. 5579	12/11/1973	NOTARIA CUARTA DE CALI	28/11/1973	5995	IX
E.P. 4406	30/09/1974	NOTARIA CUARTA DE CALI	28/11/1974	10887	IX
E.P. 192	03/02/1978	NOTARIA CUARTA DE CALI	14/02/1978	25355	IX

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Cámara de Comercio de Bogotá

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CAMARA DE COMERCIO DE CALI

FECHA: 2013/12/13 HORA: 08:22:54

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E.P. 3479 07/09/1979 NOTARIA CUARTA DE CALI 19/09/1979
34204 IX

E.P. 4274 12/12/1984 NOTARIA NOVENA DE CALI 19/12/1984
73088 IX

E.P. 1155 21/05/1985 NOTARIA SEPTIMA DE CALI 03/06/1985
76917 IX

E.P. 1553 20/06/1986 NOTARIA SEPTIMA DE CALI 08/07/1986
85997 IX

E.P. 1458 09/06/1987 NOTARIA SEPTIMA DE CALI 24/12/1987
3508 IX

E.P. 7222 14/12/1988 NOTARIA TERCERA DE CALI 19/12/1988
13847 IX

E.P. 4530 04/09/1989 NOTARIA TERCERA DE CALI 07/09/1989
21508 IX

E.P. 3865 02/08/1990 NOTARIA TERCERA DE CALI 13/08/1990
31708 IX

E.P. 8305 13/12/1995 NOTARIA TERCERA DE CALI 23/01/1996
557 IX

E.P. 4559 16/11/1999 NOTARIA TERCERA DE CALI 26/11/1999
7911 IX

E.P. 6.004 02/12/2004 NOTARIA TERCERA DE CALI 14/12/2004
13371 IX

E.P. 6241 27/12/2006 NOTARIA TERCERA DE CALI 19/01/2007
625 IX

E.P. 389 07/02/2008 NOTARIA TERCERA DE CALI 19/02/2008
1880 IX

E.P. 1907 11/05/2009 NOTARIA TERCERA DE CALI 26/05/2009
5950 IX

E.P. 3672 24/08/2009 NOTARIA TERCERA DE CALI 27/08/2009
9876 IX

ACT 116 04/03/2010 ASAMBLEA GRAL ORDINARIA 11/05/2010
5470 IX

E.P. 4421 21/10/2010 NOTARIA TERCERA DE CALI 26/10/2010
12622 IX

E.P. 1786 16/05/2011 NOTARIA TERCERA DE CALI 24/05/2011

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6318 IX

ACT 124 30/03/2012 ASAMBLEA GENERAL 31/05/2012

6645 IX

ACT 125 27/07/2012 ASAMBLEA GENERAL DE ACCIONISTAS 23/08/2012

10177 IX

ACT 127 12/12/2012 ASAMBLEA GENERAL DE ACCIONISTAS 13/12/2012

14600 IX

ACT 131 17/06/2013 ASAMBLEA GENERAL DE ACCIONISTAS 24/06/2013

7217 IX

CERTIFICA:

VIGENCIA: INDEFINIDA

CERTIFICA:

OBJETO SOCIAL. LA SOCIEDAD EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ REALIZAR, ENTRE OTROS, LAS SIGUIENTES ACTIVIDADES COMERCIALES Y EMPRESARIALES: 2.1) FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES. 2.2) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. 2.3) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPO DE PRODUCTOS VETERINARIOS. 2.4) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. 2.5) IMPORTAR CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES Y/O PRODUCTOS INTERMEDIOS O A GRANEL. PARÁGRAFO UNO: EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD; DAR Y RECIBIR EN MUTUO DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN O NO LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS, O CUALESQUIERA OTRAS EMPRESAS DE OTROS OBJETOS SOCIALES. PARÁGRAFO DOS: ADEMÁS DE LAS ACTIVIDADES REFERIDAS EN ESTE ARTICULO, Y LAS ENUNCIADAS EN EL

