US007576050B2

(12) **United States Patent**
Greig et al.

(10) **Patent No.: US 7,576,050 B2**
(45) **Date of Patent: Aug. 18, 2009**

(54) **GLP-1 EXENDIN-4 PEPTIDE ANALOGS AND USES THEREOF**

(75) Inventors: **Nigel Greig**, Phoenix, MD (US); **Josephine Egan**, Baltimore, MD (US); **Maire Doyle**, Baltimore, MD (US); **Harold Holloway**, Middle River, MD (US); **Tracy Ann Perry**, Baltimore, MD (US)

(73) Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 634 days.

(21) Appl. No.: **10/485,140**

(22) PCT Filed: **Jul. 30, 2002**

(86) PCT No.: **PCT/US02/24141**

§ 371 (c)(1),
(2), (4) Date: **Apr. 1, 2004**

(87) PCT Pub. No.: **WO03/011892**

PCT Pub. Date: **Feb. 13, 2003**

(65) **Prior Publication Data**

US 2004/0242853 A1 Dec. 2, 2004

Related U.S. Application Data

(60) Provisional application No. 60/309,076, filed on Jul. 31, 2001.

(51) **Int. Cl.**
A61K 38/26 (2006.01)
C07K 14/605 (2006.01)

(52) **U.S. Cl.** **514/2**; 514/12; 530/308; 530/324

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,710,795	A	1/1973	Higuchi et al.	
6,268,479	B1 *	7/2001	Stern et al.	530/350
6,429,197	B1	8/2002	Collidge et al.	
7,157,429	B1	1/2007	Bachovchin	514/200
2002/0115605	A1 *	8/2002	During et al.	514/12
2003/0004162	A1	1/2003	Treadway	514/228.2
2003/0050227	A1	3/2003	Kondo	514/2
2004/0018981	A1	1/2004	Dong	514/12
2005/0239854	A1	10/2005	Sugiyama	424/449

FOREIGN PATENT DOCUMENTS

EP	0658568	A1	6/1995
WO	WO 97/46584		12/1997
WO	WO 98/30231	A2	7/1998
WO	WO99/038501		8/1999
WO	WO 99/40788	A1	8/1999
WO	WO 99/43705		9/1999
WO	WO99/46283		9/1999
WO	WO99/67278		12/1999
WO	WO 0016797	A2	3/2000
WO	WO 200016797	A2	3/2000
WO	WO 00/34332		6/2000
WO	WO 00/41546	A	7/2000
WO	WO00/47219		8/2000
WO	WO 00/66142	A	11/2000
WO	WO 00/73331	A	12/2000
WO	WO 01/04156	A1	1/2001
WO	WO 01/51078	A	7/2001
WO	WO 02/46227	A	6/2002

OTHER PUBLICATIONS

Wang et al. 'Glucagon-like Peptide-1 Can Reverse the Age-related Decline in Glucose Tolerance in Rats' J. Clin. Invest. 1997 99: 2883-2889.*

Oka et al. Endogenous GLP-1 is involved in [beta]-amyloid protein induced memory impairment and hippocampal neuronal death in rats.*

Siram et al. Experimental Allergic Encephalomyelitis a Misleading Model of Multiple Sclerosis. Ann Neurol. vol. 58, pp. 939-945. 2005.*

Citron, Martin. Alzheimer's Disease: Treatments in Discovery and Development. Nature Neurroscience Supplment. vol. 5, pp. 1055-1057. Nov. 2002.*

File Medline on STN An No. 2005478947. Simmons, Zachary. "Management Strageies for Patients with Amyotrphoic Lateral Sclerosis from diagnosis Through Death." The Neurologist (Sep. 2005), vol. 11, No. 5, pp. 257-270. Abstract only.*

Azzouz, Mimoun "Gene Therapy for ALS: Progress and Prospects" Biochemical et Biophysica Acta (2006), vol. 1762 pp. 112-1127.*

Margolis et al. 'Diagonsis of Huntington Disease' Clinical Chemistry, vol. 49, No. 10 pp. 1726-1732 (2003).*

