



US008129342B2

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

(10) **Patent No.:** **US 8,129,342 B2**  
(45) **Date of Patent:** **\*Mar. 6, 2012**

(54) **HIGH PURITY LIPOPEPTIDES**

(75) Inventors: **Thomas J. Kelleher**, Thousand Oaks, CA (US); **Jan-Ji Lai**, Westborough, MA (US); **Joseph P. DeCoursey**, Boston, MA (US); **Paul D. Lynch**, Arlington, MA (US); **Maurizio Zenoni**, Ferentino Frosinone (IT); **Auro R. Tagliani**, Pavia (IT)

5,912,226	A	6/1999	Baker
5,955,509	A	9/1999	Webber
6,194,383	B1	2/2001	Hammann
6,468,967	B1	10/2002	Oleson, Jr. et al.
6,696,412	B1	2/2004	Kelleher
6,852,689	B2	2/2005	Oleson, Jr. et al.
RE39,071	E *	4/2006	Baker et al. .... 514/2.3
8,058,238	B2 *	11/2011	Kelleher et al. .... 435/886

(73) Assignee: **Cubist Pharmaceuticals, Inc.**, Lexington, MA (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.  
This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/888,233**

(22) Filed: **Sep. 22, 2010**

(65) **Prior Publication Data**  
US 2011/0207658 A1 Aug. 25, 2011

**Related U.S. Application Data**

(63) Continuation of application No. 11/739,180, filed on Apr. 24, 2007, now Pat. No. 8,058,238, which is a continuation of application No. 10/747,485, filed on Dec. 29, 2003, now abandoned, which is a continuation of application No. 09/735,191, filed on Nov. 28, 2000, now Pat. No. 6,696,412.

(60) Provisional application No. 60/177,170, filed on Jan. 20, 2000.

(51) **Int. Cl.**  
**C07K 7/50** (2006.01)  
**C07K 7/00** (2006.01)

(52) **U.S. Cl.** ..... **514/9**; 514/11; 514/2; 514/14; 530/317; 530/322; 530/344; 435/886

(58) **Field of Classification Search** ..... 514/9, 11, 514/2, 14; 530/317, 322, 344; 435/886  
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,331,594	A	5/1982	Hamill
4,482,487	A	11/1984	Abbott
4,524,135	A	6/1985	Abbott
4,537,717	A	8/1985	Abbott
RE32,310	E	12/1986	Debono
RE32,311	E	12/1986	Debono
RE32,333	E	1/1987	Hamill
RE32,455	E	7/1987	Hamill
4,800,157	A	1/1989	Eaton
4,874,843	A	10/1989	Baker
4,882,164	A	11/1989	Ferro
4,885,243	A	12/1989	Huber
5,271,935	A	12/1993	Franco
5,387,670	A	2/1995	Roy

FOREIGN PATENT DOCUMENTS

EP	095295	A1	11/1983
EP	178152	A2	4/1986
EP	294990	A2	12/1988
EP	337731	B1	10/1989
EP	386951	A2	9/1990
WO	WO 99/27954		6/1999
WO	WO 99/27957		6/1999
WO	WO 99/40113		8/1999
WO	WO 99/43700		9/1999
WO	WO 00/18419		4/2000
WO	WO 00/81419		4/2000
WO	WO 01/44271		6/2001
WO	WO 01/44272		6/2001
WO	WO 01/44274		6/2001
WO	WO 01/53330		7/2001

OTHER PUBLICATIONS

Agreement between Cubist Pharmaceuticals, Inc. and Eli Lilly and Company dated Nov. 7, 1997 (redacted form from SEC Edgar).  
Agreement between Cubist Pharmaceuticals, Inc. and Eli Lilly and Company dated Oct. 6, 2000 (redacted form from SEC Edgar).  
Assignment of US RE 39,071 from Eli Lilly and Company to Cubist Pharmaceuticals, Inc. recorded on Apr. 23, 2007 Reel/Frame: 019181/0916.  
Debono, M. et al.; "Enzymatic and Chemical Modifications of Lipopeptide Antibiotic A21978C: The Synthesis and Evaluation of Daptomycin (LY146032)," J. Antibiotics; 41; 1988; pp. 1093-1105.