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PARÁGRAFO UNO, COMO SOCIEDAD POR ACCIONES SIMPLIFICADA, PODRÁ REALIZAR CUALQUIER ACTIVIDAD LICITA DE COMERCIO EN EL PAÍS O EN EL EXTERIOR, POR EJEMPLO, Y DE MANERA ILUSTRATIVA MÁS NO LIMITATIVA, GESTIÓN DE LICENCIAS, REGISTROS DE EXPORTACIÓN O IMPORTACIÓN, CONVENIOS RELATIVOS A ESAS ACTIVIDADES Y LOS ACTOS RELACIONADOS CON EL MISMO. PARÁGRAFO TRES: POR RAZÓN DE SU OBJETO SOCIAL, LA SOCIEDAD NO PODRÁ CONSTITUIRSE GARANTE NI FIADORA DE OBLIGACIONES DISTINTAS DE LAS PROPIAS NI GARANTIZAR CON SUS BIENES OBLIGACIONES DE TERCEROS, A MENOS QUE LO AUTORICE EN CADA CASO LA ASAMBLEA GENERAL DE ACCIONISTAS CON EL VOTO DE LA TOTALIDAD DE LOS MIEMBROS PRESENTES EN LA REUNIÓN CORRESPONDIENTE.

CERTIFICA:

DIRECCIÓN Y ADMINISTRACIÓN. LA DIRECCIÓN Y ADMINISTRACIÓN DE LA SOCIEDAD ESTARÁ A CARGO DE: A) LA ASAMBLEA GENERAL DE ACCIONISTAS; Y B) LOS REPRESENTANTES LEGALES COMO SE DESCRIBEN EN SUS CARGOS Y FUNCIONES EN LOS PRESENTES ESTATUTOS.

REPRESENTACIÓN LEGAL. ADMINISTRACIÓN. LA ADMINISTRACIÓN DE LA SOCIEDAD CORRESPONDE AL REPRESENTANTE LEGAL, EL CUAL SE CLASIFICARÁ EN TRES TIPOS, TIPO A, TIPO B Y TIPO C, ASÍ COMO LA GESTIÓN Y EJECUCIÓN DE LOS NEGOCIOS Y ASUNTOS SOCIALES. LOS REPRESENTANTES LEGALES SERÁN DESIGNADOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS POR UN TÉRMINO INDEFINIDO Y SE MANTENDRÁN VIGENTES HASTA TANTO NO SEAN REMOVIDOS DE SUS CARGOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS. ADICIONALMENTE SE CREA EL CARGO DE REPRESENTANTE LEGAL PARA ASUNTOS LEGALES, ADMINISTRATIVOS Y JUDICIALES EN TODAS LAS JURISDICCIONES, EL CUAL SERÁ IGUALMENTE DESIGNADO POR LA ASAMBLEA GENERAL DE ACCIONISTAS Y CUYAS FUNCIONES SE DESCRIBEN EN EL ARTÍCULO SIGUIENTE DE ESTOS ESTATUTOS.

FUNCIONES DEL REPRESENTANTE LEGAL.- EL REPRESENTANTE LEGAL DE LA SOCIEDAD, SEA REPRESENTANTE TIPO A, B O C, ES UN MANDATARIO CON REPRESENTACIÓN, INVESTIDO DE FACULTADES EJECUTIVAS Y ADMINISTRATIVAS, Y COMO TAL, TIENE A SU CARGO LA REPRESENTACIÓN LEGAL, LA GESTIÓN COMERCIAL Y FINANCIERA, LA RESPONSABILIDAD DE LA ACCIÓN ADMINISTRATIVA, LA COORDINACIÓN Y SUPERVISIÓN GENERAL DE LA EMPRESA, LAS CUALES CUMPLIRÁ CON ARREGLO A LAS NORMAS DE ESTOS ESTATUTOS Y LAS DISPOSICIONES LEGALES, Y CON SUJECIÓN A LAS ÓRDENES E INSTRUCCIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS. AL REPRESENTANTE LEGAL LE CORRESPONDE: 1) REPRESENTAR A LA SOCIEDAD EN LOS NEGOCIOS SOCIALES SIN LÍMITE DE CUANTÍA Y ACTUACIÓN, SIEMPRE Y CUANDO ACTÚEN