Korczyn et al. 'Emerging Therapies in the Pharmacological Treatment of Parkinson's Disease' Drugs vol. 62, No. 5 pp. 775-786. 2002.*

Steinman et al. 'How to Successfully Apply Animal Studies in Experimental Allergic Encephalomyelitis to Research on Multple Sclerosis' Ann Neurol. vol. 60, pp. 12-21. 2006.*

(Continued)

Primary Examiner—Anish Gupta
(74) *Attorney, Agent, or Firm*—Ballard Spahr, LLP

(57) **ABSTRACT**

The invention relates to novel polypeptide analogues of GLP-1 and exendin-4. The polypeptide, in a preferred embodiment, is insulinotropic and long-acting. Preferably, the polypeptide's insulinotropic effect is comparable to or exceeds the effect of an equimolar amount of GLP-1 or exendin-4. The invention also relates to a method of treating a subject with diabetes, comprising administering to the subject the polypeptide of the invention in an amount that has an insulinotropic effect. The invention also relates to methods of using GLP-1, exendin-4, and polypeptide analogues thereof for neuroprotective and neurotrophic effects.

OTHER PUBLICATIONS

Patel et al. Pharmacotherapy of Cognitive Impariment in Alzheimer's Disease: A Review J. Geriatr. Psychiatry Neruol. vol. 8 pp. 81-95. 1995.*
Pinderhuges et al. Evidence-Based Apporach to Management of Feverin Patients With End-Stage Dementia. J. of Palliative Med. vol. 6, No. 3, 2003.*
Okamoto et al. "Treatment of Vascular Dementia." Ann. New York Academy of Science. pp. 507-512. 2002.*
Poewe, W. "The Need for Neuroprotective Therapies in Parkinson's Disease." Neurology. vol. 66 Supp. 4, pp. s2-s9.*
Bressler et al., "Pharmacological regulation of blood glucose levels in non-insulin-dependent diabetes mellitus," *Arch. Int. Med.* 157:836-848 (1997).
Calvo et al., "Structural characterization by affinity cross-linking of glucagon-like peptide-1 (7-36) amide receptor in rat brain," *J. Neurochem*, 64(1):299-306 (1995).
Campos et al., "Divergent tissue-specific and developmental expression of receptors for glucagons and glucagon-like peptide-1 in the mouse," *Endocrinology* 134: 2156- 64 (1994).
Chen et al., "Tissue-specific expression of unique mRNAs that encode proglucagon-derived peptides or exendin-4 in the lizard," *J. Biol. Chem*. 272: 4108-4115(1997).
De Ore et al., "The effect of GLP-1 on insulin release in young and old rats in the fasting state and during an intravenous glucose tolerance test," *J. Gernotol*. 52: B245-249 (1997).
Drucker et al., "Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line," *Proc.Natl. Acad. Sci*. 84: 3434-3438 (1987).
Egan et al. "Glucagon-like peptide-1 (7-36) amide (GLP-1) enhances insulin-stimulated glucose metabolism in 3T3-I1 adipocytes: one of several potential exrtapancreatic sites of GLP-1 action." *Endocrinology* 135: 2070-2075, 1994.
Elahi et al., "The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects," *Regul. Pep*. 51: 63-74 (1994).
Elahi, et al. "The effect of age and glucose concentration on insulin secretion by the isolated perfused pancreas." *Endocrinology* 116; 11-16, 1985.
Federal Register 63, Friday, Feb. 20, 1998, p. 8652.
Fehmann et al. "Cell and Molecular Biology of the Incretin Hormones Glucagon-Like Peptide-I and Glucose-dependent Insulin Releasing polypeptide." *Endocrine Rev*. 16:390-410, 1995.
Fehmann et al. "Cell and Molecular Biology of the Incretin Hormones Glucagon-Like Peptide-I and Glucose-Dependent Insulin Releasing Polypeptide," *Endocrine Rev*. 16: 390-410 (1995).
Fehmann et al., "Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells," *Endocrinology* 130: 159-166 (1992).
Gefel et al. "Glucagon-Like Peptide I Analogs: Effects on Insulin Secretin and Adenosine 3',5'-Monophosphate Formation." *Endocrinology* 126:2164-68, 1990.
Geula and Mesulam, "Cortical cholinergic fibers in aging and Alzheimer's disease: a morphometric study," *Neuroscience* 33: 469-81 (1989).
Ghazzi et al., "Cardiac and glycemic benefits of troglitazone treatment in NIDDM," *Diabetes* 46: 433-439. (1997).
Göke et al., "Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites," *Eur. J. Neurosci* : 2294-2300 (1995).
