J|A|C|S
COMMUNICATIONS

Published on Web 08/10/2002

Structures of the Muraymycins, Novel Peptidoglycan Biosynthesis Inhibitors

Leonard A. McDonald,* Laurel R. Barbieri, Guy T. Carter, Eileen Lenoy, Jason Lotvin, Peter J. Petersen, Marshall M. Siegel, Guy Singh, and R. Thomas Williamson

Wyeth-Research, 401 North Middletown Road, Pearl River, New York 10965

Received December 12, 2001

Guided by cell-wall specific biological assays,[1] our search for antibacterial agents from natural sources resulted in the discovery of the muraymycins, a family of novel nucleoside-lipopeptide antibiotics with a unique core structure.[2] The muraymycins are cell-wall biosynthesis inhibitors with in vitro activity and in vivo efficacy. The compounds show antimicrobial activity against Gram-positive bacteria and excellent activity against a permeable *E. coli* strain. The muraymycins were shown to inhibit MraY,[3] an enzyme that links UDP-MurNAc-pentapeptide to the C_{55} lipid carrier during bacterial cell-wall biosynthesis. We report here the structures of the muraymycin family of peptidoglycan biosynthesis inhibitors from a *Streptomyces sp.*[4] organism.

Nineteen compounds were purified from a crude complex by a combination of adsorption chromatography, ion exchange, and HPLC. These compounds fall into several series, each differing in the amino sugar or one amino acid residue. When hydroxyleucine is present in place of leucine, a variety of fatty acid esters are formed from core structures with different amino sugars. Compounds lacking the amino sugar (**A5** and **C4**) are apparently hydrolysis products formed during purification. The muraymycin core structures were established using data sets comprised of one- and two-dimensional NMR experiments including proton, carbon, COSY, TOCSY, HMQC, HMBC, and NOESY. The final structures were assembled using HMBC, ROESY, and FT-MS^n experiments. Figure 1 shows the structure of muraymycin B1, the proton and carbon assignments, and the relevant correlations used in establishing the structure. The structures of the remaining compounds (Figure 2) were established from similar data. High-resolution electrospray ionization Fourier transform ion-cyclotron-resonance mass spectrometry (FT-ICR/MS) and FT-ICR/MS^n were used to obtain the elemental compositions for muraymycin B1 and its major diagnostic fragment ions. The computed compositions were consistent with the proposed NMR-derived structure. Doubly and triply charged molecular ions were consistent with the presence of basic groups in the molecule. UV and NMR evidence supported the uracil-uronic acid-ribofuranoside substructures and placed muraymycin B1 in the nucleoside antibiotic family. The closest related structures are liposidomycin[5] and FR-900493.[6] The uronic acid, whose relative stereochemistry was established by J-configuration analysis,[7] is connected to a 2-methoxy-5-amino-5-deoxy-ribofuranoside and to a 3-aminopropyl residue that is juxtaposed to a hydroxyleucyl residue. As evidenced by the production of **C1** from **B1** via mild base hydrolysis (0.1 N NaOH), the hydroxyleucine β hydroxyl group is esterified by an 8-methyl decanoic acid. The fatty acid confers a degree of lipophilicity to the molecule, apparently contributing to its activity.

An unusual feature of the molecule is the hexahydro-2-imino-4-pyrimidylglycyl moiety, a cyclic guanidino amino acid residue

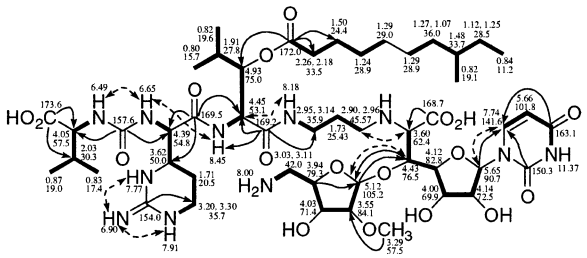


Figure 1. Structure of muraymycin B1 with COSY and TOCSY defined spin networks (bold bonds), select ROESY (dashed arrows), and HMBC (solid arrows) correlations.

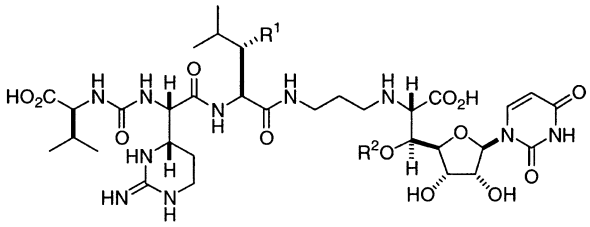


Figure 2. Structure of muraymycins (relative stereochemistry).