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DE MANERA CONJUNTA DOS REPRESENTANTES TIPO A, O UN REPRESENTANTE TIPO A CONJUNTAMENTE CON UN REPRESENTANTE TIPO B; 2) COMPROMETER A LA SOCIEDAD HASTA UNA CUANTÍA DE DOS MILLONES DE DÓLARES DE ESTADOS UNIDOS DE AMÉRICA (US$2.000.000), SIEMPRE Y CUANDO ACTÚEN DE MANERA CONJUNTA DOS REPRESENTANTES TIPO B, O UN REPRESENTANTE TIPO B CON UN REPRESENTANTE TIPO C, O DOS REPRESENTANTES TIPO C. LOS REPRESENTANTES LEGALES CUALQUIERA SEA SU TIPO, DONDE ACTÚEN DE MANERA CONJUNTA AL MENOS DOS DE ELLOS Y SIN LÍMITE DE CUANTÍA PODRÁN (I) SUSCRIBIR EN NOMBRE DE LA SOCIEDAD CONTRATOS DE DISTRIBUCIÓN DE LOS PRODUCTOS FABRICADOS, IMPORTADOS O DE TITULARIDAD DE LA SOCIEDAD, (II) PRESENTAR EN NOMBRE DE LA SOCIEDAD PROPUESTAS U OFERTAS DENTRO DE PROCESOS LICITATORIOS, INVITACIONES DIRECTAS O CUALQUIER PROCESO CONTRACTUAL. ASIMISMO, PODRÁ SUSCRIBIR LOS DOCUMENTOS QUE SE REQUIERAN PARA FORMALIZAR EL CONTRATO EN CASO DE SER ADJUDICADO A LA SOCIEDAD, (III) OTORGAR PODERES. LA OTORGACIÓN DE PODERES DE MANERA PARCIAL Y TOTAL EN NINGÚN CASO PODRÁ EXCEDER LAS CUANTÍAS ESTABLECIDAS PARA LOS DIFERENTES TIPOS DE REPRESENTANTES A, B Y C. EL OTORGAMIENTO DE PODERES ESTARÁ SUJETO A LAS REGLAS DE COMBINACIONES Y FIRMAS ESTABLECIDAS ANTERIORMENTE.

NO OBSTANTE LO ANTERIOR, EL REPRESENTANTE LEGAL CUALQUIERA SEA SU TIPO, DE FORMA INDIVIDUAL Y SIN LÍMITE DE CUANTÍA, PODRÁ FIRMAR DECLARACIONES DE CAMBIO Y DECLARACIONES DE IMPUESTOS DEL ORDEN NACIONAL Y DISTRITAL.

DE IGUAL MANERA EL REPRESENTANTE LEGAL PARA ASUNTOS LEGALES, ADMINISTRATIVOS, JUDICIALES Y EN TODAS LAS JURISDICCIONES TENDRÁ LAS SIGUIENTES FUNCIONES Y ASÍ MISMO PODRÁ OTORGAR PODERES PARA LOS SIGUIENTES ASUNTOS: 1) REPRESENTAR A LA SOCIEDAD JUDICIAL Y EXTRAJUDICIALMENTE EN TODOS LOS ASUNTOS LEGALES, JUDICIALES, ADMINISTRATIVOS, LABORALES, CIVILES, PENALES, CONTENCIOSOS ADMINISTRATIVOS Y DEMÁS ASUNTOS RELACIONADOS; 2) ADELANTAR TODO TIPO DE TRÁMITES ANTE CUALQUIER AUTORIDAD O ENTIDAD PÚBLICA; 3) REPRESENTAR A LA SOCIEDAD EN AUDIENCIAS JUDICIALES, CONCILIAR, RECIBIR, TRANSIGIR, DESISTIR Y CONFERIR PODERES EN RELACIÓN CON LAS ATRIBUCIONES AQUÍ DESCRITAS PARA DICHO REPRESENTANTE.

ADICIONALMENTE, Y CON LAS LIMITACIONES DE MONTOS ANTES EXPUESTAS, EL REPRESENTANTE LEGAL PODRÁ

1) CUMPLIR Y HACER CUMPLIR LAS DECISIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS Y HACER USO DE LA RAZÓN SOCIAL;

2) REPRESENTAR LEGALMENTE A LA SOCIEDAD CONFORME A LA LEY ANTE TODAS LAS

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AUTORIDADES CIVILES, JUDICIALES, MILITARES, SANITARIAS, DE POLICÍAS ADUANALES, POSTALES, Y DEMÁS APLICABLES.