Göke et al., "Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide in an antagonist at the Glucagon-like Peptide-1-(7-36)-amide receptor of insulin-secreting-β-cells," *J. Biol. Chem*. 268: 19650-19655 (1993).
Greig et al., "Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations." *Diabetologia* 42: 45-50 (1999).
Gromada et al. "Glucagon-Like Peptide 1(7-36) Amide Stimulates Exocytosis in Human Pancreatic β-Cells by both Proximal and Distal
Gutniak et al., "Antidiabetogenic effect of glucagon-like peptide-1 (7-36 amide) in normal subjects and patients with diabetes mellitus," *N. Eng. J. Med*. 326: 1316- 1322 (1992).
Guz et al. "Expression of murine STF-1, aputative insulin gene transcription factor, in β cells of pancreas, duodenal epithelium and pancreatic excrine and endocrine progenitors during ontogengy." *Development* 121: 11-18, 1995.
Hawes et al. "Distinct pathways of G_i-and G_α-mediated mitogen-activated protein kinase activation." *J. Biol. Chem*. 270: 17148-17153, 1995.
Holz et al. "Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like-peptide-1." *J. Boil. Chem*. 270: 17749-17757, 1995.
Hosokawa et al. "Mechanism of impaired glucose-potentiated insulin secretion in diabetic 90% pancreatectomy rats. Study using glucagonlike peptide-1 (7-37)." *J. Clin. Invest*. 97: 180-1860, 1996.
Jin et al., "Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: An Immunocytochemical Study," *J. Comp. Neurol*. 271: 519-32. (1988).
Kimura et al. "High concentrations of cholecystokinin octapeptide suppress protein kinase c activity in guinea pig pancreatic acini." *Peptides* 17: 917-925, 1996.
Lahiri et al., "Cholinesterase inhibitors, β-amyloid precursor protein and amyloid β-peptides in Alzheimer's disease," *Acta. Neurol. Scand Suppl* 176 : 60-67.(2000).
Lee, "NeuroD and Neurogenesis," *Dev. Neuroscience* 19:27-32(1997).
Malhotra et al. "Exendin-4, a new peptide from heloderma suspectum vemon, potentiates cholecystokinin-induced amylase from rat pancreatic acini." *Regul. Pept*. 41: 149-156, 1992.
Mark et al., "Amyloid β-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation." *J. Neurosci*. 17 : 1046-1054(1997).
Mashima et al. "Betacellulin and activin A. coordinately convert amylase-secreting AR42J cells into insullin-secreting cells." *J. Clin. Invest*. 97:1647-1654, 1996.
Mashima et al. "Formation of Insulin-Production Cells from pancreatic Acinar AR42J Cells by Hepatocyte Growth Factor." *Endocrinology* 137: 3969-3976, 1996.
Mattson et al., "Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular Ca^{2+} concentration, and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons," *J. Neurochem* 65 (4): 1740-1751. (1995).
Moceri et al., "Early-life risk factors and the development of Alzheimer's disease," *Neurology* 54: 415-420 (2000).
Montrose-Rafizadeh et al. "Incretin hormones regulate glucose-dependent insulin secretion in RIN 1046-38 cells: mechanism of action." *Endocrinology* 135: 589-594, 1994.
Montrose-Rafizadeh et al., "High potency antagonists of the pancreatic glucagon-like peptide-1 receptor," *J. Biol. Chem*. 272: 21201-21206 (1997).
Montrose-Rafizadeh et al., "Incretin hormones regulate glucose-dependent insulin secretion in RIN 1046-38 cells: mechanisms of action," *Endocrinology* 135: 589-594 (1994).
Montrose-Rafizadeh et al., "Overexpression of glucagon-like peptide-1 receptor in an insulin-secreting cell line enhances glucose responsiveness." *Mol, Cell Endocrinol*. 130 (1-2): 109-117. (1997).
Montrose-Rafizdeh et al. "Novel signal transduction and peptide specificity of gluccagon-like peptide receptor in 3T3-L1 adipocytes." *J. Cell. Physiol*. 172: 275-280, 1997.
Nathan et al. "Insulinotropic action of glucagonlike peptide-I- (7-37) in diabetic and nondiabetic subjects," *Diabetes Care* 15: 270-276 (1992).
Nauck et al. "Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide1 (7-36 amide) in type 2 (non-insulin dependent) diabetic patients," *Diabetologia* 36: 741-744 (1993).
Nauck et al. "Preserved incretin activity of Glucagon-like peptide 1 (7-36 amide) but not of synthetic human gastric inhibitory polypeptide in patients with Type-2 diabetes mellitus," *J. Clin. Invest*. 91: 301-307 (1993).
Naya et al. "Diabetes, defective pancreatic morphogenesis, and