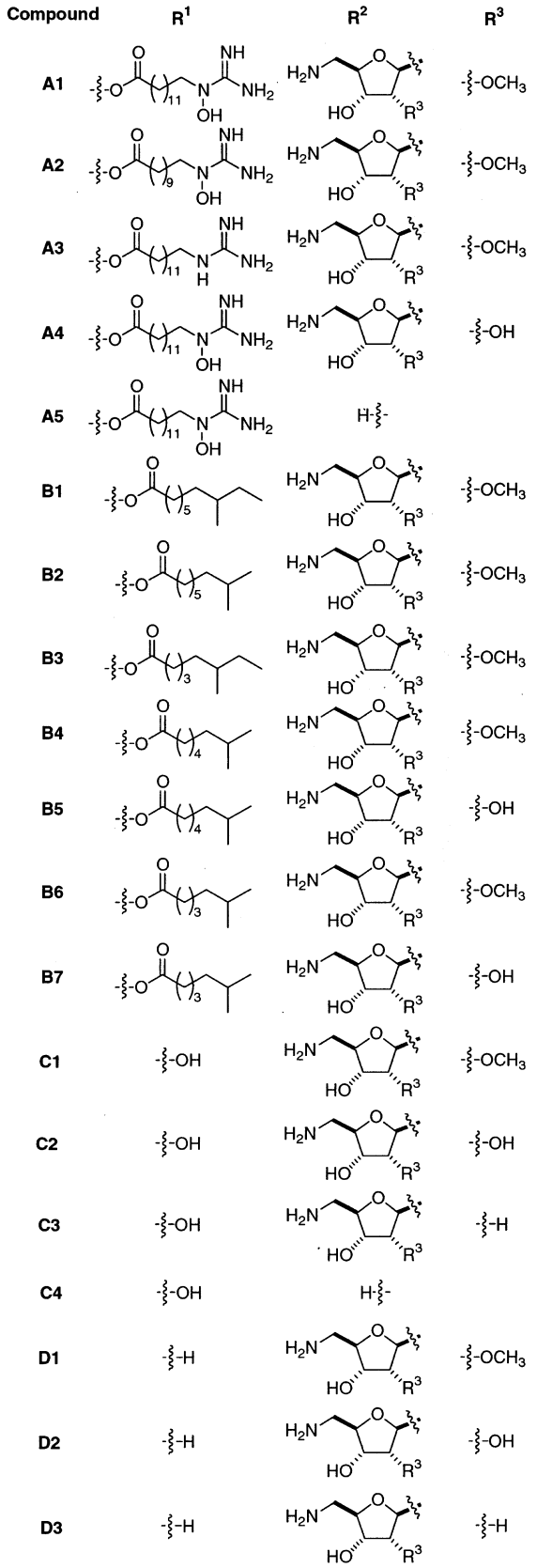
that is the source of much of the basicity. The 2S*,3S* relative stereochemistry of this residue is the same as that of L-epicapreomycidine,[8] a component of protease inhibitors such as chymostatin[9] and elastatinal.[10] Similar to those inhibitors, the muraymycins terminate in a basic amino acid-urea-amino acid motif, effectively reversing the directionality of the peptide chain. The muraymycin structures terminate in an epicapreomycidine-urea-valine moiety.

All of the muraymycins have a common peptide-appended, glycosylated uronic acid core structure. Members of this family differ primarily in the fatty acid (R^1) and the terminal amino sugar (R^2). Compound **A1**, one of the most active members of the family, showed *Staphylococcal* (MIC 2 to 16 μg/mL), *Enterococcal* (MIC 16 to >64 μg/mL), and Gram-negative (MIC 8 to >64 μg/mL) activity. Very good activity was also observed against an *E. coli* imp mutant (MIC < 0.03 μg/mL). Muraymycin A1 demonstrated efficacy in *Staphylococcus aureus* infected mice (ED_{50} 1.1 mg/kg). The presence and structure of the fatty acid (R^1) influence activity, and esters were generally more active than nonesters. Esters with charged (guanidino or hydroxyguanidino) fatty acids were most active in vitro, with increased potency observed with lengthening of the fatty acid chain.

The muraymycins are structurally and mechanistically related to uridyl peptide antibiotics such as the mureidomycins,[11] pacidamycins,[12] napsamycins,[13] liposidomycins,[5] and FR-900493[6] and its derivatives.[14] This compound class reportedly inhibits bacterial translocase,[15] an enzyme that plays a critical role in cell-wall biosynthesis. Translocase, a MraY gene product, catalyzes the transfer of the peptidoglycan precursor, phosphoryl-MurNAc-

* To whom correspondence should be addressed. E-mail: mcdonal@wyeth.com.

10.1021/ja017748h CCC: $22.00



pentapeptide, from UMP in the cytosol to the membrane bound C_{55}-undecaprenyl phosphate lipid carrier.[16] Like the mureidomycins and liposidomycins, the muraymycins inhibit translocase,[3] thereby inhibiting the transfer and attachment of *p*-MurNAc-pentapeptide to the lipid carrier. Five of the muraymycins tested inhibited MraY (lipid II formation) and peptidoglycan synthesis at the lowest concentration tested (0.027 μg/mL; see Supporting Information). These qualitative data show that the muraymycins are comparable to liposidomycin C and mureidomycin A, which were reported to inhibit *E. coli* translocase in vitro with IC_{50} values of 0.05 and 0.03 μg/mL, respectively.[17,18]

Acknowledgment. The authors are indebted to the following individuals for their contributions to various aspects of this project: D. Abbanat, V. Bernan, J. Ashcroft, A. Bailey, N. Huang, K. Janota, J. Korshalla, F. Pinho, B. Rasmussen, M. Singh, K. Tabei, M. Tischler, M. Tuckman, W. Weiss, Y. Yang, M. Young, R. Andersen, G. Kruppa, F. Koehn, G. Schlingmann, F. Kong, H. He, D. Rothstein, M. Decenzo, and M. Kuranda.