(Continued)

Primary Examiner — Chih-Min Kam

(74) Attorney, Agent, or Firm — Cubist Pharmaceuticals, Inc.

(57) **ABSTRACT**

The invention discloses highly purified daptomycin and to pharmaceutical compositions comprising this compound. The invention discloses a method of purifying daptomycin comprising the sequential steps of anion exchange chromatography, hydrophobic interaction chromatography and anion exchange chromatography. The invention also discloses a method of purifying daptomycin by modified buffer enhanced anion exchange chromatography. The invention also discloses an improved method for producing daptomycin by fermentation of *Streptomyces roseosporus*. The invention also discloses high pressure liquid chromatography methods for analysis of daptomycin purity. The invention also discloses lipopeptide micelles and methods of making the micelles. The invention also discloses methods of using lipopeptide micelles for purifying lipopeptide antibiotics, such as daptomycin. The invention also discloses using lipopeptide micelles therapeutically.

## OTHER PUBLICATIONS

- Desai, J. D., et al.; "Microbial Production of Surfactants and Their Commercial Potential," *Microbiology and Molecular Biology Review*; vol. 61; No. 1; 1997; pp. 47-64; American Society for Microbiology.
- Fostel, Jennifer M., et al.; "Emerging Novel Antifungal Agents," *DDT*; vol. 5; No. 1; Jan. 2000; pp. 25-32; Elsevier Science Ltd.
- Horowitz, Sarah, et al.; "Isolation and Characterization of a Surfactant Produced by *Bacillus licheniformis* 86," *J. Industrial Microbiol.*; 6; 1990; pp. 243-248; Society for Industrial Microbiology.
- Kirsch, Lee E., et al.; "Kinetics of the Aspartyl Transposition of Daptomycin, a Novel Lipopeptide Antibiotic," *Pharmaceutical Research*; vol. 6; No. 5; 1989; pp. 387-393; Plenum Publishing Corporation.
- Lasic, Dan D., et al.; "Novel Applications of Liposomes," *Trends Biotechnology*; vol. 16; Jul. 1998; pp. 307-321; Elsevier Science Ltd.
- Lasic, Danilo D., et al.; "Mixed Micelles in Drug Delivery," *Nature*; vol. 355; Issue No. 6357; Jan. 16, 1992; pp. 279-280.
- Lin, S. C., et al.; "General Approach for the Development of High-Performance Liquid Chromatography Methods for Biosurfactant Analysis and Purification," *J. Chromatography*; 825; 1998; pp. 149-159.
- Lin, S. C. et al.; "Recovery and Purification of the Lipopeptide Biosurfactant of *Bacillus subtilis* by Ultrafiltration," *Biotechnology Techniques*; vol. 11; No. 6; Jun. 1997; pp. 413-416; Chapman Hall.
- Miao, Vivian et al.; "Daptomycin Biosynthesis in *Streptomyces roseosporus*: Cloning and Analysis of the Gene Cluster and Revision of Peptide Stereochemistry," *Microbiology*, (2005), 151, pp. 1507-1523.
- Molloy, M. et al., Abstract, "Structure & Anhydro-Daptomycin and Iso-Daptomycin," ACS 200th Meeting, 1990.