Noma, "Overexpression of NeuroD in PC12 cells alters morphology and enhances epxression of the adenylate kinase isozyme 1 gene," *Molecular Brain Research* 67:53-63 (1999).
Orskov, "Glucagon-like peptide-1, a new hormone of the entero-insular axis," *Diabetologia* 35: 701-711 (1992).
Ott et al. "Diabetes mellitus and the risk of dementia: The Rotterdam Study," *Neurology* 53: 1937-42 (1999).
Perfetti et al. "Age-dependent reduction on insulin secretion and insullin mRNA in isolated islet from rats." *Am. J. Physiol*. 269: E983-990, 1995.
Perry et al., "Behavioural, histological and immunocytochemical consequences following 192 IgG-saporin immunolesions of the basal forebrain cholinergic System," *Brain Res. Bull*. 54: 29-48 (2001).
Ritzel et al., "Pharmacokinetic insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-Response-Relationships," *Diabetologia* 38: 720-725 (1995).
Satoh et al., "Characterization of human and rat glucagon-like peptide-1 receptors in the neurointermediate lobe: lack of coupling to either stimulation of inhibition of adenylyl cyclase," *Endocrinology* 141: 1301-9 (2000).
Shughrue et al., "Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus," *Endocrinol*. 137 (11): 5159-62 (1996).
Suzuki et al., "An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP_{717}) mutants," *Science* 264: 1336-1340. (1994).
Teitelman "Induction of beta-cell neogenesis by islet injury." *Diabetes Metabolism Rev*. 12: 91-102, 1996.
Thorens et al. "Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1." *Proc. Natl. Acad. Sci. USA* 89:8641-8645, 1992.
Thorens et al. "Glucagon-like peptide-I and the control of insulin secretion in the normal state and in NIDDM," *Diabetes* 42: 1219-1225 (1993).
Thorens et al., "Cloning and functional expression of the human islet GLP-1 receptor Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor," *Diabetes* 42: 1678-1682 (1993).
Valverde and Villanueva-Penacarrillo et al. "In vitro insulinomimetic effects of GLP-1 in liver, muscle and fat." *Acta Physiologica Scandinavica* 157:359-360, 1996.
Wang et al. "GIP regulates glucose transporters, hexokinases, and glucose-induced insulin secretion in RIN 1046-38 cells," *Mol. Cell. Endo*. 116: 81-87 (1996).
Wang et al. "Glucagon-like peptide-1 is a phsyiological incretin in rat." *J. Clin. Invest*. 95: 417-421, 1995.
Wang et al. Glucagon-like peptides-1 can reverse the age related decline in glucose tolerance in rats. *J. Clin. Invest*. 99: 2883-2889, 1997.
Wang et al., "Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells," *Endocrinology* 136: 4910-4917 (1995).
Wei et al., "Tissue-specific expression of the human receptor for glucagon-like peptide- I: brain, heart and pancreatic forms have the same deduced amino acid sequences," *FEBS Letters* 358(3): 219-224 (Jan. 30, 1995).
Widmann et al. "Desensitization and phosporylation of the glucagon-llike peptide-1 (GLP-1) receptor by GLP-1 and 4-phorbol.12-Myristate 13-acetate." *Mol. Endocrinol*. 10: 62-75, 1996.
Willms et al., "Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in Type 2 (noninsulin-dependent) diabetic patients," *Clin. Endocrinol. Metab*. 81: 327- 332 (1996).