3) ORGANIZAR LOS SERVICIOS Y LAS OFICINAS DE LA SOCIEDAD, LA CONTABILIDAD Y APLICAR LOS ESTATUTOS Y REGLAMENTOS;

4) LLEVAR A CABO LA GESTIÓN COMERCIAL Y FINANCIERA;

5) TENDRÁ LA PLENA RESPONSABILIDAD DE LA ACCIÓN ADMINISTRATIVA, LA COORDINACIÓN Y LA SUPERVISIÓN GENERAL DE LA SOCIEDAD, LAS CUALES CUMPLIRÁ CON ARREGLO A LAS NORMAS DE ESTOS ESTATUTOS;

6) COMPARECER EN LOS JUICIOS, TRANSIGIR Y COMPROMETER LOS NEGOCIOS SOCIALES DE CUALESQUIERA NATURALEZA QUE FUEREN, INTERPONER TODO GÉNERO DE RECURSOS Y CONSTITUIR APODERADO QUE LO REPRESENTEN JUDICIAL O EXTRAJUDICIALMENTE.

7) HACER DEPÓSITOS EN BANCOS Y AGENCIAS BANCARIAS, FIRMAR CHEQUES, LETRAS, PAGARÉS, GIROS, LIBRANZAS Y CUALQUIER OTRO DOCUMENTO, ASÍ COMO NEGOCIAR ESTOS INSTRUMENTOS, TENERLOS, COBRARLOS Y PAGARLOS, DESCARGARLOS, ETC.;

8) NOMBRAR Y REMOVER LIBREMENTE A LOS EMPLEADOS DISTINTOS DE LOS QUE LA ASAMBLEA GENERAL DE ACCIONISTAS TENGA ATRIBUCIONES; DE ACUERDO AL REGLAMENTO INTERNO DE LA COMPAÑÍA.

9) PRESENTAR A LA ASAMBLEA GENERAL DE ACCIONISTAS EN SUS REUNIONES ORDINARIAS, EL INFORME SOBRE LA FORMA COMO HAYA LLEVADO A CABO SU GESTIÓN E INNOVACIONES QUE CONVENGA INTRODUCIR PARA EL MEJOR SERVICIO DE SUS INTERESES;

10) SUSCRIBIR TODA CLASE DE DOCUMENTOS PÚBLICOS O PRIVADOS CON EL PROPÓSITO DE INSTRUMENTAR CUALQUIER TIPO DE ACTOS, NEGOCIOS JURÍDICOS, OPERACIONES DE CUALQUIER ÍNDOLE, Y SIEMPRE Y CUANDO NO SUPERE LA CUANTÍA PREVISTA EN ESTOS ESTATUTOS COMO LÍMITE DEL EJERCICIO DE SUS FUNCIONES Y CUANTÍAS, Y POR ENCIMA DE LA CUAL REQUIERE AUTORIZACIÓN DE LA ASAMBLEA DE ACCIONISTAS;

11) PODRÁ EJERCER LA REPRESENTACIÓN LEGAL SIN PERJUICIO DE LA MISMA FUNCIÓN ASIGNADA A LOS OTROS REPRESENTANTES LEGALES PARA OTORGAR PODERES A TERCEROS, NOMBRAR ABOGADOS Y PROCURADORES, INTERPONER RECLAMACIONES EN LA VÍA GUBERNATIVA, ANTE TRIBUNALES, JURISDICCIONES ORDINARIAS, LABORALES Y ESPECIALES, REPRESENTAR A LA SOCIEDAD ANTE ADMINISTRACIONES O HACIENDAS PÚBLICAS, PRIVADAS, NACIONALES, INTERNACIONALES Y AUTONÓMICAS, CONCURRIR A TODA CLASE DE SUBASTAS, CONCURSOS O SUMINISTROS OFICIALES O PARTICULARES, PUDIENDO POR TANTO REDACTAR, SUSCRIBIR, PRESENTAR Y, EN SU CASO, MEJORAR EN LICITACIÓN VERBAL O ESCRITA LAS OFERTAS O PROPOSICIONES PERTINENTES, ACEPTAR DONACIONES PARA LA SOCIEDAD Y ACEPTAR CON BENEFICIO DE INVENTARIO, LEGADOS,

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EJERCITAR ACCIONES O PONER EXCEPCIONES, PARA ACTUAR EN CALIDAD DE DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE;

12) RESOLVER SOBRE LAS RENUNCIAS Y LICENCIAS DE LOS EMPLEADOS DE LA COMPAÑÍA, CUYO NOMBRAMIENTO LE CORRESPONDA.