- Molloy, M. et al., Poster, "Structure & Anhydro-Daptomycin and Iso-Daptomycin," ACS 200th Meeting, 1990.
- Mulligan, Catherine N., et al., "Recovery of Biosurfactants by Ultrafiltration," *J. Chem. Tech. Biotechnology*; 47; 1990; pp. 23-29; Society of Chemical Industry; Printed in Great Britain.
- Schott, H.; "Colloidal Dispersions," Remington: The Science and Practice of Pharmacy; vol. 1; 19th Edition; 1995; pp. 252-277; Mack Publishing Company; Easton, Pennsylvania USA.
- Shaw, Duncan J.; "Liquid-Gas and Liquid-Liquid Interfaces," *Introduction to Colloid and Surface Chemistry*; 1989; pp. 64-114; 4th Edition; Butterworth-Heinemann Ltd. Great Britain.
- Sterling, John; "Membrane-Based System Combines Selective Separation with High-volume Throughput," *Genetic Engineering News*; vol. 19; No. 20; Nov. 15, 1999; pp. 1, 34.
- Supersaxo, Andreas et al.; "Mixed Micelles as Proliposomal, Lymphotropic Drug Carrier," *Pharmaceutical Research*; vol. 8; No. 10; 1991; pp. 1286-1291; Plenum Publishing Corporation.
- Sweadner, Kathleen J. et al., "Filter Removal of Endotoxin (Pyrogens) in Solution in Different States of Aggregation," *Applied and Environmental Microbiology*; vol. 34; No. 4; 1977; pp. 382-385; American Society for Microbiology; Printed in the USA.
- Tally, F.P., et al.; "Daptomycin: A Novel Agent for Gram Positive Infections," *Exp. Opin. Invest. Drugs*; 8; 1999; 1223-1238.
- Thimon, L. et al., "Surface-Active Properties of Antifungal Lipopeptides Produced by *Bacillus subtilis*," *J. Am. Oil Chem. Soc.*; 69; 1992; pp. 92-93.
- Yakimov, Michail M. et al.; "Characterization of a New Lipopeptide Surfactant Produced by Thermotolerant and Halotolerant Subsurface *Bacillus licheniformis* BAS50," *Applied and Environmental Microbiology*; vol. 61; No. 5; 1995; pp. 1706-1713; American Society for Microbiology.
- Bayer, A. et al.; LY146032 Compared with Penicillin G in Experimental Aortic Valve Endocarditis Caused by Group G *Streptococci*; *Antimicrobial Agents and Chemotherapy*, Jan. 1988, p. 141-143.
- Eliopoulos, G. M., et al.; "In Vitro and In Vivo Activity of LY 146032, a New Cyclic Lipopeptide Antibiotic," *Antimicrobial Agents and Chemotherapy*, Oct. 1986, p. 532-535.
- Ramos, M. C., "Comparison of Daptomycin, Vancomycin, and Ampicillin-Gentamicin for Treatment of Experimental Endocarditis Caused by Penicillin-Resistant *Enterococci*," *Antimicrobial Agents and Chemotherapy*; Sep. 1992, p. 1864-1869.
- U.S. Appl. No. 07/060,148, filed Jun. 10, 1987, Baker et al.
- Cubicin® (daptomycin for injection) Label 1004—Sep. 2003.
- Cubicin® (daptomycin for injection) Label 1004-1—Revised Aug. 2004.
- Cubicin® (daptomycin for injection) Label 1004-2—Revised Jun. 2005.
- Cubicin® (daptomycin for injection) Label 1004-10-1 Aug. 2010.