Yada et al. "Glucagon-like peptide-1-(7-36) amide and a rise in cyclic adenosine 3', 5'-monophosphate increase cyosolic free Ca^{2+} in rat pancreatic β-cells by enhancing Ca^{2+}channel activity." *Endocrinology* 133: 1685-1692.
Drucker, "Minireview: The glucagon-like peptides," *Endocrinology* 142:521-527 (2001).
Iwai, et al., "Effects of glucagon-like peptide-1 on LTP in β-amyloid protein (1-42)-treated hippocampal slices." *Soc. Neurosci. Abstr*. 26(12):1116 Abstract No. 420.7 (2000).
Kondo, et al., "Effects of endogenous GLP-1 on neurite elongation in rat primary cultured hippocampal neurons," *Jpn. J. Pharmacol*. 85(1):276, P-866 (2001).
Lahiri, et al., "Exendin-4 (Ex-4) revives PC12 cells from nerve growth factor (NGF)-mediated cell death and apoptosis," *Soc. Neurosci. Abstr*. 27(2):2620, Abstract No. 983.16 (2001).
Lovshin, et al. "Glucagon-like peptide (GLP)-2 action in the murine central nervous system is enhanced by elimination of GLP-1 receptor signaling," *J. Biol. Chem*. 276(24):21489-21499 (Jun. 2001).
Oka, et al., "Endogenous GLP-1 is involved in β-amyloid protein-induced memory impairment and hippocampal neuronal death in rats," *Brain Research* 878:194-198 (2000).
Oka, et al., "Behavioral studies indicate a role for glucagon-like peptide-1 in memory and learning in rat." *Society for Neuroscience* 25:1862, 742.13 (1999).
Oka, et al., Partial translation of "Effect of a GLP-1, on β-amyloid protein-induced memory impairment and hippocampal neuronal death," *The 22nd Annual Meeting of the Japanese Neuroscience Society, Program Abstract*, p. 146, I-P-207 (1999).
Perry, et al., "Evidence of GLP-1 mediated neuroprotection in an animal model of pyridoxine-induced peripheral sensory neuropathy," *Experimental Neurology* 203:293-301 (2007).
Perry, et al., "A Novel Neurotrophic Property of Glucagon-Like Peptide 1: A Promoter of Nerve Growth Factor-Mediated Differentiation in PC12 Cells," *The Journal of Pharmacology and Experimental Therapeutics* 300(3):958-966 (Mar. 2002).
Suzuki, et al., "A role of endogenous GLP-1 in amnesia and neuronal death induced by continuous I.C.V. infusion of β-amyloid protein in rat." *Jpn. J. Pharmacol*. 82(1):236P, P-468 (2000).
Doyle et al., Insertion of an N-terminal 6-Aminohexanoic Acid after the 7 Amino Acid Position of Glucagon-Like Peptide-1 Produces a Long-Acting Hypolycemic Agent, *Endocrinology* 2001, vol. 142, No. 10, pp. 4462-4468.
Ahren et al., No Correlation Between Insulin and Islet Amyloid Polypeptide After Stimulation with Glucagon-like Peptide-1 in Type 2 Diabetes, *EP Journal of Endocrinology*,1997, vol. 137, pp. 643-649.
Koide et al., "Biosynthesis of a Protein Containing A Nonprotein Amino Acid By *Escherichia coli*: L-2-Aminohexanoic Acid At Position 21 in Human Epidermal Growth Factor," *Proc. Natl. Acad. Sci. USA*., vol. 85, pp. 6237-6241, (1988).
Doyle et al., Insertion of an N-terminal 6-Aminohexanoic Acid after the 7 Amino Acid Position of Glucagon-Like Peptide-1 Produces a Long-Acting Hypolycemic Agent, *Endocrinology* 2001, vol. 142, No. 10, pp. 4462-4468.
Ahren et al., No Correlation Between Insulin and Islet Amyloid Polypeptide After Stimulation with Glucagon-like Peptide-1 in Type 2 Diabetes, *EP Journal of Endocrinology*, 1997, vol. 137, pp. 643-649.