13) ORDENAR LOS PAGOS A REALIZAR POR LA SOCIEDAD PARA QUE EL TESORERO O QUIEN HAGA SUS VECES, EXPIDA LAS ÓRDENES DE PAGO USANDO EL MEDIO MÁS EXPEDITO PARA CONTAR CON LA CELERIDAD QUE LOS NEGOCIOS SOCIALES EXIGEN; TODO ELLO DENTRO DE LAS LIMITACIONES ESTABLECIDAS EN ESTOS ESTATUTOS.

14) LOS REPRESENTANTES TIPO B Y C NO PODRÁN VENDER O AUTORIZAR LA VENTA DE LOS INTANGIBLES DE LA COMPAÑÍA.

15) LAS DEMÁS QUE LE CORRESPONDAN CONFORME A LA LEY Y A LOS ESTATUTOS.

SON FUNCIONES DEL REPRESENTANTE LEGAL PARA ASUNTOS LEGALES, ADMINISTRATIVOS Y JUDICIALES EN TODAS LAS JURISDICCIONES LAS SIGUIENTES: 1) REPRESENTAR A LA SOCIEDAD JUDICIAL Y EXTRAJUDICIALMENTE EN TODOS LOS ASUNTOS JUDICIALES, ADMINISTRATIVOS, LABORALES, CIVILES, PENALES, CONTENCIOSOS ADMINISTRATIVOS, Y DEMÁS ASUNTOS RELACIONADOS; 2) ADELANTAR TODO TIPO DE TRÁMITES ANTE CUALQUIER AUTORIDAD O ENTIDAD PÚBLICA; 3) REPRESENTAR A LA SOCIEDAD EN AUDIENCIAS JUDICIALES, CONCILIAR, RECIBIR, TRANSIGIR, DESISTIR Y CONFERIR PODERES EN RELACIÓN CON LAS ATRIBUCIONES AQUÍ DESCRITAS.

CERTIFICA:

DOCUMENTO: ACTA No. 127 DEL 12 DE DICIEMBRE DE 2012
ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS
INSCRIPCION: 14 DE DICIEMBRE DE 2012 No. 14639 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REPRESENTANTE LEGAL TIPO A
ALEJANDRO WEINSTEIN MANIEU
PPTE.5920870-5
REPRESENTANTE LEGAL TIPO A
ALEJANDRO WEINSTEIN CRENOVICH
PPTE.2228962-4
REPRESENTANTE LEGAL TIPO A
NICOLAS WEINSTEIN MANIEU
PPTE.5920871-3
REPRESENTANTE LEGAL TIPO A

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NICOLAS WEINSTEIN DIAZ
PPTE.15376690-8
REPRESENTANTE LEGAL TIPO B
PATRICIO VARGAS MUÑOZ
PPTE.12232715-9
REPRESENTANTE LEGAL TIPO B
VICTOR JULIO CHUNCO
C.E.439831
REPRESENTANTE LEGAL TIPO B
AGUSTIN EGUIGUREN
PPTE.11472453-K
REPRESENTANTE LEGAL TIPO C
LUIS PALACIOS
C.C.319788
REPRESENTANTE LEGAL TIPO C
FRANCISCO JAVIER PICART LAURENZ
C.E.399661
REPRESENTANTE LEGAL TIPO C
JULIO ESPINOZA OVALLE
PPTE.10931698-9

CERTIFICA:

DOCUMENTO: ACTA No. 131 DEL 17 DE JUNIO DE 2013
ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS
INSCRIPCION: 24 DE JUNIO DE 2013 No. 7218 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REPRESENTANTE LEGAL PARA ASUNTOS LEGALES, ADMINISTRATIVOS Y JUDICIALES
TITO NOE PARRA MURILLO
C.C.79579680

CERTIFICA:

DOCUMENTO: ACTA No. 129 DEL 21 DE MARZO DE 2013
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 29 DE AGOSTO DE 2013 No. 10088 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REPRESENTANTE LEGAL TIPO C
VICTOR JULIO CHUNCO

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CERTIFICADO NACIONAL

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C.E.439831

CERTIFICA:

DOCUMENTO: ACTA No. 130 DEL 15 DE MAYO DE 2013
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 28 DE MAYO DE 2013 No. 6165 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REVISOR FISCAL FIRMA
BDO AUDIT S.A.
NIT.860600063-9

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 21 DE MAYO DE 2013
ORIGEN: BDO AUDIT S.A.
INSCRIPCION: 28 DE MAYO DE 2013 No. 6166 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REVISOR FISCAL PRINCIPAL
GIOVANNI MOSQUERA RAYO
C.C.16739734

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 06 DE NOVIEMBRE DE 2013
ORIGEN: BDO AUDIT S.A.
INSCRIPCION: 20 DE NOVIEMBRE DE 2013 No. 13443 DEL LIBRO IX
FUE(RON) NOMBRADO(S):
REVISOR FISCAL SUPLENTE
CARLOS ANDRES BUENO PORTILLA
C.C.16506322

CERTIFICA:

CAPITAL AUTORIZADO: $40,000,000,224
NUMERO DE ACCIONES: 3,333,333,352
VALOR NOMINAL: $12
CAPITAL SUSCRITO: $24,140,480,940
NUMERO DE ACCIONES: 2,011,706,745
VALOR NOMINAL: $12
CAPITAL PAGADO: $24,140,480,940
NUMERO DE ACCIONES: 2,011,706,745
VALOR NOMINAL: $12

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CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 03 DE DICIEMBRE DE 2009
INSCRIPCIÓN: 28 DE DICIEMBRE DE 2009 BAJO EL NRO. 14869 DEL LIBRO IX
CONSTA LA SITUACIÓN DE CONTROL:
CONTROLANTE: DORAL INVESTMENTS INTERNATIONAL INC
DOMICILIO: PROVINCIA DE PANAMA
ACTIVIDAD: OTRAS ACTIVIDADES EMPRESARIALES
CONTROLADA: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.
NIT: 890301463-8
DOMICILIO: CALI VALLE
NACIONALIDAD: COLOMBIANA
ACTIVIDAD: LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETETICOS, COLORANTES DE PERFUMERÍA Y AFINES.
PRESUPUESTO DE CONTROL: POSEE PARTICIPACIÓN ACCIONARIA MAYOR AL 50% A PARTIR DEL 10 DE NOVIEMBRE DE 2009.

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 30 DE ABRIL DE 2004
INSCRIPCIÓN: 03 DE MAYO DE 2004 No. 4859 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 29 DE ABRIL DE 2005
INSCRIPCIÓN: 06 DE MAYO DE 2005 No. 4983 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 15 DE JUNIO DE 2006
INSCRIPCIÓN: 21 DE JUNIO DE 2006 No. 7526 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 01 DE NOVIEMBRE DE 2006
INSCRIPCIÓN: 28 DE NOVIEMBRE DE 2006 No. 13380 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2006
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2006 No. 14301 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2007
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2007 No. 13604 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE OCTUBRE DE 2009
INSCRIPCIÓN: 22 DE OCTUBRE DE 2009 No. 12216 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 23 DE FEBRERO DE 2010
INSCRIPCIÓN: 25 DE FEBRERO DE 2010 No. 2203 DEL LIBRO IX

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DOCUMENTO: DOCUMENTO PRIVADO DEL 27 DE ABRIL DE 2010
INSCRIPCIÓN: 28 DE ABRIL DE 2010 NRO. 4911 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 10 DE JUNIO DE 2010
INSCRIPCION: 17 DE JUNIO DE 2010 NRO. 7195 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 25 DE AGOSTO DE 2010
INSCRIPCION: 07 DE SEPTIEMBRE DE 2010 BAJO EL NRO. 10541 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 14 DE JUNIO DEL 2011
INSCRIPCION: 22 DE JUNIO DE 2011 BAJO EL NRO. 7772 LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 02 DE MAYO DE 2012
INSCRIPCION: 03 DE MAYO DEL 2012 NRO. 5421 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 01 DE JUNIO DE 2012
INSCRIPCIÓN: 05 DE JUNIO DEL 2012 NRO. 6825 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 22 DE AGOSTO DE 2012
INSCRIPCIÓN: 31 DE AGOSTO DEL 2012 NRO. 10510 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 15 DE NOVIEMBRE DE 2012
INSCRIPCION: 21 DE NOVIEMBRE DE 2012 No. 13665 DEL LIBRO IX
CONSTA EL SIGUIENTE GRUPO EMPRESARIAL:
CONTROLANTE
LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S
NIT. 890301463-8
DOMICILIO:CALI VALLE
NACIONALIDAD:COLOMBIA
ACTIVIDAD: A) LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETETICOS, COLORANTES DE PERFUMERÍA Y AFINES. B) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. C) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPOS DE PRODUCTOS VETERINARIOS. D) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. EN DESARROLLO DE SU OBJETO SOCIAL PUEDE LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD, DAR Y RECIBIR A MUTUO COMERCIAL DINERO Y CELEBRAR LOS