\* cited by examiner

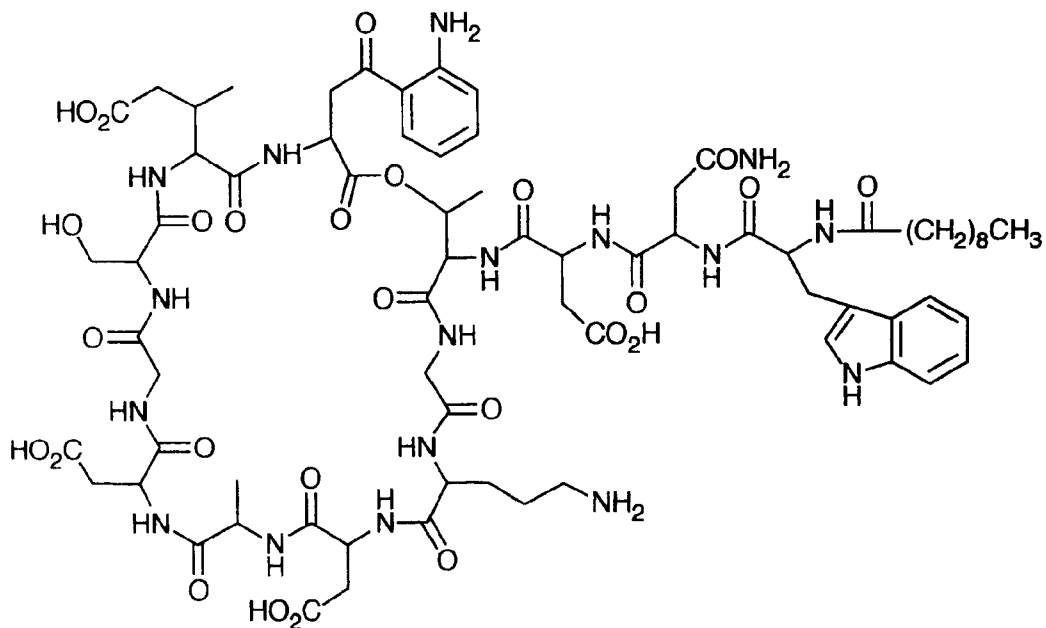


Fig. 1

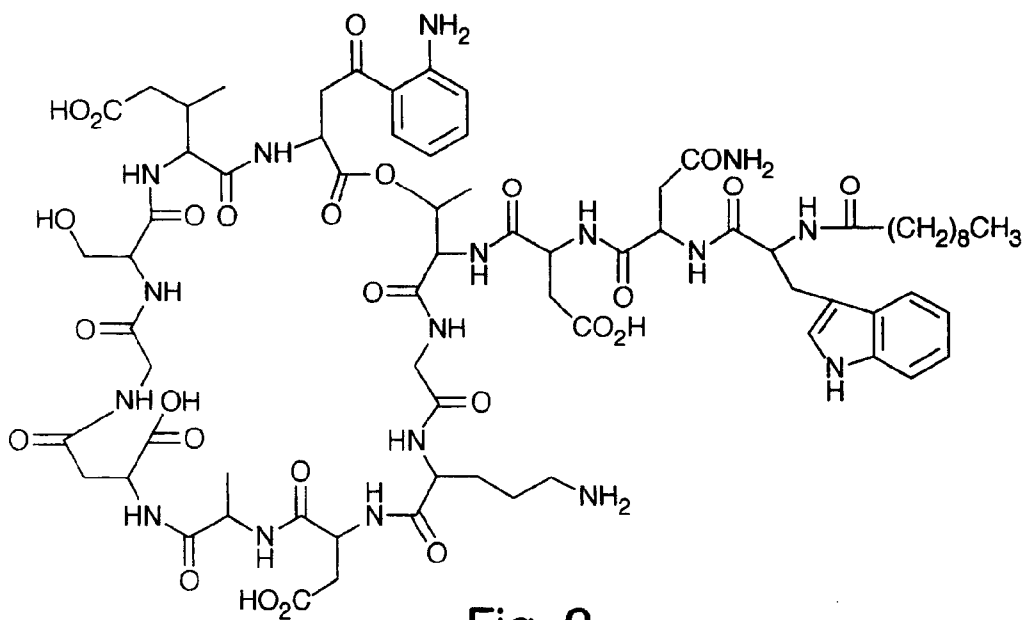


Fig. 2