* cited by examiner

Figure 1

Amino acid sequence of GLP-1 (7-36) (SEQ ID NO:3), exendin-4 (SEQ ID NO:2), and novel peptide analogues

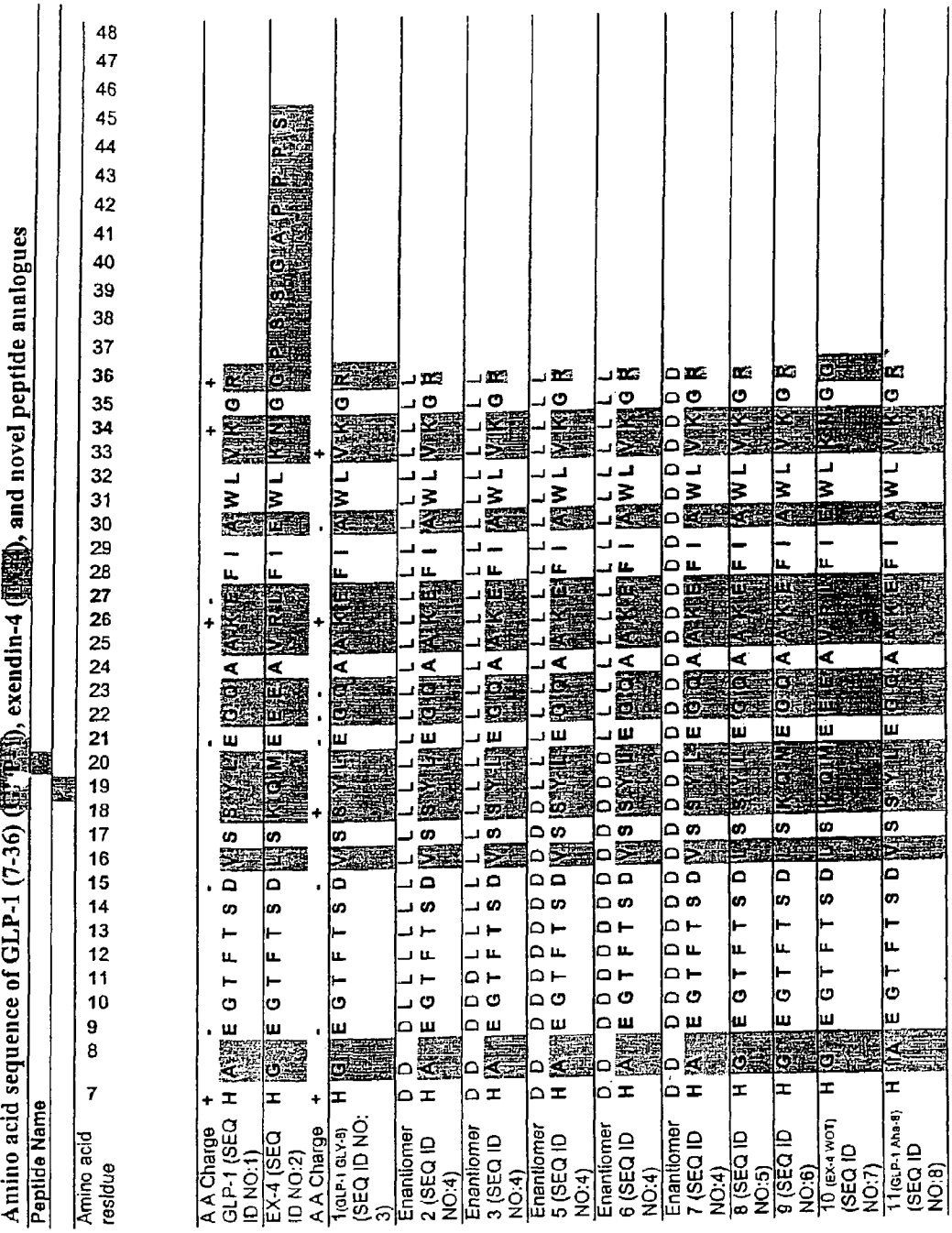
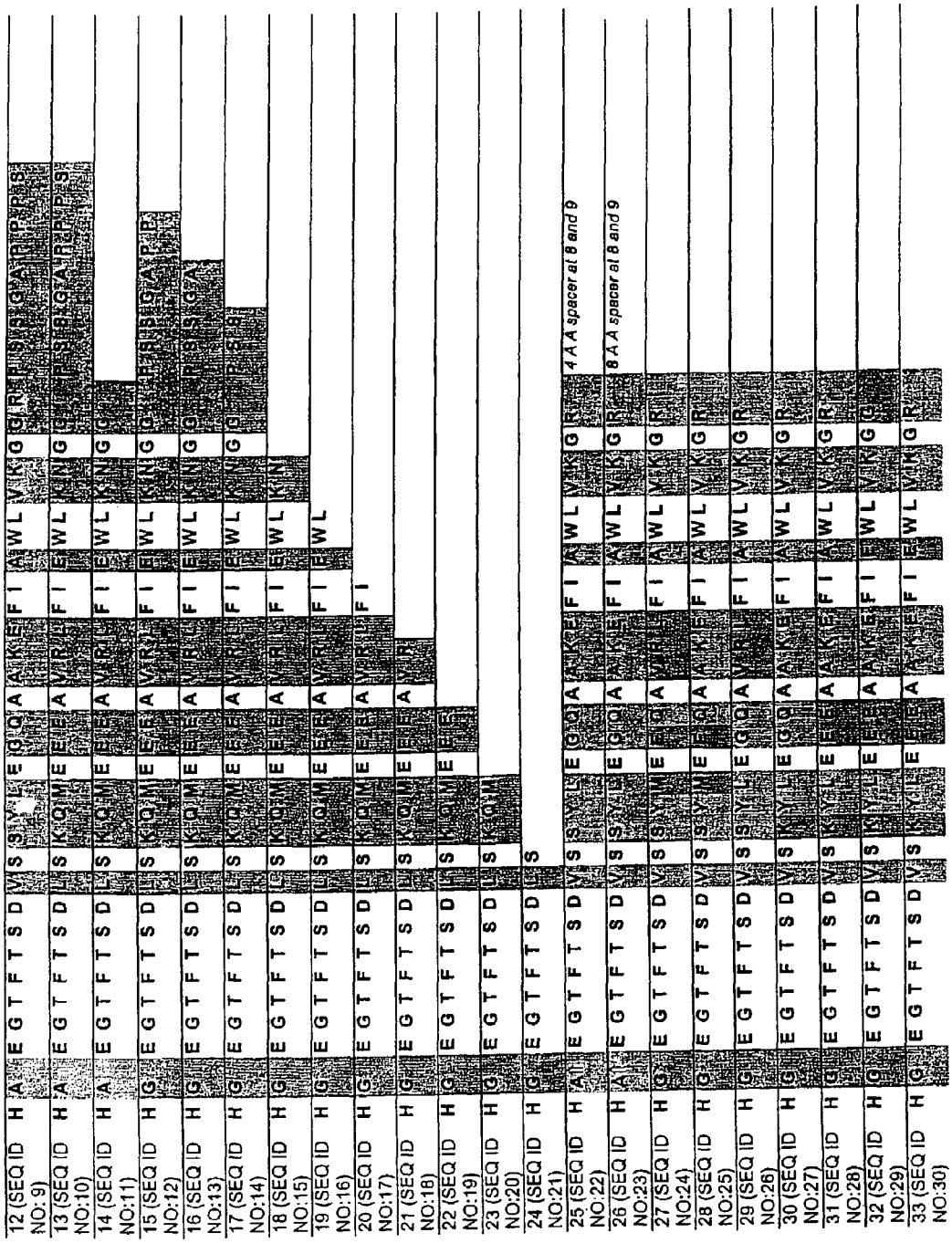


Figure 1 (cont.)



DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.