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CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS.

SUBORDINADA

AMERICAN GENERICS S A S

NIT. 800223214-9

DOMICILIO:CALI VALLE

NACIONALIDAD:COLOMBIA

PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LAFRANCOL INTERNACIONAL S.A.S.

NIT. 815003912-2

DOMICILIO:PALMIRA VALLE

NACIONALIDAD:COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: LA SOCIEDAD EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ REALIZAR, ENTRE OTROS, LAS SIGUIENTES ACTIVIDADES COMERCIALES Y EMPRESARIALES DESDE LA ZONA FRANCA CUMPLIENDO LAS NORMAS DEL RÉGIMEN FRANCO: 1) LA PRESTACIÓN DE TODO TIPO DE SERVICIOS ASI COMO FABRICAR, DISTRIBUIR, VENDER, COMPRAR, IMPORTAR Y EXPORTAR TODO TIPO DE MATERIAS PRIMAS, PRODUCTOS TERMINADOS Y SEMITERMINADOS RELACIONADOS CON LA INDUSTRIA DE ALIMENTOS FUNCIONALES, COSMÉTICA Y FARMACÉUTICA. 2) DESARROLLAR Y EJECUTAR EN CUALQUIER FORMA O MODO SERVICIOS LOGÍSTICOS, CONTRATAR DIRECTAMENTE O MEDIANTE CONTRATO EXTERNO TODAS Y CADA UNA DE SUS ACTIVIDADES O SERVICIOS. 3) CELEBRAR CUALQUIER TIPO DE CONTRATO DE FABRICACIÓN DE MAQUILA CON ENTIDADES COLOMBIANAS O EXTRANJERAS.

SUBORDINADA

LAFRANCOL PERÚ S.R.L.

DOMICILIO:LIMA

NACIONALIDAD:PERU

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LABORATORIOS PAULY PHARMACEUTICAL S.A.S.

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NIT. 800097402-6
DOMICILIO:CALI VALLE
NACIONALIDAD:COLOMBIA
PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.
ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.
SUBORDINADA
LAFRANCOL GUATEMALA S.A.
NIT. 4088707
DOMICILIO:GUATEMALA
NACIONALIDAD:GUATEMALA
PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.
ACTIVIDAD: PRODUCCIÓN, FABRICACIÓN, EXPORTACIÓN Y DISTRIBUCIÓN DE PRODUCTOS FARMACÉUTICOS Y/O ALIMENTICIOS Y NATURALES DE CONSUMO HUMANO Y LA COMERCIALIZACIÓN DE TODA CLASE DE MERCANCÍAS POR CUENTA PROPIA O EN NOMBRE DE TERCEROS.
SUBORDINADA
DISTRIBUCIONES UQUIFA S A S
NIT. 800182021-7
DOMICILIO:CALI VALLE
NACIONALIDAD:COLOMBIA
PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.
ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS Y ALIMENTOS FUNCIONALES DE CONSUMO HUMANO.
SUBORDINADA
LABORATORIOS NATURMEDIK S A S
NIT. 800226363-1
DOMICILIO:CALI VALLE
NACIONALIDAD:COLOMBIA
PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.
ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.
SUBORDINADA
LAFRANCOL DOMINICANA S.A.S.
DOMICILIO:SANTO DOMINGO REPÚBLICA DOMINICANA

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Cámara de Comercio de Bogotá