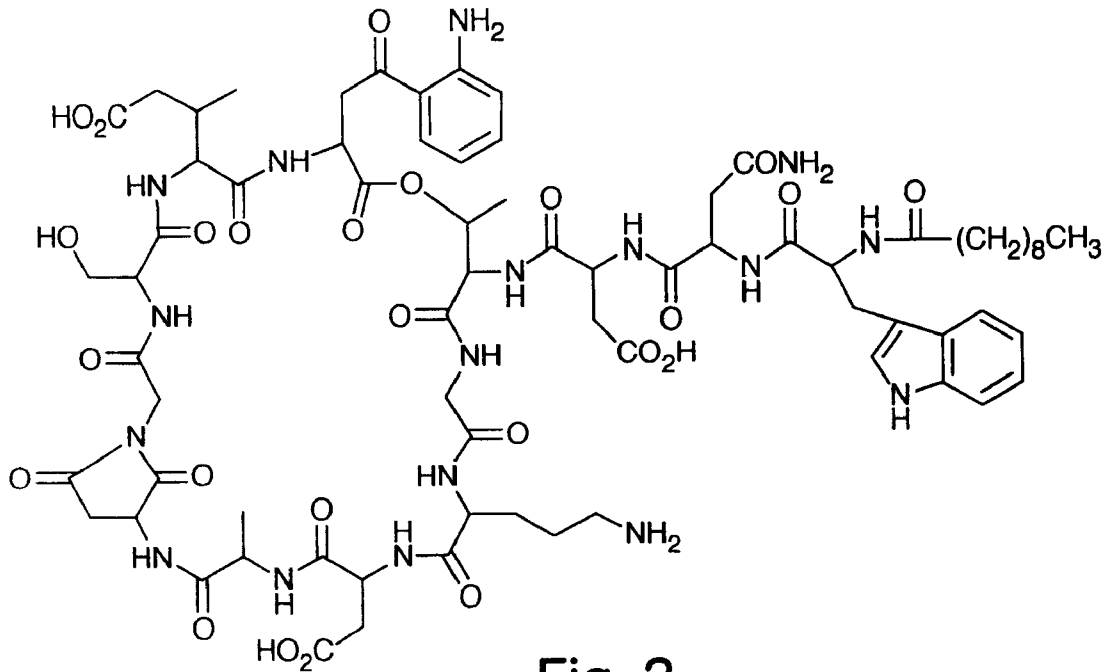


Fig. 3

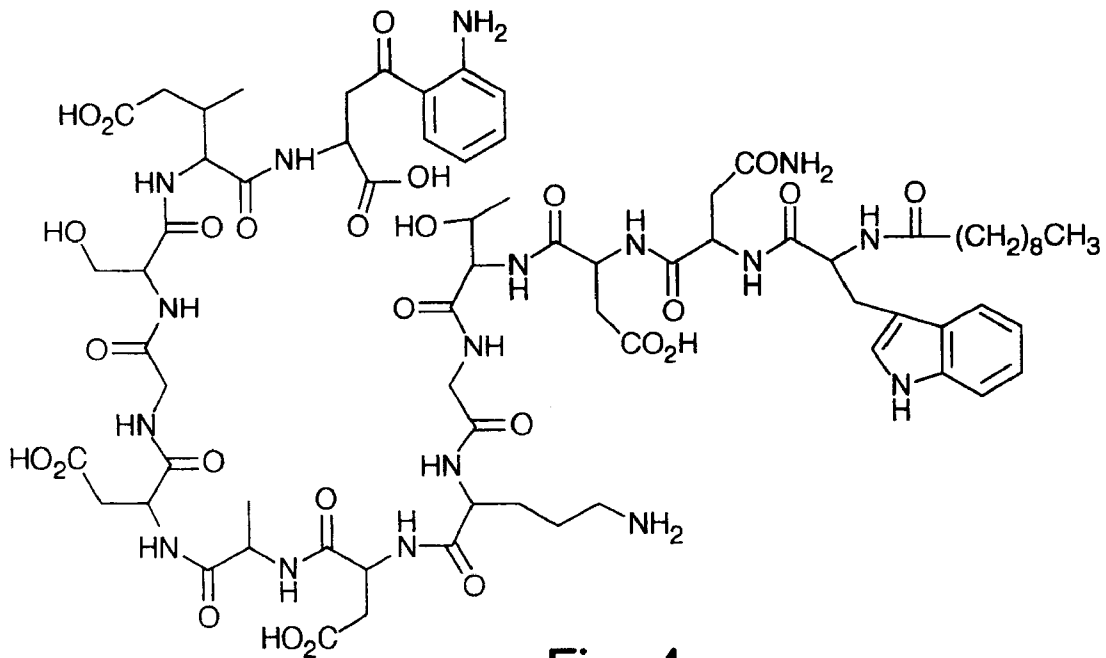


Fig. 4

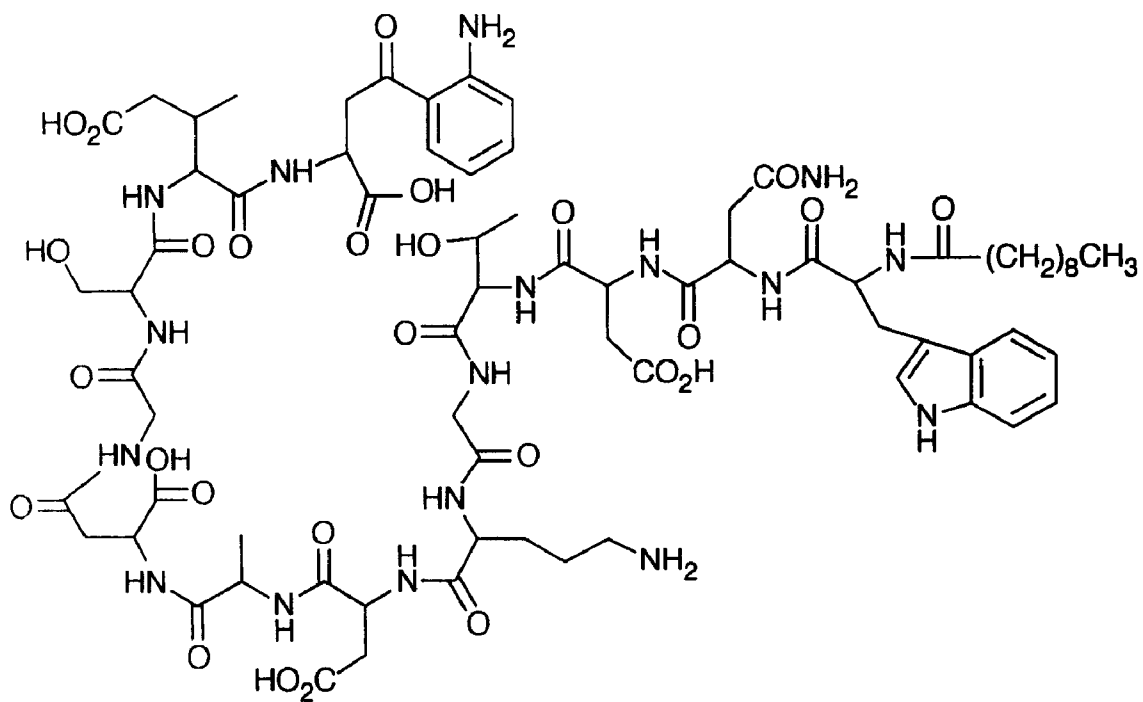


Fig. 5

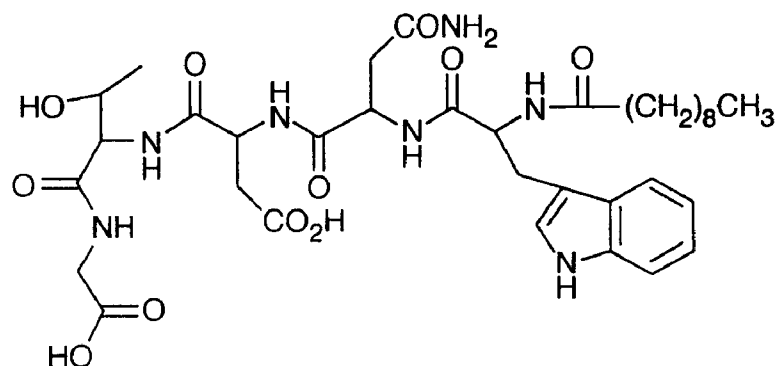


Fig. 6

# 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.