Supporting Information Available: Complete NMR data set, table of chemical shifts, and structure determination discussion for muraymycin B1. Data showing inhibition of lipid II and peptidoglycan formation by muraymycins. Proton spectra for each compound (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rasmussen, B. A.; McDonald, L.; Petersen, P. J.; Rothstein, D.; Singh, G.; Yang, Y.; DeCenzo, M.; Kurdnda, M. Abstract of Papers, 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL; American Society for Microbiology: Washington, DC, 2001; Abstract 1162.

(2) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Lenoy, E.; Lotvin, J.; Petersen, P. J.; Siegel, M. M.; Singh, G. Abstract of Papers, 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL; American Society for Microbiology: Washington, DC, 2001; Abstract 1159.

(3) Singh, G.; Yang, Y.; Rasmussen, B.; Petersen, P.; McDonald, L.; Yamashita, A.; Lin, Y.; Norton, E.; Francisco, G.; Li, Z.; Barbieri, L. Abstract of Papers, 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL; American Society for Microbiology: Washington, DC, 2001; Abstract 1163. Inhibition of lipid II and peptidoglycan formation were determined using a TLC methodology adapted from the method described by Mengin-Lecreaulx et al. (Mengin-Lecreulx, D.; Texier, L.; Rousseau, M.; Van Heijenoort, J. *J. Bacteriol.* **1991**, *173*, 4625−4636.). In brief, the MurG biochemical assay utilized *E. coli* membranes to catalyze the late steps in cell wall biosynthesis including *MraY*, the phospho-MurNAc pentapeptide translocase and MurG, the UDP-*N*-acetylglucosaminyl transferase. The formation of lipid II was assessed using radiolabeled UDP-*N*-acetylglucosamine. *E. coli* membranes, compound, UDP-MurNAc pentapeptide, and [^{14}C]-UDP-*N*-acetylglucosamine were incubated, the reaction was terminated by boiling, and samples of reaction mixture were analyzed by TLC. TLC plates were exposed to film, and the inhibition of lipid II and peptidoglycan formation was monitored by comparing the sample area to the control area.

(4) Bernan, V.; Lotvin, J.; Greenstein, M. Abstract of Papers, 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL; American Society for Microbiology: Washington, DC, 2001; Abstract 1158.

(5) Dini, C.; Drochon, N.; Guillot, J. C.; Mauvais, P.; Walter, P.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 533−536.

(6) Ochi, K.; Ezaki, M.; Iwani, M.; Komori, T.; Kohsaka, M. Ep Patent 333177, 1989.

(7) Matsumori, M.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.

(8) Teshima, T.; Konishi, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 508−511.

(9) Tatsuta, K.; Mikami, N.; Fujimoto, K.; Umezawa, S.; Umezawa, H.; Aoyagi, T. *J. Antibiot.* **1973**, *26*, 625−646.

(10) Okura, A.; Morishima, H.; Takita, T.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1975**, *28*, 337−339.

(11) Bozzoli, A.; Kazmierski, W.; Kennedy, G.; Pasquarello, A.; Pecunioso, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2759−2763.

(12) Fronko, R. M.; Lee, J. C.; Galazzo, J. G.; Chamberland, S.; Malouin, F.; Lee, M. D. *J. Antibiot.* **2000**, *53*, 1405−1410.

(13) Chatterjee, S.; Nadkarni, S. R.; Vijayakumar, E. K. S.; Patel, M. V.; Ganguli, B. N.; Fehlhaber, H. W.; Vertesy, L. *J. Antibiot.* **1994**, *47*, 595−8.

(14) Yoshida, Y.; Yamanaka, H.; Sakane, K. Jp Patent 05078385, 1993.

(15) Brandish, P. E.; Kimura, K.-I.; Inukai, M.; Southgate, R.; Lonsdale, J. T.; Bugg, T. D. H. *Antimicrob. Agents Chemother.* **1996**, *40*, 1640−1644.

(16) Boyle, D. S.; Donachie, W. D. *J. Bacteriol.* **1998**, *180*, 6429−6432.

(17) Kimura, K.; Miyata, N.; Kawanishi, G.; Kamio, Y.; Izaki, K.; Isono, K. *Agric. Biol. Chem.* **1989**, *53*, 1811−1815.

(18) Inukai, M.; Isono, F.; Takatsuki, A. *Antimicrob. Agents Chemother.* **1993**, *37*, 980−983.

JA017748H