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2013/12/13 HORA: 08:22:54

OPERACION: 02LJS1213021 PAGINA: 15

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NACIONALIDAD:REPÚBLICA DOMINICANA
PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222.
ACTIVIDAD:FABRICACIÓN DE PRODUCTOS FARMACÉUTICOS.
SUBORDINADA
FOCUS PHARMACEUTICAL S.A.S.
NIT: 900.104.555-8
DOMICILIO: CALI
NACIONALIDAD: COLOMBIANA
PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995. LA FECHA DE LA PRIMERA TRANSACCION COMERCIAL FUE EN ENERO DE 2010 Y LA CAPITALIZACION SE HIZO EN ENERO DE 2010.
ACTIVIDAD: EL OBJETO PRINCIPAL DE LA SOCIEDAD ES EL DESARROLLO, FABRICACIÓN O PRODUCCIÓN, DIRECTAMENTE O A TRAVÉS DE TERCEROS EN CUALQUIER MODALIDAD NEGOCIAL, LA COMERCIALIZACIÓN Y DISTRIBUCIÓN, NACIONAL O INTERNACIONAL, DE TODA CLASE DE ARTÍCULOS DE GÉNERO FARMACÉUTICO, ESPECÍFICAMENTE DE MEDICAMENTOS ESA ACTIVIDAD PRINCIPAL CONLLEVA LA HABILITACIÓN PARA DESARROLLAR TODA OTRA CONEXA O NECESARIA, INCLUYENDO DE MANERA ESPECIAL PERO NO LIMITATIVA, LA INVESTIGACIÓN FARMACÉUTICA, EL DESARROLLO DE PRODUCTOS MÉDICOS Y FARMACÉUTICOS, EQUIPOS MÉDICOS, TRATAMIENTOS ALTERNATIVOS Y EN GENERAL, TODO AQUELLO VINCULADO A LA PROTECCIÓN DE LA SALUD HUMANA.
PODRÁ EFECTUAR LA INVERSIÓN EN BIENES INMUEBLES, URBANOS O RURALES Y LA ADQUISICIÓN, ADMINISTRACIÓN, ARRENDAMIENTO, GRAVAMEN Y ENAJENACIÓN DE LOS MISMOS.
LA INVERSIÓN DE FONDOS PROPIOS, EN BIENES INMUEBLES, BONOS, VALORES BURSÁTILES Y PARTES DE INTERÉS EN SOCIEDADES COMERCIALES, ASÍ COMO LA NEGOCIACIÓN DE TODA CLASE DE DERECHOS DE CRÉDITO.

CERTIFICA:

SE DEJA CONSTANCIA ASI MISMO, QUE LOS REPRESENTANTES LEGALES TIPO C ANTES DESIGNADOS DEBERÁN ACTUAR SIEMPRE DE LA SIGUIENTE FORMA:
JUAN CAMILO PALACIO CON JAVIER PICART; O
LUIS PALACIOS CON ROBERTO VENTURA; O
JULIO ESPINOZA CON JUAN CAMILO PALACIO; O
JULIO ESPINOZA CON LUIS PALACIOS; O
JULIO ESPINOZA CON JAVIER PICART

CERTIFICA:

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2013/12/13 HORA: 08:22:54

OPERACION: 02LJS1213021 PAGINA: 16

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QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.5234- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL
UBICADO EN: ---K 1 46 84 DE CALI
RENOVO : POR EL AÑO 2013

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.870576- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIOS FRANCO COLOMBIANO LAFRANCOL SAS
UBICADO EN: CL. 36A NRO. 4 05 DE CALI
FECHA MATRICULA : 02 DE MAYO DE 2013

CERTIFICA:

QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 19 DE MARZO DE 2013 .

CERTIFICA:

QUE NO FIGURAN OTRAS INSCRIPCIONES QUE MODIFIQUEN TOTAL O PARCIALMENTE EL PRESENTE CERTIFICADO.

LOS ACTOS ADMINISTRATIVOS DE REGISTRO QUEDAN EN FIRME DIEZ (10) DIAS HABILES DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO DENTRO DE DICHO TERMINO NO SEAN OBJETO DE RECURSOS.

DE CONFORMIDAD CON EL DECRETO 2150 DE 1.995 Y LA AUTORIZACION IMPARTIDA POR LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO LA FIRMA MECANICA QUE APARECE A CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES.

DADO EN CALI A LOS 13 DIAS DEL MES DE DICIEMBRE DEL AÑO 2013 HORA: 08:56:56 AM